Elasmobranch Husbandry Manual:

Captive Care of Sharks, Rays, and their Relatives

Mark Smith, Doug Warmolts, Dennis Thoney, and Robert Hueter (Editors)

A Special Publication of the Ohio Biological Survey, Inc. 2004

ISBN-13: 978-0-86727-152-3
ISBN-10: 0-86727-152-3
LC#: 2004115835

The Elasmobranch Husbandry Manual:

Captive Care of Sharks, Rays and their Relatives

Editors

Mark Smith
Doug Warmolts
Dennis Thoney
Robert Hueter

Published by
Ohio Biological Survey, Inc.

Columbus, Ohio 43221-0370 **2004**

Ohio Biological Survey

Special Publication

ISBN-13: 978-0-86727-152-3
ISBN-10: 0-86727-152-3
Library of Congress Number: 2004115835

Publication Director

Brian J. Armitage

Editorial Committee

Barbara K. Andreas, Ph. D., Cuyahoga Community College & Kent State University
Brian J. Armitage, Ph. D., Ohio Biological Survey
Benjamin A. Foote, Ph. D., Kent State University (Emeritus)
Jane L. Forsyth, Ph. D., Bowling Green State University (Emeritus)
Eric H. Metzler, B.S., The Ohio Lepidopterists
Scott M. Moody, Ph. D., Ohio University
David H. Stansbery, Ph. D., The Ohio State University (Emeritus)
Ronald L. Stuckey, Ph. D., The Ohio State University (Emeritus)
Elliot J. Tramer, Ph. D., The University of Toledo

Literature Citation

Smith, M., D. Warmolts, D. Thoney, and R. Hueter (editors). 2004. The Elasmobranch Husbandry Manual: Captive Care of Sharks, Rays and their Relatives. Special Publication of the Ohio Biological Survey. xv + 589 p.

Cover and Title Page
Illustration by Rolf Williams, The National Marine Aquarium, Rope Walk, Coxside, Plymouth, PL4 0LF United Kingdom

Distributor
Ohio Biological Survey, P.O. Box 21370, Columbus, Ohio 43221-0370 U.S.A.

Copyright
© 2004 by the Ohio Biological Survey

All rights reserved. No part of this publication may be reproduced, stored in a computerized system, or published in any form or in any manner, including electronic, mechanical, reprographic, or photographic, without prior written permission from the publishers, Ohio Biological Survey, P.O. Box 21370, Columbus, Ohio 43221-0370 U.S.A.

Layout and Design: Brian J. Armitage, Ohio Biological Survey
Printing: The Ohio State University, Printing Services, Columbus, Ohio

Ohio Biological Survey
P.O. Box 21370
Columbus, OH 43221-0370
<ohiobiosurvey@sbcglobal.net>
www.ohiobiologicalsurvey.org

11-2004—1.5M

FOREWORD

More than half a century ago, the largest shark and ray species were placed on public display. In 1934, the Mito Aquarium in Japan held a whale shark for 122 days, and in 1951, the Marine Biological Station in al-Ghardaqa, Egypt, presented a 10-foot-wide manta ray that had been captured in the Red Sea (Clark, 1953; Clark, 1963). These great wonders were viewed in large, open-water systems where the sea had been netted or penned off to form embayments that were large enough for the fish to swim in, but not large enough to supply the enormous amount of planktonic food they required.

It was not until the 1980's that Senzo Uchida in Okinawa, Japan, succeeded in keeping these creatures alive and healthy for years, feeding them in a closed environment—a giant oceanarium—where they could be viewed in all their magnificence. Hundreds of smaller species of sharks, skates, rays and chimeras are now maintained in over one hundred large public aquariums and in marine laboratories for display and study of their methods of reproduction, feeding habits, and behavioral interactions. Some grow so well they outstrip their enclosures and must be netted and transported back into the sea.

We have come a long way in learning to maintain healthy elasmobranchs. This book reports the latest advances for keeping these marvelous and little-understood fishes on display for the public to see and scholars to study alive, in contrast to the many great illustrated tomes on the detailed anatomy of elasmobranchs based upon dissections of dead specimens.

It is a personal pleasure for me to write the foreword to this book. In the early days at Mote Marine Laboratory (called Cape Haze Marine Laboratory in the 1950's), we first studied large elasmobranchs, especially sharks, in open stockade-built "pens" in the bay next to our laboratory pier on the west coast of Florida. We appreciated the easy maintenance of having fresh seawater wash in and out of our big (70 ft x 40 ft) "Skinner Box," and first discovered to our amazement the individuality of our sharks and rays, their gentleness and their ability to learn and make visual discriminations (Clark, 1959). Our lemon and tiger sharks had their babies in our pens. We "walked" and force-fed many newly caught sharks just to keep them alive. But we were at the mercy of weather changes, winter chills, and red tides. We noted that our captive sharks detected and reacted differently to the lowest concentrations of the red tide organism before bathers at nearby beaches started coughing from onshore breezes.

One of the most difficult types of sharks for us to keep alive were the several species of hammerheads. We could not even bring them back alive from the nearby Gulf of Mexico where we set our lines. Only the small bonnetheads, netted by fishermen, would live briefly in our pens. Today, great hammerheads are swimming and feeding at Mote Marine Laboratory in two large research aquariums, attesting to our great strides in keeping them alive and well. And Senzo Uchida now keeps several healthy whale sharks and manta rays together in one of the world's largest oceanariums. What we will learn from these captive creatures will be incredible.

It was an honor to open the 1st International Elasmobranch Husbandry Symposium in Orlando, Florida, in October 2001, and now to introduce this book that compiles the results of the Symposium.

Eugenie Clark
Center for Shark Research
Mote Marine Laboratory
1600 Ken Thompson Parkway,
Sarasota, FL 34236, USA
October 2004

REFERENCES

Clark, E. 1953. Lady with a Spear. Harper and Brothers, New York. 225 p.
Clark, E. 1959. Instrumental conditioning in lemon sharks. Science 130(3369): 217-218.
Clark, E. 1963. The maintenance of sharks in captivity, with a report on their instrumental conditioning. In P.W. Gilbert (ed.), *Sharks and Survival*, Heath and Co., Boston. p. 115-149.

INTRODUCTION

Sharks and rays are an important attraction for public aquariums where they provide an interesting and invaluable educational tool. Elasmobranchs are also maintained in public aquariums and marine laboratories for the purposes of scientific investigation. Much of what we know about these inscrutable animals has been learned through observing them in aquaria.

Elasmobranchs exhibit a K-selected life history strategy, characterized by low fecundity, slow growth rates, and late sexual maturity. Unfortunately, this life history strategy makes sharks and rays susceptible to overexploitation. Reproduction of elasmobranchs in aquariums is poorly understood and is frequently restricted by the physical limitations of facilities. In addition, unless appropriate husbandry practices are adopted, elasmobranch survivorship in aquariums can be lower than in their natural habitat.

As a basic conservation measure, the elasmobranch caretaker community needs to increase its level of peer review, constantly exchange information, and continually update prevailing husbandry practices. In addition, it should provide assistance to new and developing facilities, where less than ideal husbandry protocols may be adopted through lack of training or readily available information.

Until the present day there has been no handbook enumerating the captive care of sharks and rays. Information has been available in scientific journals, the gray literature, and predominantly within the memories of experienced aquarium veterans, but it has been typically scattered and difficult to access. It seems incredible that the husbandry of such an important and charismatic group of animals has not been more comprehensively addressed in the literature. *The Elasmobranch Husbandry Manual: Captive Care of Sharks, Rays and their Relatives* attempts a first step toward addressing this oversight.

The development of the *Manual* was slightly unorthodox and merits some description. It began as a bullet list of husbandry topics, tabled and discussed at the 1999 Regional Aquatic Workshop in Minneapolis, Minnesota (USA). This list was then fine-tuned over ensuing months by a steering committee established at the same meeting. The initial premise was to generate an exhaustive list of elasmobranch husbandry topics and then solicit contributions to match those topics from individuals considered to be leaders in their respective fields. As the *Manual* was conceived to be a conservation initiative, participation was to be, and indeed remained, entirely voluntary.

As a catalyst to the development of the *Manual* the 1st International Elasmobranch Husbandry Symposium was held in Orlando, Florida (USA), between the 3rd and 7th of October in 2001. The first three days of the Symposium included invited papers, representing the formal chapters of the *Manual*, and an additional day was made available for the presentation of voluntary contributions and the discussion of a plan of action. Bringing together ~180 learned individuals from 16 countries, the Symposium provided an opportunity to exchange information about the husbandry of elasmobranchs and to conduct an informal peer review of the contributions made by invited speakers. Following the Symposium, invited contributions were then peer-reviewed in a more formal manner and the result is the *Manual* you are now reading.

The ultimate objective of the *Manual* was to produce a single-reference handbook that could be used as a guide to the captive care of elasmobranchs, assisting in the development of new exhibits, aiding the training of husbandry personnel, and answering specific husbandry questions about this important taxonomic group. In addition, it was a project objective to make the *Manual* available free-of-charge, via the World Wide Web, allowing anyone who might work with elasmobranchs ready access to the information. The resulting website is to be used as a forum to distribute the *Manual*, to post *Manual* updates, and to provide additional information and husbandry tools useful to elasmobranch caretakers.

A number of articles presented at the 1st International Elasmobranch Husbandry Symposium were deemed to be of lesser immediate relevance and were not included in the *Manual*. These articles, in combination with archive articles from previous issues of *Drum and Croaker,* have been compiled by Peter J. Mohan (editor of *Drum and Croaker*) and published as *The Shark Supplement: 40 Years of Elasmobranch Husbandry Science, Speculation, and Apocrypha* (*Drum and Croaker Special Edition No. 2*). This supplement may be accessed through either the *Manual* or the *Drum and Croaker* websites.

Aquariology is an emerging science and many experienced aquarium professionals have little formal scientific training, yet many of these individuals have years of valuable hands-on experience. Conversely, many workers who actively cooperate with public aquariums are professional academics and respected leaders in their respective fields. The *Manual* brings together contributions from both ends of this spectrum. This process has given the *Manual* an inclusive and, at times, a slightly eclectic feel. Rather than detract from the merit of individual contributions, or indeed the broad coverage of the manual, we believe that this unique characteristic enhances the accessibility and ultimately the applicability of the *Manual*. It was always considered that the *Manual* would serve, in part, as a bridge between pure science and applied aquariology, and we trust that this goal has been achieved.

The editors,

Mark Smith
Director
cosestudi
302 Dakota Wool Stores,
88 Macquarie Street,
Newstead, QLD, 4006,
AUSTRALIA
T ++ 61 0 732 542 096
E mark@cosestudi.com

Doug Warmolts
Asst. Director of Living Collections
Columbus Zoo & Aquarium
9990 Riverside Dr. Box 400,
Powell, Ohio 43065, USA
T ++1 614 724 3524
E doug.warmolts@columbuszoo.org

Dennis Thoney
Associate Director
Bodega Marine Laboratory
University of California, Davis
P.O. Box 247
Bodega Bay, CA 94923-0247, USA
T ++1 707 875 2211
E dathoney@ucdavis.edu

Robert Hueter
Director
Center for Shark Research
Mote Marine Laboratory
1600 Ken Thompson Parkway,
Sarasota, FL 34236, USA
T ++1 941 388 4441
E rhueter@mote.org

ACKNOWLEDGEMENTS

Numerous individuals and organizations greatly assisted with the development of the *Manual* and indeed the project could never have been completed without the generous contribution of their time and resources. We would like to thank the following individuals:

Project steering committee

George Benz, Ilze Berzins, Greg Charbeneau, Joe Choromanski, Jerry Crow, Jane Davis, Ray Davis, Beth Firchau, Pat Garratt, Suzanne Gendron, Alan Henningsen, John Hewitt, Robert Hueter, Max Janse, Allan Marshall, Tony McEwan, Pete Mohan, Dave Powell, Juan Romero, Juan Sabalones, Mike Shaw, Mark Smith, Frank Steslow, Dennis Thoney, Gary Violetta, Doug Warmolts, and Marty Wisner.

Project sponsors

A very special thanks to the David and Lucile Packard Foundation, without whom the *Manual* could not have been possible, and additional thanks to these other project sponsors: Chester Zoo, Columbus Zoo and Aquarium, Florida Aquarium, International Design for the Environment and Associates, The Living Seas, Monterey Bay Aquarium, Mote Marine Laboratory, National Aquarium in Baltimore, National Marine Aquarium, Núcleo de Pesquisa e Estudo em Chondrichthyes, Oceanário de Lisboa, Ripley Aquariums (a division of Ripley Entertainment, Inc.), SeaWorld Orlando, and Shark Reef Mandalay Bay.

I D E A International Design for the Environment Associates, Inc.

Symposium organizers and moderators

Doug Warmolts, Mark Smith, George Benz, Ilze Berzins, Joe Choromanski, Jerry Crow, Greg Charbeneau, Kevin Curlee, Jane Davis, Ray Davis, Becky Ellsworth, Beth Firchau, Sarah Fowler, Suzanne Gendron, Alan Henningsen, Robert Hueter, Allan Marshall, Pete Mohan, Frank Murru, Mafalda Sousa Pires, Dave Powell, Juan Romero, Juan Sabalones, Dennis Thoney, Tom Schmid, Gary Violetta, Marty Wisner, and Ken Yates.

Manual reviewers

Brian Armitage, Jackson Andrews, George Benz, Ilze Berzins, Greg Cailliet, Mary Camhi, José Castro, Greg Charbeneau, Joe Choromanski, Geremy Cliff, Jerry Crow, Kevin Curlee, Jane Davis, Ray Davis, Heidi Dewar, Carol Farmer, Beth Firchau, Sonja Fordham, Sarah Fowler, Jim Gelsleichter, Suzanne Gendron, Carrie Goertz, Ken Goldman, Marty Greenwald, Joe Groff, Perry Hampton, Jay Hemdale, Alan Henningsen, Robert Hueter, Robin James, Ray Jones, Carl Luer, Holly Martel Bourbon, Tony McEwan, Steve Menzies, Pete Mohan, Henry Mollet, John Morrissey, Mike Murray, Frank Murru, Jack Musick, John O'Sullivan, Dave Powell, Paula Powell, Sarah Poynton, Alison Davidson nee Scarratt, Peter Scott, Mike Shaw, Mahmood Shivij, Mark Smith, Andy Stamper, Frank Steslow, Scott Terrell, Dennis Thoney, Gary Violetta, Gerard Visser, Mike Walsh, Hans Walters, Brent Whitaker, Rolf Williams, Marty Wisner, Reid Withrow, and Ken Yates.

Additional support

A special thanks to Mike Shaw for copy editing the *Manual* and to Pete Mohan for editing the references throughout. Thank you to Brian Armitage and the Ohio Biological Survey, and a special thanks to Gordon McGregor Reid and Heather Koldewey. Thank you to Rolf Williams and Juan Romero who were responsible for illustrations and photographs not supplied by the authors.

Additional support for the project was provided by the following individuals: Jackson Andrews, Greg Bell, George Benz, Ilze Berzins, Kevin Bonifas, Carlos Bohorquez, Andrew Camoens, Ellen Carpenter, Jeffrey Carrier, José Castro, Greg Charbeneau, Natasha Christie, Joe Choromanski, Frederick Chua, Eugenie Clark, João Pedro Correia, Jerry Crow, Mike Crumpler, Ray Davis, Andy Dehart, Kevin Feldheim, Beth Firchau, Sarah Fowler, Rod Garner, Suzanne Gendron, Carrie Goertz, Manoel Mateus Bueno Gonzalez, Joe Groff, Randy Hamilton, Gary Hannon, Bobbie Headley, Kendall Heard, Silvio Heidler, Alan Henningsen, John Hewitt, Diane Hockman, Jack Jewell, Ray Jones, Kay Kunze, David Lai, Claude Le Milinaire, Jeff Mahon, Alan Marshall, Tom Mattix, Cindy Melchiorre, Steve Miller, Pete Mohan, Frank Murru, Julie Packard, João Falcato Pereira, Nuno Pereira, Glen Pittenger, Dave Powell, Susanne Riddle, Robert Rinker, Juan Romero, John Rupp, Paul Russell, Juan Sabalones, Tom Schmid, Lee Simmons, Jennifer Sowash, Mark Stetter, Scott Terrel, Gary Violetta, Matt Walker, Mike Walsh, Nancy Walters, Rolf Williams, Ken Yates, and Forrest Young.

DISCLAIMER

*The Elasmobranch Husbandry Manual: Captive Care of Sharks, Rays and their Relatives i*s intended to present the current scientific and experiential understanding of the captive care of elasmobranchs in aquarium or research laboratory settings. Some contributions lend themselves to scientific rigor, where material presented is supported by peer-reviewed literature. Other contributions are based, out of necessity, on the collective experience of professional aquarists, because relevant scientific literature is scant or non-existent. The contributors and editors cannot be, and are not, legally, financially or in any other way, responsible for the application of techniques described within the *Manual*. When undertaking any procedures or techniques outlined in the *Manual*, it is up to individual workers to assess the unique circumstances of their situation, apply common sense, and subsequently apply any procedures or techniques at their own risk. In all cases, the reader of this *Manual* is cautioned not to use this handbook as an exact step-by-step guide, but rather as a starting reference point for further case-specific research.

ELASMOBRANCH PLAN OF ACTION

During the 1st International Elasmobranch Husbandry Symposium a plan of action for the elasmobranch caretaker community was discussed and developed. The premise of the plan of action was that it could be used by regional taxon advisory groups and individual institutions when prioritizing objectives, collection plans, programs to be funded, etc. In particular, the plan of action had four primary objectives: (1) assist in the understanding, protection, and recovery of threatened shark, skate, and ray species worldwide; (2) improve the husbandry of sharks, skates, and rays maintained in captivity; (3) provide quality conservation and research project opportunities for public aquariums; and (4) establish the public aquarium community as a significant player in elasmobranch conservation. These objectives were to be more specifically addressed through seven areas of focus: (1) legislation, permitting and collection; (2) husbandry; (3) veterinary care; (4) captive breeding; (5) re-introductions; (6) research; and (7) education, outreach and advocacy. For the reader's reference, the plan of action is presented in its original form. The reader should note that the plan of action is a living document and that some of the identified action items are in progress or indeed have been completed since the Symposium.

Legislation, permitting, and collection

1. Public aquariums should be familiar with the current conservation status of any species proposed for display by regularly consulting such resources as the World Conservation Union's (IUCN) Red List of Threatened Species™ (www.redlist.org).

2. Public aquariums should be familiar with relevant legislation and permitting requirements, at all levels, by regularly consulting such international resources as the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES) (www.cites.org) and the Convention on Migratory Species (CMS) (www.cms.int), as well as national and state agencies, such as the National Marine Fisheries Service (NMFS) (www.nmfs.noaa.gov) and the Florida Fish and Wildlife Conservation Commission (FFWCC) (www.myfwc.com).

3. Public aquariums should never "export" demand for a threatened species (e.g., *Pristis* spp.) to regions where legal protection for that species is inadequate.

4. Public aquariums should ensure that third-party commercial collectors, acquiring animals on their behalf, always meet permitting requirements and use appropriate collection and transport techniques.

5. Public aquariums should communicate effectively with permitting agencies, not only by adhering to required reporting schedules but by building an ongoing healthy rapport with local authorities. Communications should include: (1) an exchange of information about both the conservation value of public aquariums and their specific needs; and (2) feedback about the observed status of permitted species (e.g., observed frequency in the wild, behavior in captivity, etc.).

6. Public aquariums should communicate information about commercial collectors, acquisition techniques, and permitting agencies.

7. Priority legislation, permitting, and collection objectives:

    a. Develop a comprehensive species list showing correct nomenclature, current conservation status, and relevant governing legislation.

    b. Develop a review protocol for potential commercial collectors and suppliers

    c. Develop a database of apposite commercial collectors and suppliers.

    d. Develop an elasmobranch acquisition protocol—i.e., adapt the existing American Zoo and Aquarium Association (AZA) (www.aza.org) acquisition policy.

    e. Monitor the development of the Marine Aquarium Council (MAC) (www.aquariumcouncil.org). Support the development of a supplier certification scheme and include relevant aspects within the elasmobranch acquisition protocol.

Husbandry

1. Public aquariums should ensure that husbandry personnel are fully conversant with basic husbandry techniques.

2. Public aquariums should question the application of routine husbandry procedures and ensure that they understand the rational behind their continued use. Don't adopt the old adage of "…it's been done that way for years…", as original justification may be flawed or no longer relevant.

3. Public aquariums should communicate more effectively about elasmobranch husbandry experiences. Potentially useful data should be channeled to appropriate research and data-storage institutions.

4. Public aquariums should maintain standardized, long-term, and accurate husbandry records. Techniques for industry-wide communication of large data series should be developed.

5. Publish! Relevant elasmobranch husbandry observations should be published in peer-reviewed scientific journals and the gray literature (e.g., *Zoo Biology*, *Drum and Croaker*, etc.).

6. Priority husbandry objectives:

    a. Establish an elasmobranch husbandry specialist group (focusing on nutrition, record-keeping standards, etc.).

    b. Develop a handbook of elasmobranch husbandry techniques.

    c. Develop a data bank of husbandry information, including water quality parameters, nutrition, etc.

    d. Standardize record-keeping and data exchange techniques.

    e. Develop a multi-disciplinary program for a flagship, conservation-dependent, species—e.g., the sand tiger shark (*Carcharias taurus*). Generate a model list of research questions, subdivide the work, and determine sources of funding. Aspects of such a program could include: (1) investigating the cause of spinal deformities; (2) establishing "normal" blood parameters; (3) investigating reproductive hormones and cues; (4) developing a collaborative breeding program; (5) investigating global genetic variation; and (6) investigating the status of wild populations.

Veterinary care

1. Public aquariums should ensure that husbandry personnel are fully conversant with basic veterinary practices.

2. Tissue and blood samples (from routine examinations, biopsies, specimen losses, etc.) should be taken and analysed, wherever possible, to build a database of "normal" parameters.

3. Public aquariums should communicate more effectively about veterinary experiences. Potentially useful data should be channeled to appropriate research and data-storage institutions. A secure mode of information sharing with academics, to protect institutions and data ownership, should be developed. One-on-one interactions between public aquariums and academic institutions is encouraged.

4. Public aquariums should maintain standardized, long-term, and accurate veterinary records. Techniques for industry-wide communication of large data series should be developed.

5. Publish! Relevant veterinary observations should be published in peer-reviewed scientific journals and the gray literature (e.g., *Zoo Biology*, *Drum and Croaker*, etc.).

6. Priority veterinary care objectives:

    a. Establish a veterinary specialist group to focus on pharmaceutical use, blood parameter “norms”, tissue sampling techniques, etc.

    b. Develop a data bank of veterinary information, including: (1) pathology—symptoms, causative agents, and treatments; (2) hematology and blood chemistry—wild and captive “norms”; (3) pharmaceuticals—dosages, efficacy, and species sensitivity; (4) photo-imaging—clinical, diagnostic, histological, and microbiological; and (5) standardized record-keeping and data exchange techniques.

Captive breeding:

1. Public aquariums intending to develop a captive breeding program should consider which species represent a conservation priority, specifically: (1) is the species listed as endangered or critically endangered on the IUCN Red List of Threatened Species™?; (2) is the species regionally endemic, little studied, or even undescribed, and at risk of losing its habitat?; (3) is the species in demand for public aquariums—e.g., sand tiger sharks, zebra sharks (*Stegostoma fasciatum*), spotted eagle rays (*Aetobatus narinari*), etc.?; and (4) does the aquarium have the requisite expertise?

2. Public aquariums should consider the longer-term objectives of the breeding program, specifically: (1) will breeding and inter-aquarium distribution of the species reduce pressure on wild populations?; (2) will the breeding program contribute toward the collective knowledge of elasmobranch reproduction?; (3) is the intention to breed a pool of animals for future release into the wild and if so is this a fitting objective (refer to re-introductions below)?

3. Public aquariums should discourage the breeding of common species excess to current requirements. Consider usage of surplus animals for invasive reproduction research (e.g., organ development studies, etc.).

4. Priority captive breeding objectives:

    a. Establish a captive breeding specialist group.

    b. Develop a databank of captive breeding information detailing relevant aspects of species successfully reproduced, or exhibiting reproductive behavior, in public aquariums.

    c. Establish zoological studbooks for those species that have bred successfully in captivity and that require a management program.

    d. Develop a common system of identification to track individual animals within a breeding metapopulation.

    e. Establish a centralized breeding facility to support the development of collaborative breeding programs for key species (e.g., sand tiger sharks, zebra sharks, etc.).

    f. Establish a tissue bank as a resource for reproduction studies. Support genetic and hormonal research by making available tissue samples for appropriate projects.

Re-introductions

1. Draft and adopt a re-introduction policy consistent with IUCN Re-introduction Specialist Group (RSG) (www.iucnsscrsg.org) guidelines—i.e., to not release elasmobranchs into the wild, with the exception of coastal public aquariums and marine laboratories that have open systems and short-term specimen retention times, and to never release exotic species. Develop a corresponding rigorous re-introduction protocol.

It should be clear that the release of elasmobranchs as a solution for surplus and unwanted animals is not acceptable.

Research

1. Public aquariums should encourage research. The cost-benefits of research activities need to be clearly explained and justified to aquarium management (e.g., improved husbandry practices; improved conservation policies and performance; improved education programs, etc.).

2. Public aquariums developing institutional research programs should ensure that the following issues have been considered and are clearly established for each project: (1) what will the study accomplish?; (2) why does the study need to be undertaken?; (3) how much will the study cost?; (4) how long will the study take?; (5) who will undertake the study and are they qualified?; (6) is the study duplicating effort elsewhere?; and (7) will the study integrate smoothly with a wider inter-institutional research effort? These issues are particularly important if you wish to attract funding.

3. Public aquariums should take advantage of their innate resources (i.e., infrastructure, human, etc.) and focus investigations within their area(s) of expertise.

4. Public aquariums should develop investigations in concert with existing research and conservation efforts currently undertaken by academia.

5. Public aquariums should encourage the collection and dissemination of data for both rare species and those species targeted by conservation and management programs (e.g., *Pristis* spp.).

6. Public aquariums should optimize the value of interns by maintaining a list of valuable projects that can be undertaken during their tenure.

7. Priority research objectives:

    a. Establish a research specialist group.

    b. Establish an independent academic review committee.

    c. Establish a mechanism for systematically evaluating, selecting, and implementing quality research projects that may be supported and funded by the AZA's Conservation Endowment Fund, the European Union, etc.

    d. Establish a database of ongoing research projects undertaken by member institutions of the various regional zoological associations—e.g., the AZA, the European Association of Zoos and Aquaria (EAZA) (www.eaza.net), the Australasian Regional Association of Zoological Parks and Aquaria (ARAZPA) (www.arazpa.org.au), etc.

    e. Develop a list of future research priorities oriented toward one or more of the following: (1) improved elasmobranch captive management (e.g., nutrition, water quality, exhibit design, enrichment, etc.); (2) elasmobranch captive breeding programs; (3) *in situ* or *ex situ* conservation efforts; (4) recovery of endangered wild elasmobranch populations; and (5) improved education, outreach, and advocacy techniques.

Education, outreach and advocacy:

1. Public aquariums must establish and preserve education as a fundamental aspect of their mission. Public aquariums should identify education priorities related to elasmobranchs and integrate them into their educational program where appropriate.

2. Public aquariums should be aware of, and contribute toward, existing and developing conservation and management strategies on an international and domestic level (e.g., CITES, IUCN, MAC, etc.). Public aquariums should directly apply and disseminate information about same.

3. Public aquariums should improve links with other public aquariums, academia, and government agencies, to ensure possession of up-to-the-moment information about all aspects of elasmobranch conservation. Better communication should be sought through attendance at relevant meetings (e.g., the annual meetings of the American Elasmobranch Society (AES) (http://www.flmnh.ufl.edu/fish/organizations/aes/aes.htm), the Regional Aquatic Workshop, the European Union of Aquarium Curators (EUAC) (www.euac.org), the European Elasmobranch Association (EEA) (www.eulasmo.org), etc.), participation on list servers (e.g., Elasmo-L), and exchange of peer-reviewed publications, etc.

4. Public aquariums should be proactive about using the media for education and advocacy purposes.

5. Public aquariums should promote and support the activities of the IUCN Shark Specialist Group (SSG) (http://www.flmnh.ufl.edu/fish/organizations/ssg/ssg.htm) and *Shark News*, the official organ of the SSG.

6. Public aquariums should promote and support MAC and discourage hobbyists from acquiring threatened elasmobranchs (or those species that will out-grow exhibits).

7. Priority education, outreach, and advocacy objectives:

    a. Establish an education specialist group.

    b. Develop a comprehensive educational package for distribution to all public aquariums (e.g., an update of the IUCN SSG slide presentation *Sharks in Danger*). Issues covered by the educational package should include: K-selected life history, overfishing, finning, shark attack, responsible trade practices (e.g., retail outlets, hobbyists, and the MAC certification scheme), ongoing research projects (e.g., biomedical)., etc.

    c. Develop techniques for improved public access to elasmobranchs (e.g., touch-pools); increasing educational opportunities and augmenting the uptake of conservation messages. Develop suitable guidelines for same.

TABLE OF CONTENTS

The Elasmobranch Husbandry Manual: Captive Care of Sharks, Rays and their Relatives, pages 1-14.
© 2004 Ohio Biological Survey

Chapter 1
Elasmobranchs in the Public Aquarium: 1860 to 1930

THOMAS J. KOOB

The Center for Research in Skeletal Development and Pediatric Orthopaedics,
Skeletal Biology Section, Shriners Hospitals for Children,
Tampa, FL 33612, USA.
E-mail: tkoob@shctampa.usf.edu

Abstract: Elasmobranchs have been exhibited in public aquariums and marine biological stations since their inception in the 1860's. Some of these institutions were remarkably successful at maintaining elasmobranchs in captivity, in some cases holding specimens for many years. These early aquariums developed capture and transportation techniques, water quality parameters, feeding regimens, and display methods for various species of elasmobranchs. Many of the husbandry techniques developed are still used today. Public aquariums and marine biological stations provided some of the first opportunities to observe and document the biology of elasmobranchs (e.g., feeding, mating, and egg-laying behavior).

It was early in 1873 and the Brighton Aquarium was about to open to the public when the manager and naturalist, Henry Lee, was called to the main display tank (volume: 189 m^3) to see the following: "…one of the nursehounds [*Scyliorhinus stellaris*] had hanging from her, close to her body, an egg which had just been extruded. I was [delighted] to have the opportunity of observing an operation which has been the subject of speculation and conflicting opinion … for five hours [the shark] swam around … generally near the surface … appearing neither to care for, nor to be incommoded by, the appended egg … She began to rub herself heavily along the shingle at the bottom of the tank, and to endeavor to free herself of her encumbrance by vigorous contortions of the body and rapid muscular motion of the tail. In readiness for such an event … I had previously ordered to be prepared some artificial gorgonians, made of the twigs of a birch broom, and fastened firmly, in the shape of a little bush, to a heavy stone. One of these I now lowered into the tank, close to the parturient fish … in about a half hour she began to reconnoiter my sham gorgonian, swam round it twice, and then, seemingly satisfied that it would suit her purpose, deliberately tried to make a way through the midst of the little bush near its root. At this part, however, the sticks of the birch broom were [stiff] … and she failed to drive a heading into them; but, with wonderful intelligence, she rose higher and higher, and at last succeeded in separating with her nose the upper and more pliant twigs, and forced a passage for herself through the brushwood. Resting for a second, she, with a quick undulation of the [hind] portion of her body, entangled the tendrils at the first presented end of the egg amongst the branches, and sailing through and around the upper and slighter part of the little tree, dragged from her body the tendrils at the other end of the egg, and with them another egg, similarly furnished. The moment this second egg had passed from the orifice, the mother fish gently sank towards the bottom, and curling herself in the form of a ring—nose and tail meeting, and partially overlapping—encircled the base of the bush, and with its stem as an axis, revolved around it fourteen times, winding from her body the tendrils of the last produced end of the second egg … As soon as this was completed she swam slowly away, and gave no further attention to her embryo progeny…"

While this behavior was observed several times at other aquariums before the close of the century, it was only brought to the attention of scientific circles over 100 years later (Castro et al., 1988). In the mid 1800's, although egg cases had been found entwined around algae, corals, shells, and rocks, either at low tide or in the strand after storms, there was uncertainty about how they got there. Was it by currents and simple chance that

the tendrils became entangled, were the tendrils like climbing vines that curled around an object when contact was made, or did the female actively moor the eggs? Lee, amongst others, was convinced that such a secure and orderly attachment could only be effected by the parent fish, not only intentionally mooring the egg but choosing a specific locality. So when the Brighton Aquarium was built, it provided the opportunity to answer the question. Of particular interest to the aquarist, Lee was not only observing the animals, many hours into the night on some occasions, but he had prepared a sham gorgonian. Here, in 1873, was an aquarist competent by the standards of today.

This incident gives a good sense of how successful aquariums had become at maintaining elasmobranchs within a decade of the opening of the first public facility. Granted, dogfish are small, temperate, and sedentary species, surviving in aquariums relatively easily, but these animals appeared to be in good health as they were readily laying eggs. Already, capture, transport, feeding, and water quality control techniques were being developed for elasmobranch exhibits.

EARLY AQUARIUMS

The Brighton Aquarium was not the first public aquarium to open, nor was it the first to hold elasmobranchs in captivity. With the popular success of the aquarium at the Zoological Society's Crystal Palace in London, as well as the burgeoning popularity of parlor aquariums in the 1850's (see Taylor, 1993, for an excellent entry into the literature pertaining to parlor aquariums), interest in public displays of marine life, particularly large displays open to the general public, increased enormously. However, before 1860, aquariums were rather static affairs where the water was exchanged only infrequently, and by hand at that.

The development of the modern aquarium originated in the pioneering designs of William Alford Lloyd, an Englishman with both vision and the wherewithal to realize it. He recognized that a supply of clean, circulating seawater was essential to the health of the organisms. Today the design seems relatively simple (although many aquariums used a similar system well into the 20th century): seawater was pumped or trucked from the nearest sea into a basement holding tank or cistern, from there it was pumped to a level above the display tanks, and then fed through pipes to individual tanks below. The stream of water was arranged so as to aerate the water as it entered each tank. The water was exchanged several times each day, returning to the reservoir where it was clarified by sedimentation and filtration. Lloyd designed the public aquariums in Paris (1860), Hamburg (1864), Hannover (1866), and Berlin (1869). It was largely through his efforts that aquariums became fashionable to a degree that cities vied with one another in their efforts to build comparable facilities. This rapid rise in the number of aquariums in the mid- to late-19th century mirrors the burgeoning of public aquariums worldwide over the last two decades of the 20th century, and for the same reason: The public was and is fascinated by marine life.

Most of the early aquariums incorporated a grotto-like design. To enter the display, the visitor walked into a darkened cavern that was meant to give the impression of descending into the sea. The artificial rock walls were festooned with replicas of the sea floor to increase the illusion. The only light that entered the grotto came from the tanks, which were usually illuminated from above by natural light.

It is not our intent here to review the history of aquariums as such (for recent accounts see Taylor, 1993; and McCosker, 1999). We are principally concerned with public aquariums that displayed elasmobranchs. Identifying early public aquariums that maintained captive elasmobranchs is difficult at best. Records were rarely kept, even well into the second half of the 20th century, and historical documents relating the operation of the aquariums are non-existent or difficult, if not impossible, to find. What follows is therefore incomplete and is based primarily on anecdotal accounts and reports by scientists who visited aquariums for pleasure and instruction or used them for research. Nevertheless, there is ample evidence to conclude that elasmobranchs were persistent residents in the earliest aquariums and remained a staple of display throughout the early development of the public aquarium. Moreover, many novel observations on the biology of elasmobranchs were made for the first time by watchful attendants.

Determination of species throughout this chapter is based on identifications appearing in the original reports. For many of the common species (e.g., the spiny dogfish, *Squalus acanthias*, and the small spotted catshark, *Scyliorhinus canicula*), there is little doubt about their validity. For others,

tracing synonymy and ascertaining geographical distribution was necessary to determine probable species assignments. Two sources were used to identify species: Mould and McEachran (1977) and Compagno (1999).

In what follows, mention will be made of a few exclusively public aquariums that undoubtedly displayed elasmobranchs. Many others have been excluded, not because they lacked the expertise to maintain elasmobranchs, but, regrettably, because we have been unable to locate sufficient information on species held. A summary of aquariums known or suspected of exhibiting elasmobranchs has been provided in Table 1.1.

Aquarium, Boulevard Montmartre, Paris

In 1867, Henry Lee, the same gentleman who was soon to become the manager of the Brighton Aquarium, visited the new aquarium in the Boulevard Montmartre, Paris. He pronounced it the best he had ever seen, far outshining the newly erected aquarium at the Park of the Exhibition Universelle (1867), as well as the better known aquarium at the Jardin d' Acclimatation. Once through the turnstile, the visitor descended into an artificial cave bristling with plaster stalactites. Plate glass-fronted tanks fitted out with well situated rock work and lighted from above were placed at eye level making every object in the tank easily visible. The tanks were stocked with numerous species of sea anemones, prawns, lobsters, crabs, cuttlefish, conger eels, plaice, skates, and two species of dogfish (the spiny dogfish and the nursehound). In addition, the aquarium displayed the eggs of dogfish and skates artificially attached to the corners of the rocks.

Berlin Aquarium

The Berlin Aquarium deserves notice here, in that it was one of the earliest to open (1869) and "…its success has been remarkable … there has been

Table 1.1. Public aquariums displaying elasmobranchs between 1860 and 1930, showing elasmobranch groups displayed.

Aquarium	Opened	Displayed
Hamburg Aquarium (Germany)	1864	sharks
Aquarium, Boulevard Montmarte, Paris (France)	1867	sharks
Berlin Aquarium (Germany)	1869	sharks
Blackpool Aquarium (UK)	1873	unknown
Brighton Aquarium (UK)	1873	sharks, skates
Stazione Zoologica, Naples (Italy)	1873	sharks, skates, and rays
Aquarium, Crystal Palace (UK)	1874	sharks
Manchester Aquarium (UK)	1876	unknown
Frankfurt Aquarium (Germany)	1877	sharks, skates, and rays
Amsterdam Aquarium (Netherlands)	1884	unknown
New York Aquarium (USA)	1896	sharks, skates
Musée Océanographique (Monaco)	1899	sharks, skates, and rays
Honolulu Aquarium (USA)	1906	unknown
Belle Island Aquarium (USA)	1906	sharks, rays
Boston Aquarium (USA)	1914	sharks, skates
Birch Aquarium at Scripps (USA)	1918	sharks

no other aquarium in Europe which has appealed to a greater number of people…" (Dean, 1896). The visitor entered first through the serpent gallery with its terrariums and wire cages containing tarantulas, turtles, lizards, and snakes. From there, the visitor descended through a cavernous opening into rough-cut rock grottos, one after the other, connected by darkened stone-arched passageways. Aquariums were placed in the walls of the passageways and grottos. One feature of the Berlin Aquarium, that presaged some modern displays, was the fact that animals were grouped according to the region they inhabited. One tank held animals from the North Sea; another, animals from the Mediterranean Sea; and yet another, species from the Baltic Sea (Dean, 1894; Dean, 1896).

Few records have been found that relate the species of elasmobranchs held in the Berlin Aquarium. However, we do know that Fr. Kopsch, of the 1st Anatomical Institute of Berlin, studied embryonic development of smallspotted catsharks using animals held at the Aquarium (Kopsch, 1897). The Aquarium had several females who laid approximately 80 eggs in the tanks. Spawning took place only in June and July. Kopsch reported that the eggs could be successfully incubated, although he warned against touching the eggs too often. Based on his experience, he recommended hanging them by the tendrils so that the wider end of the egg was hanging downwards. Some of these eggs were successfully hatched and the hatchlings were raised for at least five months. They were fed chopped cephalopod meat (Kopsch, 1897).

Frankfurt Aquarium, Zoologischer Garten

The Frankfurt Aquarium, erected on the grounds of the Zoologischer Garten, opened its doors to the public in 1877. It contained 91 exhibition tanks, ranging in size from 10 to 500 liters. The tanks were fed by a recirculating water supply housed in a tower built to resemble a castle ruin. Innovative for the time, there were four separate water systems. Not only was it possible to circulate both fresh and salt water, it was possible to regulate water temperature in the tanks. There were cold and warm freshwater tanks and cold and warm saltwater tanks, allowing exhibition of a remarkable diversity of fishes, including over 75 teleost species, and six species of elasmobranchs. On permanent display were smallspotted catsharks, tope (*Galeorhinus galeus*), and angelsharks (*Squatina squatina*). Species occasionally exhibited were spiny dogfish, common torpedo (*Torpedo torpedo*), and thornback rays (*Raja clavata*).

Brighton Aquarium

The Brighton Aquarium deserves further mention, on the one hand because of its success with captive elasmobranchs, and on the other, because Henry Lee reported many of his observations on sharks and skates in the popular literature (primarily in *Land and Water*, in which he wrote a regular column entitled Aquarium Notes). Brighton was a splendid location for a new aquarium. It was an extremely popular seaside resort to which Londoners flocked by carriage and rail for rejuvenation by the sea. No better place to erect a public attraction could be found. The Aquarium was situated on one of the most conspicuous points of the town. Moreover, its entrance, at the intersection of the two most popular promenades, the Madeira Road and the Marine Parade, could hardly fail to beckon the holiday traveler.

The Aquarium's location, on the English Channel, close to fresh seawater and rich fishing grounds, contributed to its early accomplishments. Seawater was pumped directly from the Channel into five reservoirs of 1,900 m^3. From there it was distributed to over 50 tanks of varying size, totaling 171 linear meters of viewing. Glass-fronted tanks lined the central corridor (218 m x 31 m), an elegant arrangement resembling an early Italian palace with its groined arches of brick and terra cotta (Figure 1.1). The largest tank measured 31 meters in length. Elasmobranchs regularly on display included the nursehound, the "…rough hound…" or smallspotted catshark, the "…picked dog…" or spiny dogfish, the "…thornback skate…" (presumably the thornback ray), and the spotted skate (*Raja montagui*).

Not unlike these fishes in modern aquariums, the catsharks were laying eggs by the hundreds. Lee fastened the eggs to sham gorgonians in the tanks and placed them so embryonic development could be observed by the Aquarium visitors, a common practice today. He noted that advanced embryos "…were inconveniently cramped for room…" and that they would beat their tails against one end of the capsule thirty times a minute, which he believed was a means of opening the hatching slit. He succeeded in incubating eggs to hatching and determined that the incubation period in the aquarium was about six months. He raised them

Figure 1.1. Interior of the Brighton Aquarium (1873), showing the arrangement of the display tanks.

for at least five months and was captivated "…to see the greedy little puppies take their meals of fish-sausage-meat…". Lee managed to incubate and hatch skate eggs laid in the aquarium. It seems likely that visitors to the Brighton Aquarium were as much enthralled with the eggs and hatchlings as the modern aquarium visitor.

Musée Océanographique, Monaco

H.R.H. Prince Albert I of Monaco is best known for his oceanographic research, which he carried out every summer aboard his personal yacht in the Atlantic, from the Azores to Spitzbergen, and for the creation of the Musée Océanographique in Monaco in 1899 (Schlee, 1973). Prince Albert's diverse scientific curiosity led him to study ocean currents, fauna in the intermediate depths, bathymetry, and marine meteorology. But his passion was promoting the emerging science of oceanography. "…He was, in fact, the epitome of oceanography's early benefactors, for his projects—inventive, unorthodox, and often dramatic—stirred interest in all aspects of the new science and were often designed to further and encourage the work of others…" (Schlee, 1973). The Prince was dedicated to education, and in 1906 founded the Institute Océanographique in Paris with the explicit objective of providing a venue to teach oceanography. The crowning achievement of Prince Albert's contributions to oceanography was the Musée Océanographique in Monaco. Here he gathered and exhibited the tools of the oceanographer, many of which he himself designed, and preserved specimens of marine life from his own collections and from those of scientists he brought along on his journeys. His design included laboratories for visiting scientists, a library, conference rooms, and access to collecting vessels. The Musée was open to the public as a way to promote oceanography and as a means to educate the populace, who had by then developed an interest in marine science. To make the experience all the more rewarding he built a public aquarium and stocked it with fish, ordinary and exotic, from around the world.

The Musée and Aquarium could not have been located in a better place for access to seawater. The promontory of Monaco juts well out into the sea, and the steep cliffs on which the building is perched slope abruptly into deep water. For this

reason, a flow-through system was used to supply fresh seawater to the aquariums. Water was drawn from two meters depth and pumped to a reservoir 13 meters above the Aquarium.

When first opened in 1905, the Aquarium was located in the sub-basement of the Musée (Figure 1.2). The Aquarium consisted of 49 tanks of various styles. On display were a variety of marine life forms, and the visitor's attention was drawn to the special attributes of each. Starfish, sea anemones, tube worms, and octopus were among the myriad invertebrates inhabiting the tanks. The changing colors of the cuttlefish were pointed out to the visitor. One tank was set up with "…mutilated…" starfish and lobsters to show the visitor how these animals could regenerate severed members. Sea bream, mullet, perch, eels, flounder, and sole were just a few of the types of fishes displayed. Elasmobranchs were permanent residents as well, including smallspotted catshark, tope, stingrays, and several unnamed species of skate.

New York Aquarium

The Aquarium in New York was established by the city in 1896 in the old Castle Garden building in Battery Park at the foot of Broadway (Bridges, 1974). This building, originally erected as a defensive battery during the War of 1812, and later employed for various social and entertainment functions, and finally an Emigrant Landing Station, was chosen as the site of the new aquarium, not because it was well suited, but because the city was trying to find a way to salvage a fiscal nightmare. The first few years of operation met with complete failure. The public could not be admitted due to the dangers of structural collapse. Something needed to be done if it was ever to succeed. Management was transferred in 1902 to the New York Zoological Society, which successfully operated the Aquarium, despite great financial difficulties, until it was relocated in 1941.

The main floor of the exhibition Aquarium, a circular room with a diameter of 69 meters, consisted of seven large floor pools, 94 large wall tanks, and 30 smaller tanks (Figure 1.3). Both fresh and salt water were pumped to the tanks. Freshwater was supplied by the city water system, while seawater was brought in by tank steamer. There was a heating and chilling system for maintaining appropriate water temperatures. The seawater system was a closed, recirculating one that pumped water from the 380 m^3 reservoir to the tanks, and returned water through sand filters. This system worked so effectively that the water

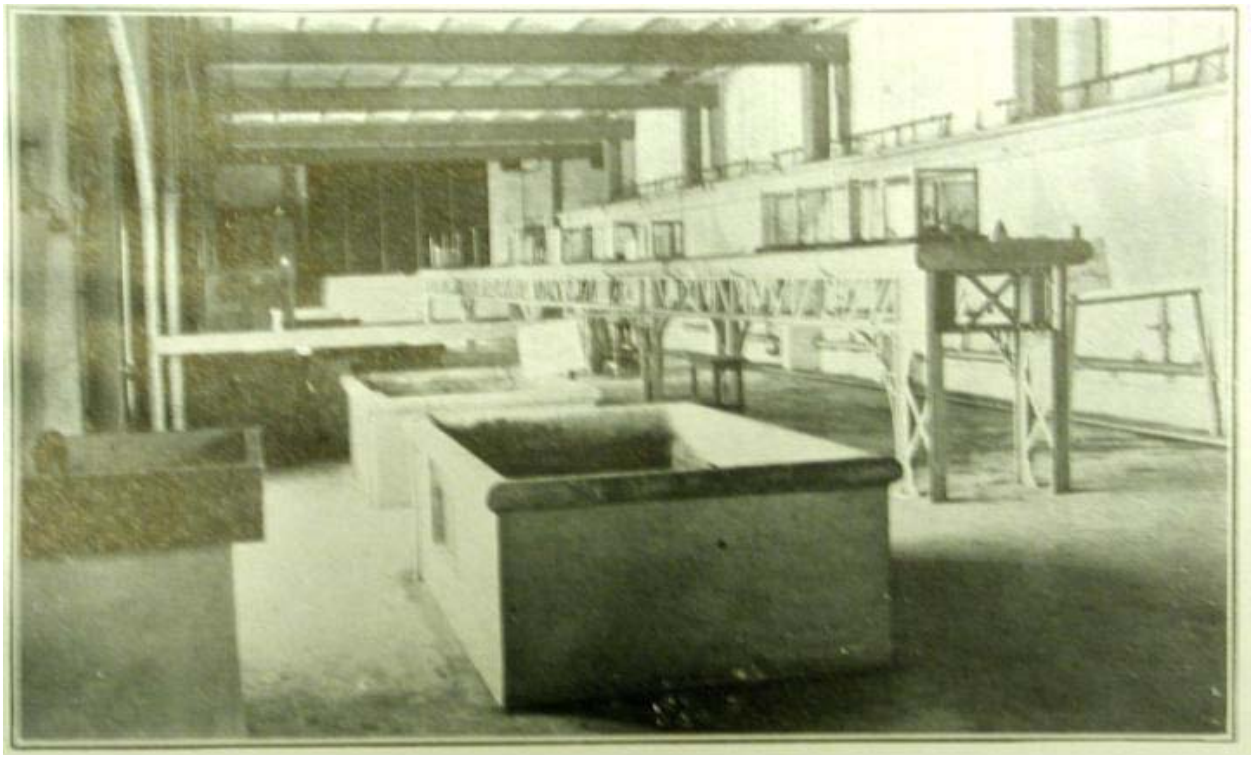


Figure 1.2. The original aquarium room of the Musée Océanographique (1905). Reproduced from Kofoid (1910).

Figure 1.3. Interior of the New York Aquarium (1896), showing the arrangement of display tanks. Reproduced from Townsend (1928).

brought to the Aquarium in 1907 was still in use over 20 years later.

The New York Aquarium was renowned for the diversity of fishes on display (Bridges, 1974). The recirculating water system was instrumental in this success. But in no small part this success was a result of the Zoological Society's expeditions, which returned with scores of fishes from around the world. During the first 20 years of its existence, the Aquarium exhibited over 350 different kinds of fishes, including 118 freshwater forms, 129 tropical marine species, and 111 northern marine species. In addition to the exhibition tanks, the Aquarium maintained 26 large reserve tanks for fishes not on display (Figure 1.4).

The Aquarium had great success maintaining elasmobranchs in captivity, although, of course, not with all species. Elasmobranchs regularly exhibited included dusky smooth-hound (*Mustelus canis*), spiny dogfish, little skate (*Raja erinacea* = *Leucoraja erinacea*), barndoor skate (*Raja laevis* = *Dipturus laevis*), winter skate (*Raja ocellata* = *Leucoraja ocellata*), roughtail stingray (*Dasyatis centroura*), electric ray (*Torpedo nobiliana*), cownose ray (*Rhinoptera bonasus*), and smooth butterfly ray (*Gymnura micrura*). Large specimens of the nurse shark (*Ginglymostoma cirratum*) did not survive long, but

Figure 1.4. The attendant's corridor behind the display tanks at the New York Aquarium (1896), showing some of the reserve tanks holding fishes not on display. Reproduced from Townsend (1928).

smaller ones lived for up to two years. Smooth hammerheads (*Sphyrna zygaena*) were exhibited, but only for short periods, and a 2.1 m blue shark (*Prionace glauca*) was held for three weeks. Perhaps of more than passing interest, the New York Aquarium kept a large sand tiger shark (*Carcharias taurus*) and displayed the fish for many years (Figure 1.5).

EXPOSITIONS

Many temporary aquariums were set up at expositions and fairs, and since a large number of people visited these events and elasmobranchs were often on display, they deserve mention here. The U.S. Commission of Fish and Fisheries customarily operated relatively large aquariums at American industrial expositions. At the world fairs of Chicago, Atlanta, St. Louis, Buffalo, Omaha, Charleston, and Nashville, the aquariums attracted more visitors than any of the other exhibits. Only one will be described here as a typical example (for more see Taylor, 1993).

The World's Columbian Exposition in Chicago, on the shores of Lake Michigan, in 1893, was an immense success, attracting millions of Americans during its six months of operation. Of the U.S. government displays, the Commission of Fish and Fisheries occupied a prominent position (Bean, 1896). The aquarium was housed in the east wing of the Fish Commission building. It was a circular structure, 38 meters in diameter, containing tanks of various sizes, one third of which were devoted to saltwater forms. It was initially proposed to concoct artificial seawater from bitter water, natural sea salt, and lime. However, preliminary experiments carried out with this mix at the Commission's office in Washington concluded that it was potentially deleterious. Natural seawater (250 m^3) was brought in from North Carolina. The seawater was circulated to the tanks from a reservoir under the building. It returned to the reservoir through sand and gravel filters. The aquariums were aerated with compressed air forced through rubber tubing plugged with basswood.

The Exposition aquarium displayed marine species from both coasts and the Gulf of Mexico. Several species of elasmobranchs were among those exhibited. The tanks were stocked with two stingrays, 4 sand sharks, 24 dogfish, and 36 skates.

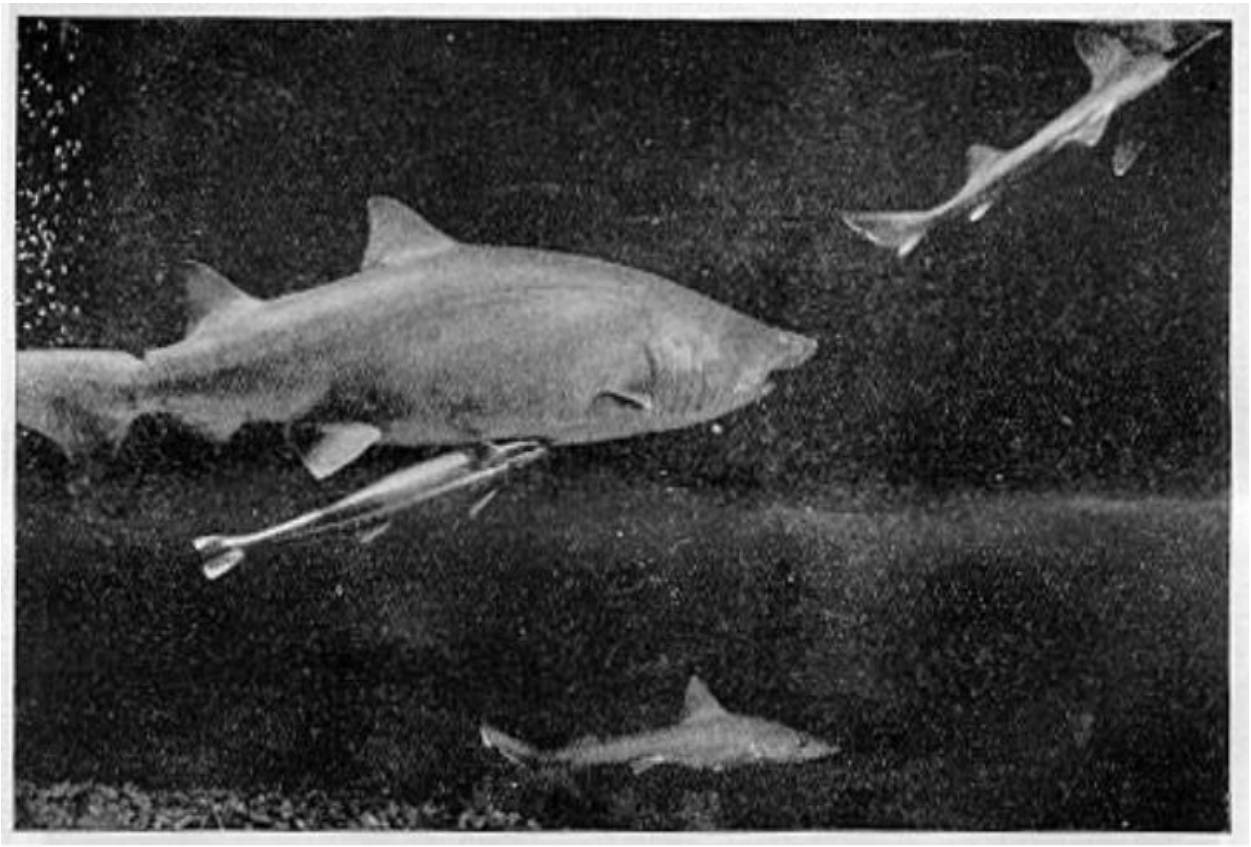


Figure 1.5. Sand tiger shark (*Carcharias taurus*) successfully maintained at the New York Aquarium (1896) for many years. Reproduced from Townsend (1928).

MARINE STATIONS

The latter half of the 19th century witnessed the rapid development of marine stations, particularly in Europe. The principal purpose of these stations was teaching and research, allowing students and professors at land-locked universities the opportunity to study marine life by the shore. They provided specimens of marine plants and animals to universities for study. Since their founders regarded education of the public as an important mission, many of the larger marine stations incorporated an exhibition aquarium. Several of these stations deserve mention for their success with captive elasmobranchs.

Plymouth Laboratory

At a meeting that took place at the Royal Society in 1884 it was decided that a provisional council would be formed to address scientific investigation of problems related to the fisheries. The council's plan, under the direction of T. H. Huxley and aided in large part by Sir Ray Lankester, was to raise funds to build a laboratory. A generous outpouring of donations followed, enough to build the Plymouth Laboratory (Marine Biological Association) within four years. It opened its doors in 1888 and began its investigation of the seas immediately. The principal mission of the station was research, offering its facilities to competent scientists who would conduct their own investigations with materials supplied by the station. Fisheries research remained the primary focus during the early years. This focus would, of course, slowly change as the nature of biological investigation evolved during the first decades of the 20th century.

The Laboratory was well designed to facilitate the study of marine organisms. The main laboratory occupied one of the two floors. Laboratories for individual investigators lined a central area that held the research aquariums. Several larger rooms for physiology, chemistry, photography, and general work were available to all resident researchers. Aside from research, the Laboratory was involved in instruction, and held courses for university students during holidays.

The Plymouth Laboratory operated a public aquarium consisting of one large room (10 m x 21 m; Figure 1.6), located on the ground floor below the research laboratory. The larger exhibition aquariums were arranged on either side

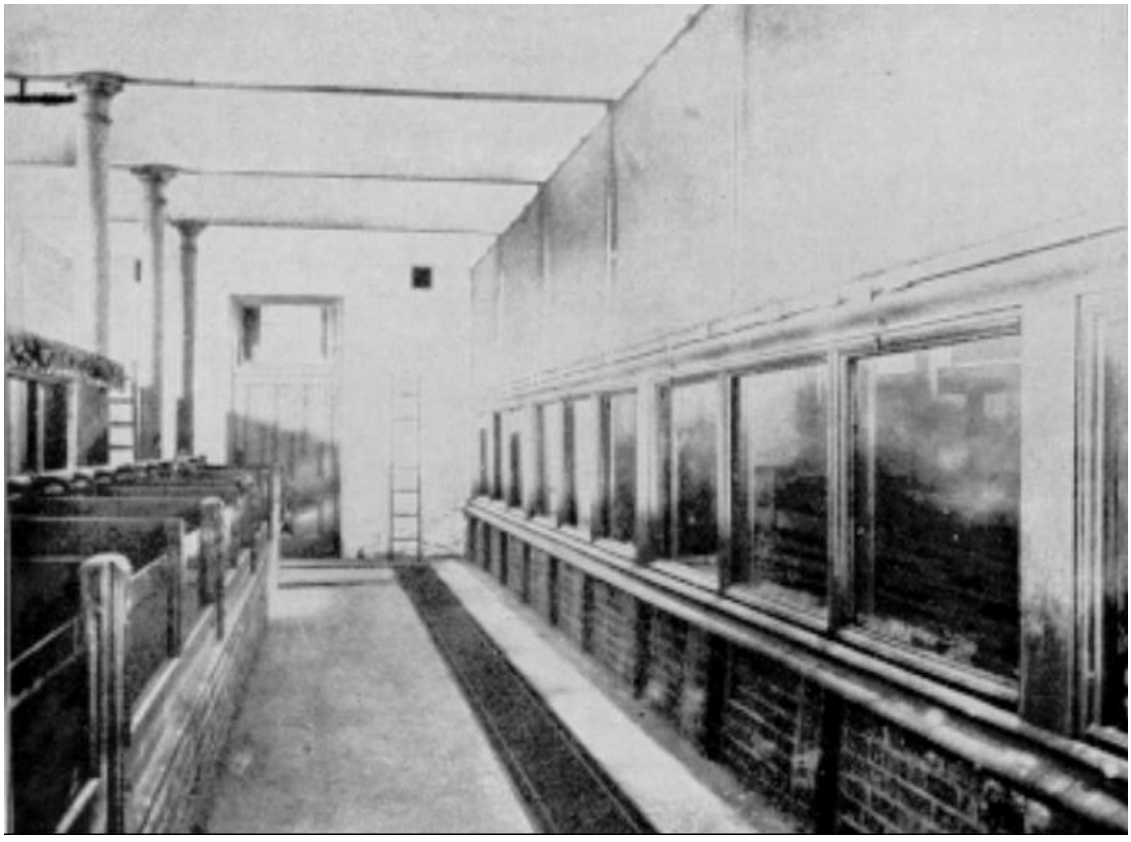


Figure 1.6. The exhibition aquarium room of the Plymouth Laboratory (1888). Reproduced from Dean (1894).

and varied in size from 1.5-10.7 m long x 1.2-1.5 m deep. The largest tank was 9.0 m long x 2.7 m wide x 1.5 m deep. Down the middle of the room were arranged five narrow tanks which allowed viewing from both sides. Seawater was distributed to the tanks from one of two reservoirs containing water pumped from near-shore waters. The reservoirs were used alternately each week, depending on the conditions of the water. The Aquarium took advantage of the extremely rich collecting grounds along the rocky Devonshire coast. The displays were well supplied with local marine fauna, including sharks and skates.

Robert S. Clark, naturalist at the Plymouth Laboratory, was interested in the locally abundant population of skates. Little was known of their life history and growth at the time (and remains poorly understood to this day). Given that these animals were commercially fished (scores were regularly landed at the Plymouth fish quay) and one of the missions of the Laboratory was fisheries investigation, it is not too surprising that Clark embarked on a study of their reproduction and growth. The resulting monograph was the first of its kind (Clark, 1926). Clark used the tanks in the public aquarium for many of his observations. His research was possible mainly because of the aquarium facilities. To list just a few of his accomplishments, he deduced that female skates stored sperm; he determined incubation periods for six species under artificial conditions (and demonstrated that these closely matched incubation periods in local natural habitats); he reported on embryonic-assisted aeration of the capsule via slits and the specialized tail appendage; and, he determined embryonic growth rates as well as neonate growth subsequent to hatching. Many of his observations would not be repeated until late in the 20th century at an institution similar in design and mission to the Plymouth Laboratory (Luer and Gilbert, 1985).

Royal Prussian Biological Station

Helgoland, a tiny island in the North Sea, 60 kilometers from the German mainland, attracted biologists interested in marine life. Alexander von Humboldt, Johannes Mueller, Rudolph Leuckart, Ernst Haeckel, Anton Dohrn were but a few of the great German biologists who studied there in the 19th century. These researchers came because of the extremely rich marine fauna and flora in the pristine rocky flats and near-shore shallow waters. Following the cession of Helgoland to Germany by England in 1892, momentum to build a biological station on the island grew rapidly. The Emperor became interested in the prospect of a biological station on German soil and commissioned representatives of the government, the Prussian Academy of Sciences, the German Fisheries Society, and the Berlin Aquarium to draw up plans for the station. The Biological Institute at Helgoland opened in 1892 under financial support from the state. The government obligated the facility to provide for research on all aspects of local marine life, courses of instruction on the biology of the sea, supply of marine specimens to scientific institutions and public aquariums, investigation of fisheries and the culture of food fishes, and investigation of the physiography and oceanography of the North Sea. Aquarium facilities were an obvious necessity, but only a few small tanks with running seawater were available during the first 10 years of operation.

Near the turn of the century a wealthy patron from Frankfurt, who regularly visited the island on holiday, offered substantial funds to erect an exhibition aquarium. The Prussian Culture Ministry, which was in charge of the Institute, accepted the offer and construction began in 1901. The new aquarium building was completed in 1902. The building and its operation were so well designed and successful at maintaining animals in captivity that it bears further description.

The Aquarium was two stories with a basement and attic, and was located on Viktoria Strasse, 25 meters from the waterfront and scarcely above high tide. It resembled a three-storied basilica with central nave and two aisles, plus a corner tower for seawater reservoirs. Lighting came through a glass roof above both the nave and aisles. The entrance hall and U-shaped exhibition hall were constructed in the usual grotto style with painted black walls. Light entered through the aquariums lining the outside walls and through a light-well above the two central rows of aquariums. Light for the service corridor behind the perimeter aquariums came through small windows in the wall. The floor above the exhibition hall contained three small investigation rooms, opening into the central well, which housed small research aquariums.

Despite the fact that waters surrounding the island were free of contamination, they were often turbid, especially after storms, and thus filtration and a closed recirculating system were necessary to ensure clarity in the exhibition tanks as well as the research aquariums. Water was pumped from 70 meters off-shore into the basement storage

tanks, from where it was lifted to the header tanks in the tower. From the header tanks, water was distributed to the exhibition and research tanks located on the two floors below, by gravity. Aeration was accomplished by jetting the water into each aquarium. Water exited the tanks through vertical pipes, which led to sand and gravel filter beds, before entering the basement storage reservoir.

The Aquarium was primarily an educational institution based on the Institute's scientific goals, but the architecture was designed with the public in mind and incorporated exhibition tanks. The largest aquarium measured 2.54 m long x 1.84 m wide x 1.75 m deep. The walls were 12.5 cm thick. Like those of the modern aquarium, the exhibition displays were meant to educate the viewer. The tanks were stocked primarily with locally abundant and carefully selected marine fauna and flora. The displays included food fishes, invertebrates, characteristic faunistic assemblages (e.g., *Zostera* spp. beds), and rock and sand fauna, together showing the range and variety of marine life.

Among the regular inhabitants of the aquarium, in one of the larger tanks equipped with a sand bottom, were elasmobranchs (Ehrenbaum, 1910). Species on display included thornback rays, skate (*Raja batis* = *Dipturus batis*), spiny dogfish, smooth-hound (*Mustelus mustelus*), dusky smoothhound, smallspotted catshark, tope, and occasionally large stingrays. The sharks were problematic in that they rarely fed in captivity and often injured themselves by running into objects, generally dying after a short period. Smooth-hounds and catsharks survived best in the Aquarium. Catshark eggs were regularly displayed. These eggs were not obtained from resident animals, but rather were received from the Plymouth Laboratory. Despite being open during summer holiday months only, the Aquarium was a tremendous success admitting 16,000 visitors a year.

Zoological Station, Rovigno

One year after its opening in 1869, the Berlin Aquarium established a marine station in Trieste, principally for the collection and shipment of marine plants and animals to the Berlin Aquarium. In 1892, the station was removed to Rovigno on the Istrian Coast of the Adriatic Sea, on the south shore of the Bay of Istria directly on the Val di Bora, 15 meters from the strand line. The purpose of the Station remained primarily one of collection and shipment of specimens for aquarium display, but was later expanded to supply living and preserved material

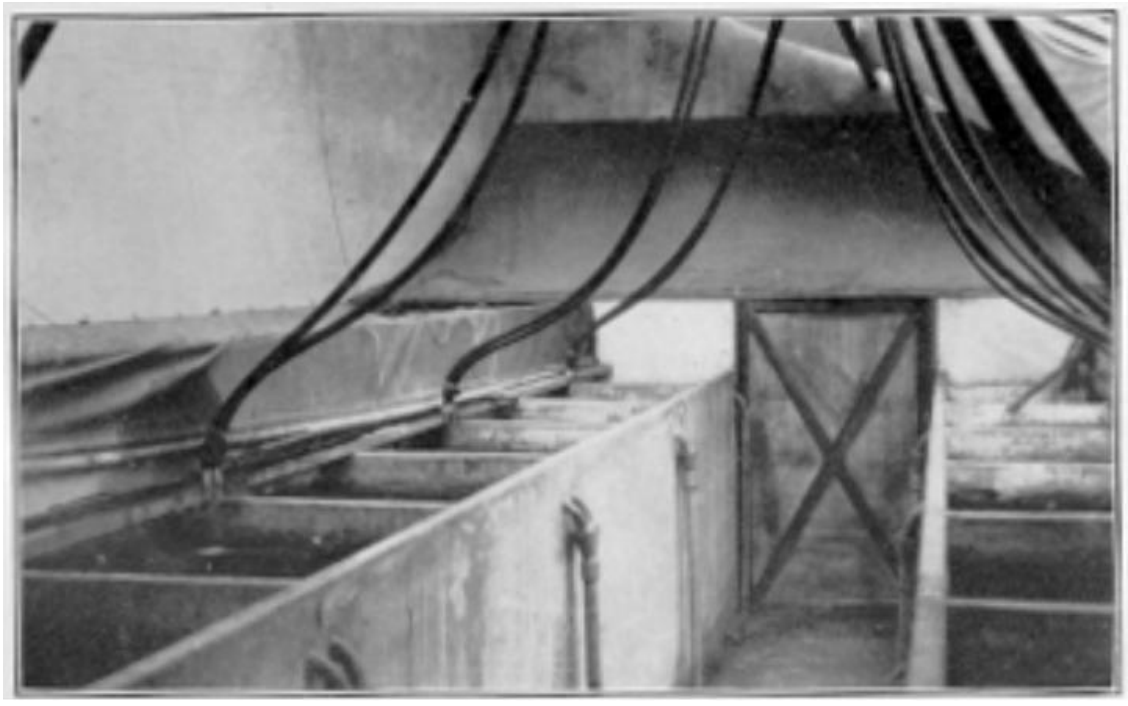


Figure 1.7. The attendant's corridor behind the display tanks at the exhibition aquarium of the Zoological Station, Rovigno (1892). Reproduced from Kofoid (1910).

to German universities, at cost. The Station was available to competent investigators of all nationalities for research.

A small public aquarium was built in a remodeled greenhouse adjacent to the main Zoological Station building. The grotto-like design was typical for the period. Aquariums (18) were arranged in a rectangle around a central corridor for the attendants (Figure 1.7).

Between 1895 and 1897, Fr. Kopsch, Assistant at the 1st Anatomical Institute in Berlin, spent several periods of time during different seasons at the Zoological Station. He was interested in fish egg development in general, but went to Rovigno to study the development of the eggs of smallspotted catsharks (Kopsch, 1897). While not readily available near Rovigno, the fish could be caught by hook and line in large numbers farther out to sea and transported back to the Station by steamship. The specimens were kept on board in a fish container until 60-90 were collected. During the 24-hour collecting trip, or within a few days of arrival, some of the animals would die, more in the summer than the winter. Kopsch's experience led him to conclude that the sudden transfer of fishes from deeper cold water to warmer surface water was harmful, since fish caught in the summer stopped eating and depositing eggs when placed in tanks at the Station. The aquarium system at the Rovigno Station facilitated Kopsch's work in that egg-laying females could be kept alive for months. During one season (February to May) ~400 eggs were laid in his tanks by 50 females. While he used many of these eggs for embryological studies, others were incubated to hatching. Based on these studies, he recognized that development was temperature dependent and carried out experiments at the Station to examine this relationship. He proposed using a system of degree-days, much like that of the commercial fish growers, for delineating the stage of any particular dogfish embryo.

Stazione Zoologica, Naples

Best among the marine stations established during the late 19th century was the Stazione Zoologica Napoli: "…foremost in the extent and completeness of its material equipment and in the wealth of opportunities it offers, inspiring in its history and unparalleled in its growth, unsurpassed in its contributions to biological science, profound in its influence upon the course of development of modern biology, and powerful in its stimulus to the establishment of biological stations elsewhere, stands the zoological station of Naples, the peer and leader of them all…" (Kofoid, 1910).

Much has been written about the history of the Naples Zoological Station. We will not review this history other than to give a brief account and refer those interested to several excellent published treatments (Openheimer 1980; Groeben, 1984; Groeben, 1985; www1). Anton Dohrn created the Zoological Station with one overriding goal—to prove Darwin's theory of evolution. He believed the study of marine organisms would provide the proof without doubt. He first went to Sicily in 1868 because the Strait of Messina was famous for the richness of fauna and flora. However, the financial difficulties of building and maintaining a laboratory there were too great and Dohrn began to think of other locations. Naples seemed to him a perfect location: It was an important commercial and tourist center; it was located directly on the sea; the local fauna were abundant; and, it was a dynamic fishing center. It took all his diplomatic skills and stubborn persistence to convince the city authorities, who were none too favorable to the idea, to grant him the use of a plot of land near the waterfront. He built the Station almost entirely from his personal fortune. It was according to his design that a magnificent building apropos the ancient city of Naples was constructed. As is well known, it soon became the Mecca for scientists wishing to study marine biology.

Dohrn recognized early on that in order to operate a research station a regular source of income would be necessary. In 1870, just after visiting the public aquariums in Hamburg and Berlin, he had an idea how to support the Station. He would build a public aquarium and charge an entrance fee. He explained to his friends "…I am going to establish in Naples a large aquarium for the public … The tuff for the grottoes can be bought in masses from Vesuvius, fresh seawater is constantly available on the doorstep, and the animals occur by the million in the sea; all can be done very cheaply. No dying animals. Hurrah, it's a marvelous idea! I have already calculated that for 120 visitors daily for nine months of the year I can have profits running and everything. And how many more will come? And in rainy weather! You must congratulate me, the idea is ready money, freedom, independence and a nice home for my dear friends in Naples…" (quoted in Groeben, 1984). Thus was born the Naples Aquarium; it opened its doors to the public in 1873.

Exhibition aquariums (18) were set in the walls (1.75-11 m long x 3 m wide x 1.5 m deep). Six centrally located tanks measured 4 m long x 1 m wide x 1 m deep. Seawater (65 m^3) was pumped directly to the tanks from a basement reservoir every day in summer. The Naples Aquarium was known for the variety and beauty of the animal life displayed, and the exceptional quality of the exhibits. Only local fauna were displayed, but even that was species-rich, with nearly two hundred genera exhibited during the year. The echinoderm tank was reputed to be outstanding. Other excellent exhibits included pelagic coelenterates and mollusks, octopus and squid, brilliantly colored tube worms, moray eels, and a diversity of local fishes, including the most diverse display of elasmobranchs for any aquarium of the period.

In 1879, Richard Schmidtlein published an account of the elasmobranchs exhibited in the Aquarium. Many of his observations are of interest in documenting how well the Naples Aquarium did at maintaining elasmobranchs in captivity, as well as pointing out that it had difficulties with certain species. Moreover, Schmidtlein made a variety of novel observations on the behavior and biology of the animals under his care. Persistent inhabitants of the aquariums were smallspotted catshark, nursehound, angelshark, marbled electric ray (*Torpedo marmorata*), torpedo, and several species of skates. Less frequently, the Aquarium displayed tope, smoothhound, angular roughshark (*Oxynotus centrina*), and pelagic stingray (*Pteroplatytrygon violacea*) (Schmidtlein, 1879).

Schmidtlein was captivated by the catsharks. He watched them day and night. He noted that during the day they would lie together motionless in the darkest corner of the aquarium, but at night they swam actively around the tank. "...Hunger invigorates them and a few kilograms of sardines thrown into the tank sets all of them in motion. Nervously, with their snouts close to the bottom, they search around. Their behavior demonstrates clearly not their eyes but their well developed sense of smell guides them in their search for food. Cruising closely by the sardine, the shark first does not notice it, however, having passed it by almost a body's length it moves around by a swift beat of its tail and usually finds the sardine after a brief, hectic search, swallowing it after a few chewing movements...". He witnessed copulation and described it "...more a fight than love play. The male grabs the female's pectoral fin, and they now roll together in the sand as if seriously fighting...". He saw the females oviposit eggs; witnessed development through the transparent capsule; and, successfully incubated them to hatching. However, he could not get the hatchlings to feed and they died soon thereafter. Tope were more difficult to keep alive at the Aquarium, continually running into objects and causing extensive trauma to their sensitive snouts. He had better luck with their eggs, which he incubated to hatching. Smoothhound were difficult to maintain in the tanks, primarily because they would not feed and survived only two weeks. However, he did witness a birth. On two occasions the aquarium received angular roughsharks, though neither survived more than three weeks. Angelsharks were a different story, they were relatively easy to maintain and readily accepted food placed directly in front of the snout.

The two species of electric rays adjusted well to captivity, swimming almost exclusively at night, and spending the greater part of the day buried in the sand. He pointed out that they swam not with their wings, but by strong beats of their muscular tail. He noticed that the electrical discharge was used both for prey capture and for defense. They often found co-inhabitants of the tank, especially *Gobius* spp. and *Blennius* spp., belly-up on the surface, mouth agape. He observed a young catshark approach a torpedo, suddenly shoot upward and frantically swim about the tank. Several times he saw an octopus enwrapping a torpedo in its tentacles, become startled and speed away.

One other short-lived inhabitant bears mentioning. The aquarium received a large pelagic stingray, which it kept alive for a month. It was an adult female that gave birth to four healthy offspring. Unfortunately the young did not survive more than a few days, refusing food and sustaining multiple injuries by repeatedly colliding with rocks. The female on the other hand adapted quite quickly to the confines of the tank. It swam incessantly, and once a particular path around the rocky ledges was found, it repeated this course precisely, sometimes for days. It was a favorite of the visitors, enthralled by its graceful movements. Sadly, it succumbed to starvation, as it would not eat, and attempts to force feed it were entirely unsuccessful.

CONCLUSION

While sparse in extent and in most cases short on details, the facts enumerated here clearly show that elasmobranchs were commonly exhibited in

public aquariums since their inception in the 1860's. Some of these institutions were remarkably successful at maintaining elasmobranchs in captivity, in some cases for many years. They worked out capture and transportation techniques, water quality issues, feeding regimens, and display methods. Moreover, watchful attendants made novel observations on the biology of elasmobranchs, including diurnal activity, feeding behavior, mating, and egg-laying behavior. The aquarists of the day used many of the same techniques to exhibit these fishes as are still used today.

EPILOGUE

In the mid 1920's, Charles Townsend, Director of the New York Aquarium, sent out a questionnaire to existing aquariums worldwide. Based on the returns, he estimated that there were 45-50 aquariums in operation at the time (Townsend, 1928). Many aquariums (32) sent back details of the operation of their facilities. Over 13 million visitors a year were entering aquariums worldwide. One is left to wonder how the public responded to seeing sharks, skates, and rays eye-to-eye.

ACKNOWLEDGEMENTS

The author thanks the organizers of the symposium, Mark Smith and Doug Warmolts, for the kind invitation to contribute to the EHM. This history could not have been completed without Magdalena Koob-Emunds. Much of the early descriptions and reports of elasmobranchs in captivity during the 19th century appeared in the German literature. She untiringly translated the difficult prose of the period, corresponded with archivists searching for source material, and provided invaluable editorial assistance. To her I owe considerable thanks for her painstaking efforts, as well as the patience in dealing with so diverse a project.

REFERENCES

Bean, T. H. 1896. Report of the representative of the United States Fish Commission at the World's Columbian Exposition. *In:* Report of the Commissioner for the Year Ending June 30, 1894, Part XX, United States Commission of Fish and Fisheries, p. 177-196. U.S. Government Printing Office, Washington, D. C.

Bridges, W. 1974. Gathering of Animals: An Unconventional History of the New York Zoological Society. Harper & Row Publishers, New York. 518 p.

Castro, J. I., P. M. Bubucis, and N. A. Overstrom. 1988. The reproductive biology of the chain dogfish, *Scyliorhinus retifer*. Copeia 1988: 740-746.

Clark, R. S. 1926. Rays and skates (Raiae). No. 1. Egg capsules and young. Journal of the Marine Biological Association 12: 577-643.

Compagno, L. 1999. A Checklist of Living Elasmobranchs. *In:* Sharks, Skates, and Rays: The Biology of Elasmobranchs, p. 471-498. W. C. Hamlett (ed.). The Johns Hopkins University Press, Baltimore, Maryland.

Dean, B. 1893. Notes on marine laboratories of Europe. American Naturalist 27: 625-706.

Dean, B. 1894. The marine biological stations of Europe. Annual Report of the Smithsonian Institute 1893: 505-519.

Dean, B. 1896. Public aquariums in Europe. Popular Science Monthly 50: 13-27.

Ehrenbaum, E. 1910. Das Aquarium der Biologischen Anstalt auf Helgoland. Internationale Revue der gesammten Hydrobiologie 3: 418-446.

Groeben, C. 1984. The Naples Zoological Station and Woods Hole. Oceanus 27: 60-69.

Groeben, C. 1985. Anton Dohrn—the statesman of Darwinism. Biological, Bulletin 168: 4-25.

Kofoid, C. A. 1910. The biological stations of Europe. Bulletin of the U.S. Bureau of Education, 440, 360 pp.

Kopsch, F. 1897. Ueber die Ei-Ablage von Scyllium canicula in dem Aquarium der zoologischen Station zu Rovigno. Biologische Centralblatt 17: 885-893.

Luer, C. A. and P. W. Gilbert. 1985. Mating behavior, egg deposition, incubation period, and hatching in clearnose skate, *Raja eglanteria*. Environmental Biology of Fishes 13: 161-171.

McCosker, J. E. 1999. The History of the Steinhart Aquarium: A Very Fishy Tale. The Donning Company, Virginia Beach, Virginia. 160 p.

Mould, B. and J. D. McEachran. 1997. A Revision of Garman's Nomenclature. *In:* The Plagiostomia (Sharks, Skates and Rays), p. xii – xxxv. S. Garman (ed.). Benthic Press, Los Angeles, California.

Openheimer, J. M. 1980. Some historical backgrounds for the establishment of the Stazione Zoologica at Naples. *In:* Oceanography: The Past, p. 179-187. M. Sears and D. Merriman (eds.). Springer, New York.

Schlee, S. 1973. A History of Oceanography. Robert Hale and Company, London, England. 398 p.

Schmidtlein, R. 1879. Beobachtungen über die Lebensweise einiger Seethiere innerhalb der Aquarien der Zoologischen Station Mittbeilungen. Mitteilungen der Zoologischen Station Neapel 1: 1-27.

Taylor, L. 1993. Aquariums: Windows to Nature. Prentice Hall, New York. 170 p.

Townsend, C. H. 1928. The public aquarium: Its construction, equipment and management. Bureau of Fisheries Document No. 1045; Appendix VII to the Report of the United States Commissioner of Fisheries for 1928, 249-337 pp.

INTERNET RESOURCES

www1: www.szn.it/acty99web/acty014.htm.

The Elasmobranch Husbandry Manual: Captive Care of Sharks, Rays and their Relatives, pages 15-23.
© 2004 Ohio Biological Survey

Chapter 2
Species Selection and Compatibility

ANDY DEHART

National Aquarium in Baltimore,
Pier 3 / 501 E. Pratt St,
Baltimore, MD 21202, USA.
E-mail: adehart@aqua.org

Abstract: The process of determining which species of elasmobranchs to obtain for an existing or new exhibit can be challenging. Species selection and compatibility are important aspects to consider when planning an elasmobranch display. The key factors in formulating a species list include exhibit goal, system design, species availability, species compatibility, and species potential for reproduction. When formulating a species list, it is prudent to discuss detailed species requirements and traits with an institution that already displays the animals in question.

The process of determining which species of elasmobranchs to obtain for an existing or new exhibit can be challenging. The interaction of each species with other elasmobranchs and other taxa (e.g., teleosts) is an important factor that must be considered. Generally speaking, these decisions should be based on the trials and experiences of other public aquariums, hobbyists, and researchers. There are roughly 400 species of sharks and 500 species of rays and skates (Compagno, 1999). According to the American Elasmobranch Society (AES) captive elasmobranch census, only about 150-200 species have been kept successfully in captivity. While aquariums are always trying to obtain and maintain new species, most of the information available on elasmobranchs in captivity is based on a relatively small number of species. This chapter is intended to serve as a guide for determining which species to select for an exhibit and their compatibility with other elasmobranchs, as well as other taxa.

SPECIES SELECTION

Elasmobranchs require unique husbandry methods for their long-term captive survival. Institutions or individuals planning to obtain and display elasmobranchs must consider these requirements from the outset of exhibit development. There are five key factors to consider when adding elasmobranchs to an existing exhibit, or indeed when designing a new exhibit: exhibit goal, exhibit design, species availability, species compatibility, and species potential for reproduction.

Exhibit goal

The first step in determining what species of elasmobranchs to select is to create a clear exhibit goal or objective. One possibility is to design an exhibit themed around a given habitat. This type of exhibit is generally a multi-taxa display with elasmobranchs, teleosts, and sometimes sea turtles. An example would be a large Atlantic coral reef habitat with several species of sharks, rays, and many species of reef fishes. A second common design theme is taxonomic, i.e., a display designed specifically around a taxonomic group such as sharks or rays. Frequently, these displays are not geographically accurate, but they are successful at showing the large variation within a given group of animals. An additional display type combines elements from both of the above. This third display type contains similar taxonomic species from a broad geographical region, such as an exhibit showing sharks from the Atlantic Ocean. In this type of display it may be possible to present two shark species that usually live in different habitats and are rarely seen together in the wild. Having a clear objective for a display makes the selection of target species more manageable.

Exhibit design

Exhibit design is the single most important factor to consider when deciding the species of elasmobranchs to obtain. Exhibit size, shape, volume, and depth, are all areas to closely assess. To swim correctly, many elasmobranchs require an extensive, uninterrupted, horizontal swimming dimension (Stoskopf, 1993). Exhibit rockwork and décor is another important consideration. Some species, like the scalloped hammerhead shark (*Sphyrna lewini*), frequently injure their head and eyes on rough, rocky outcroppings (Violetta, pers. com.). In this case, the exhibit should be designed with large, open swimming areas, smooth décor, and rounded tank walls to prevent abrasions. The tiger shark (*Galeocerdo cuvier*), on the other hand, will orient its body along the outer walls of a display and constantly abrade its pectoral fins and lower caudal lobe on the smooth concrete surfaces (Crow and Hewitt, 1988; Dehart and Stoops, 1998). For this species, an exhibit should have rough rockwork protruding in an irregular fashion from all the tank walls, keeping the shark swimming in the middle of the exhibit away from obstructions.

Clearly the natural behavior and swimming patterns for each species should be used as a guide to determine whether or not it can be kept in an exhibit. The more closely an aquarium can mimic the animal's natural habitat, in both swimming area and structure, the better the animal's health will be. Obviously pelagic animals should be maintained in extremely large, open exhibits, while sedentary, benthic animals should be kept in a system with appropriate substrate such as sand or gravel. Exhibit design is discussed in more detail in Chapter 5 of this manual.

Species availability

In recent years the ability to obtain certain shark species is becoming increasingly difficult. Availability, or the lack thereof, often plays a role in determining a species list. Elasmobranchs can be collected by the staff of the aquarium or university, within the local area, or purchased through commercial collectors. Regardless of the method chosen, it is imperative to obtain all proper permits from local, federal, and international authorities before acquiring specimens.

There are distinct advantages for a facility that can collect its own specimens, but the institution must have the resources and be in the right locale. This method generally implies a greater cost per animal and is frequently time consuming, but offers a chance for staff to get into the field, hand select individual specimens, and view the natural habitat of the species firsthand. Collecting methods are described in Chapter 7 of this manual.

There are many good commercial collectors who specialize in acquiring elasmobranchs. When dealing with a commercial collector ensure that they have all the appropriate permits. It is a good practice to check with other aquariums to verify a collector's credentials and experience. Permitting issues are discussed at length in Chapter 3 of this manual.

Another possible source for specimens is through surplus lists. For example, the American Zoo and Aquarium Association (AZA) releases a monthly surplus list to all member institutions. These animals are frequently donated to other AZA member institutions at no cost other than shipping. This is a great method for exchanging animals (and experiences) with other facilities and decreases the demand for wild-caught specimens.

Species compatibility

Compatibility refers to the interaction between an elasmobranch species and the other organisms within an exhibit. There are compatibility considerations both within and between elasmobranch species, and with bony fishes and invertebrates. Many species, such as the wobbegong shark (*Eucrossorhinus* spp. and *Orectolobus* spp.), have a tendency to eat almost any tank inhabitant that will fit in their mouths. The compatibility of individual species of elasmobranchs is discussed below in the section entitled "Species description."

Bony fishes and invertebrates will often be preyed upon in a community-style display. Bony fishes are the normal prey items of many elasmobranchs. It is therefore only natural that elasmobranchs in captivity will continue to feed on live display specimens from time to time. Predation can be minimized by selecting certain species of elasmobranchs that do well in a multi-taxa environment, and by feeding these specimens frequently. Providing places where smaller organisms can hide also helps reduce losses through predation.

Some shark species are even aggressive toward other sharks. One such example is the lemon shark (*Negaprion brevirostris*), which has been known to harass other species such as sand tiger sharks (*Carcharias taurus*) and sandbar sharks (*Carcharhinus plumbeus*). Sand tiger sharks, in turn, are piscivorous and will often consume smaller sharks on exhibit such as whitetip reef sharks (*Triaenodon obesus*) and blacknose sharks (*Carcharhinus acronotus*) (Smith, pers. com.; Thoney, pers. com.). Fortunately, only a few species display such behaviors. During reproductive cycles, typically non-aggressive individuals can become more aggressive (e.g., sand tiger sharks) (Gordon, 1993). Maintaining sharks in groups comprised of similar-sized animals will minimize aggression towards smaller individuals.

The compatibilities of different species have been summarized in Table 2.1. This matrix can be used as a rough guideline to determine the suitability of mixing different species within an exhibit. Size differences between elasmobranchs and other tank inhabitants is a key factor when dealing with compatibility and predation, but exhibit size and shape, species traits, etc., can play an important role. Specimens, within a species, will not always display the same or predictable behavior. Careful planning, research, and communication with other facilities will improve your chances of successfully maintaining a variety of shark, ray, and fish species within a single display.

The great white (*Carcharodon carcharias*), tiger, whale (*Rhincodon typus*), oceanic whitetip (*Carcharhinus longimanus*), blue (*Prionace glauca*), scalloped hammerhead, and great hammerhead (*Sphyrna mokarran*) sharks have specialized exhibit requirements (e.g., very large exhibit dimensions in the horizontal plane) and compatibility constraints, and communication with experienced institutions is strongly urged before attempting to maintain these species.

Species potential for reproduction

If captive breeding is considered an important objective for target elasmobranch species, reproductive behavior and physiology must be considered when formulating the species list. Captive reproduction of elasmobranchs is covered more completely in Chapters 16 and 17 of this manual.

SPECIES DESCRIPTION

This section provides a brief description of the most commonly held elasmobranchs, as well as a few key signature species which have proven difficult to maintain. Several volumes could be filled with a detailed description of all the elasmobranch species held in captivity, so this is an unavoidably broad overview. Species were selected using the AES captive elasmobranch censuses from 1997, 2000, and 2001. Table 2.2 summarizes the maximum size, hardiness, availability, compatibility, and geographical range of each species. A rating system is used for hardiness, availability, and compatibility. Not all specimens of a given species will necessarily behave in an established manner. Juveniles and adults are often different in terms of hardiness and compatibility.

Hardiness

The hardiness of a species describes how well it adapts to the rigors of the captive environment and is ranked on a scale of one to four as follows:

1. *Adapts readily* - Typically acclimates with ease to a new environment, has few problems adjusting to eating in captivity, and survives quarantine well.
2. *Adapts well* - Can be difficult to transport, but generally adapts well to captivity.
3. *Delicate* - Eventually acclimates to captivity, but may take longer to start eating, or have special quarantine requirements.
4. *Difficult* - These species are hard to maintain in captivity for an extended period of time. They frequently have trouble adapting to a confined environment, have trouble feeding in captivity, and often have chronic medical problems.

Availability

Availability describes how difficult the species is to obtain and is ranked on a scale of one to three as follows:

1. *Easy* - Frequently bred in captivity or is readily available in the wild.
2. *Average* - Not usually captive bred, but fairly abundant and available in the wild.
3. *Difficult* - Difficult to obtain, even in the wild, and often subject to government restrictions on their collection.

Table 2.1. Matrix showing the compatibility of different elasmobranch species. Select a species from the left-hand column (LHC) and compare to species on adjacent columns to the right. Specimen size differences, exhibit size, and inter- and intra-species variation will modify species compatibility. Key: **a** = target species (LHC) may prey upon or be aggressive toward compared species; **p** = target species (LHC) may be preyed on, or harassed by, compared species; **h** = target species (LHC) may be subject to harassment by teleosts; and **t** = target species (LHC) and compared species require different water temperature regimes.

	Carcharias taurus	*Carcharhinus acronotus*	*Carcharhinus leucas*	*Carcharhinus limbatus*	*Carcharhinus longimanus*	*Carcharhinus melanopterus*	*Carcharhinus perezi*	*Carcharhinus plumbeus*	*Carcharodon carcharias*	*Cephaloscyllium ventriosum*	*Chiloscyllium plagiosum*	*Chiloscyllium punctatum*	*Eucrossorhinus dasypogon*	*Galeocerdo cuvier*	*Ginglymostoma cirratum*	*Hemiscyllium ocellatum*	*Heterodontus francisci*	*Heterodontus portusjacksoni*	*Mustelus canis*
Carcharias taurus		**a**								**t**	**a**	**a**	**a**			**a**	**t**	**t**	**a**
Carcharhinus acronotus	**p**		**p**						**p**	**t**	**a**	**a**		**p**		**a**	**t**	**t**	
Carcharhinus leucas		**a**		**a**		**a**				**t**	**a**	**a**	**a**			**a**	**t**	**t**	**a**
Carcharhinus limbatus			**p**						**p**	**t**	**a**	**a**		**p**		**a**	**t**	**t**	
Carcharhinus longimanus																			
Carcharhinus melanopterus			**p**						**p**	**t**				**p**		**a**	**t**	**t**	**t**
Carcharhinus perezi										**t**	**a**	**a**				**a**	**t**	**t**	**a**
Carcharhinus plumbeus										**t**	**a**	**a**				**a**	**t**	**t**	**a**
Carcharodon carcharias		**a**		**a**		**a**				**a**	**a**	**a**	**a**			**a**	**a**	**a**	**a**
Cephaloscyllium ventriosum	**t**	**t**	**t**	**t**		**t**	**t**	**t**	**p**		**t**	**t**	**t**	**t**	**t**	**t**			
Chiloscyllium plagiosum	**p**		**p**						**p**	**t**							**t**	**t**	
Chiloscyllium punctatum	**p**		**p**						**p**	**t**							**t**	**t**	
Eucrossorhinus dasypogon	**p**		**p**						**p**	**t**						**a**	**t**	**t**	
Galeocerdo cuvier										**t**							**t**	**t**	**a**
Ginglymostoma cirratum										**t**							**t**	**t**	
Hemiscyllium ocellatum			**p**						**p**	**t**			**p**				**t**	**t**	
Heterodontus francisci	**t**	**t**	**t**	**t**		**t**	**t**	**t**	**p**		**t**	**t**	**t**	**t**	**t**	**t**			
Heterodontus portusjacksoni	**t**	**t**	**t**	**t**		**t**	**t**	**t**	**p**		**t**	**t**	**t**	**t**	**t**	**t**			
Mustelus canis	**p**		**p**			**t**	**t**	**t**	**p**					**p**					
Negaprion brevirostris										**t**	**a**	**a**	**a**			**a**	**t**	**t**	**a**
Notorynchus cepedianus	**t**	**t**	**t**	**t**		**t**	**t**	**t**			**t**	**t**	**t**	**t**	**t**	**t**			
Orectolobus japonicus			**p**						**p**	**t**	**a**	**a**				**a**	**t**	**t**	
Orectolobus maculatus			**p**						**p**	**t**	**a**	**a**				**a**	**t**	**t**	
Orectolobus ornatus			**p**						**p**	**t**	**a**	**a**				**a**	**t**	**t**	
Prionace glauca	**t**	**t**	**t**	**t**		**t**	**t**	**t**	**p**		**t**	**t**	**t**	**t**	**t**	**t**			
Rhincodon typus										**t**							**t**	**t**	
Scyliorhinus retifer	**t**	**t**	**t**	**t**		**t**	**t**	**t**	**p**		**t**	**t**	**t**	**t**	**t**	**t**			
Scyliorhinus stellaris	**t**	**t**	**t**	**t**		**t**	**t**	**t**	**p**		**t**	**t**	**t**	**t**	**t**	**t**			
Sphyrna lewini									**p**	**t**							**t**	**t**	**a**
Sphyrna mokarran																			**a**
Sphyrna tiburo			**p**						**p**	**t**							**t**	**t**	
Squalus acanthias			**p**			**t**	**t**	**p**	**p**				**p**						
Stegostoma fasciatum										**t**							**t**	**t**	
Triaenodon obesus			**p**							**t**							**t**	**t**	
Triakis semifasciata	**t**	**t**	**t**	**t**		**t**	**t**	**t**	**p**		**t**	**t**	**t**	**t**	**t**	**t**			
Benthic batoids			**p**						**p**										
Pelagic batoids			**p**						**p**										

Table 2.1 (continued). Matrix showing the compatibility of different elasmobranch species. Select a species from the left-hand column (LHC) and compare to species on adjacent columns to the right. Specimen size differences, exhibit size, and inter- and intra-species variation will modify species compatibility. Key: **a** = target species (LHC) may prey upon or be aggressive toward compared species; **p** = target species (LHC) may be preyed on, or harassed by, compared species; **h** = target species (LHC) may be subject to harassment by teleosts; and **t** = target species (LHC) and compared species require different water temperature regimes.

	Negaprion brevirostris	*Notorynchus cepedianus*	*Orectolobus japonicus*	*Orectolobus maculatus*	*Orectolobus ornatus*	*Prionace glauca*	*Rhincodon typus*	*Scyliorhinus retifer*	*Scyliorhinus stellaris*	*Sphyrna lewini*	*Sphyrna mokarran*	*Sphyrna tiburo*	*Squalus acanthias*	*Stegostoma fasciatum*	*Triaenodon obesus*	*Triakis semifasciata*	Benthic batoids	Pelagic batoids	Teleosts
Carcharias taurus		t	a	a	a			t	t			a	a			t	a	a	a
Carcharhinus acronotus	p	t				t		t	t							t			
Carcharhinus leucas		t	a	a	a	t		t	t			a	a		a	t	a	a	a
Carcharhinus limbatus	p	t				t		t	t							t			
Carcharhinus longimanus																	a	a	a
Carcharhinus melanopterus	p	t				t		t	t							t			
Carcharhinus perezi		t				t		t	t				a			t			
Carcharhinus plumbeus		t				t		t	t				a			t			
Carcharodon carcharias			a	a	a	a		a	a	a		a	a			a	a	a	a
Cephaloscyllium ventriosum	t		t	t	t		t			t	t	t		t	t				
Chiloscyllium plagiosum	p	t				t		t	t	p	p					t			h
Chiloscyllium punctatum	p	t				t		t	t	p	p					t			h
Eucrossorhinus dasypogon	p	t				t		t	t							t			a
Galeocerdo cuvier		t				t		t	t			a	a			t	a	a	a
Ginglymostoma cirratum		t				t		t	t							t			
Hemiscyllium ocellatum	p	t	p	p	p	t		t	t	p	p				p	t			h
Heterodontus francisci	t		t	t	t		t			t	t	t	t	t	t				h
Heterodontus portusjacksoni	t		t	t	t		t			t		t	t	t	t				h
Mustelus canis	p									p	p				p				
Negaprion brevirostris		t	a	a	a	t		t	t			a	a			t	a	a	a
Notorynchus cepedianus	t		t	t	t		t		a	t	t	t	t	t	t				a
Orectolobus japonicus	p	t				t		t	t							t			a
Orectolobus maculatus	p	t				t		t	t							t			a
Orectolobus ornatus	p	t				t		t	t							t			a
Prionace glauca	t		t	t	t		t			t	t	t		t	t				
Rhincodon typus		t				t		t	t							t			
Scyliorhinus retifer	t		t	t	t		t			t		t		t	t				h
Scyliorhinus stellaris	t	p	t	t	t	p	t			t		t		t	t				
Sphyrna lewini		t				t		t	t				a			t	a	a	a
Sphyrna mokarran													a			t	a	a	a
Sphyrna tiburo	p	t				t		t	t							t			
Squalus acanthias	p																		h
Stegostoma fasciatum		t				t		t	t							t			
Triaenodon obesus		t				t		t	t							t			a
Triakis semifasciata	t		t	t	t		t			t		t		t	t				
Benthic batoids	p									p									h
Pelagic batoids	p									p									

Compatibility

Compatibility describes the interaction of a target species with other inhabitants of an exhibit. This system pertains not only to their interaction with other elasmobranchs, but with bony fishes, invertebrates, and turtles as well. Compatibility is ranked on a scale of one to five as follows:

1. *No compatibility problems* - Good with other elasmobranchs and in a multi-taxa exhibit.

2. *Sedentary, bottom dwelling* - These species can have their fins or eyes picked by some teleosts such as butterflyfish and angelfish (especially *Chaetodon* spp., *Heniochus* spp., *Holacanthus* spp., and *Pomacanthus* spp). Other-wise, these species do well in multi-taxa exhibits.

3. *Timid, non-aggressive* - These species do not do well with other species of elasmobranchs of equal or larger size.

4. *Aggressive towards teleosts* - These species will harass and frequently eat teleosts, but interact well with other elasmobranch species.

5. *Aggressive towards others* - These species will harass and frequently eat smaller tank inhabitants (e.g., teleosts, rays, etc.). They will commonly bite other elasmobranch species. These species have larger space requirements than others.

CONCLUSIONS

The information in this chapter is to be used only as a guide. The elasmobranchs described represent some of the most common species held in captivity, as well as a few key signature species. When planning to acquire elasmobranchs for an existing or new display, it is prudent to discuss detailed species requirements and traits with an institution that already displays the species. The factors that need to be considered are exhibit goal, exhibit design, species availability, species compatibility, and whether or not there is a plan for breeding. The AES captive elasmobranch census is a good information source for finding institutions experienced with a specific species.

ACKNOWLEDGEMENTS

I would like to thank the National Aquarium, Baltimore, USA for its editorial and staffing assistance during the writing of this chapter. Special thanks to Valerie Lounsbury for her guidance. John Ballard, Manny Ezcurra, Alan Henningsen, Joe Keyon, Christopher Paparo, and Gary Violetta provided valuable biological information.

REFERENCES

Carlson, J. K, Cortés, E. and Johnson, A. G. 1999. Age and growth of the blacknose shark, *Carcharhinus acronotus*, in the eastern Gulf of Mexico. Copeia 1999: 684-691.

Castro, J. I. 2000. The biology of the nurse shark, *Ginglymostoma cirratum*, off the Florida East coast and the Bahama Islands. Environmental Biology of Fishes 58: 1-22.

Compagno, L. J. V. 1984. Sharks of the World. FAO Species Catalogue. Vol 4. Sharks of the world. An annotated and illustrated catalogue of shark species known to date. Part 1. Hexanchiforms to Lamniformes; and Part 2. Carcharhiniformes. FAO Fisheries Synopsis, (125) Vol.4. 655 pp.

Compagno, L. J. V. 1999. Systematics and body form. *In* Sharks, Skates, and Rays: The Biology of Elasmobranch Fishes (Ed. Hamlett, W. C.) Baltimore: John Hopkins University Press. 1-42 pp.

Crow, G. L. and Hewitt J. D. 1988. Longevity records for captive tiger sharks, *Galeocerdo cuvier*, with notes on behavior and management. International Zoo Yearbook 27: 238-240.

Dehart, A. H. and Stoops, G. L. 1998. Husbandry observations, and treatment of fungal infection in a tiger shark, *Galeocerdo cuvier*. Drum and Croaker 29: 9-13.

Froese, R. and Pauly, D. (Eds.) 2000. Fishbase 2000: concepts, design and data sources. Los Baños, Laguna, Philippines: ICLARM. 344 pp.

Gordon, I. 1993. Pre-copulatory behaviour of captive sand tiger shark, *Carcharias taurus*. Environmental Biology of Fishes 38: 159-164.

Last, P. R. and Stevens, J. D. 1994. Sharks and Rays of Australia, Australia: CSIRO. 513 pp.

Mollet, H. F., Ezcurra, J. M. and O'Sullivan, J. B. 2002. Captive biology of the pelagic stingray, *Dasyatis violacea* (Bonaparte, 1832). Marine and Freshwater Research 53: 531-541.

Snelson, F. F., Jr., Williams-Hooper, S. E. and Schmid, T. H. 1988. Reproduction and ecology of the Atlantic stingray, *Dasyatis sabina*, in Florida coastal lagoons. Copeia 1988: 729-739.

Stoskopf, M. K. 1993. Fish Medicine. (Ed. M. K. Stoskopf) Philadelphia: W. B. Saunders Company, Harcourt Brace Jovanovich, Inc. 882 pp.

PERSONAL COMMUNICATIONS

Smith, M. 2002. Oceanário de Lisboa, 1990-005 Lisboa, Portugal.

Thoney, D. 2002. Humboldt State University, California 95570, USA.

Violetta, G. 2001. SeaWorld Florida, Orlando, FL 32821, USA.

Table 2.2. A brief description of elasmobranchs commonly held in aquaria and some key signature species that have proven difficult to maintain. Species were selected using the American Elasmobranch Society (AES) captive census from 1997, 2000, and 2001. All biological data was taken from Compagno (1984), and Froese and Pauly (2000), for the sharks and batoids respectively, except: 1 Carlson et al., 1999; 2 Last and Stevens, 1994; 3 Castro, 2000; 4 Snelson et al., 1988; 5 Mollet et al., 2002. Hardiness: (1) adapts readily; (2) adapts well; (3) delicate; and (4) difficult. Availability: (1) easy; (2) average; and (3) difficult. Compatibility: (1) No compatibility problems; (2) sedentary, bottom dwelling; (3) timid, non-aggressive; (4) aggressive towards teleosts; (5) aggressive towards others; and (-) unknown. Please refer to body text for a more detailed description of the indices for hardiness, availability, and compatibility.

Species name	Common name	Maximum Size	Hardiness	Availability	Compatibility	Range	Description
Aetobatus narinari	spotted eagle ray	180 cm DW	3	2	1	Circumglobal in tropical waters.	Difficult to get through quarantine. Require more care than other rays.
Carcharhinus acronotus	blacknose shark	137 cm TL[1]	3	2	1	Western Atlantic in coastal temperate and tropical waters.	Delicate through quarantine, but hardy once acclimated.
Carcharhinus leucas	bull shark	340 cm TL	2	2	5	Circumglobal, in tropical and subtropical waters. Also occurs in fresh water.	Very aggressive species. Should be handled with care. Will eat rays and sharks.
Carcharhinus limbatus	blacktip shark	255 cm TL	3	2	1	Circumglobal, in tropical and subtropical continental waters.	Difficult to transport.
Carcharhinus longimanus	oceanic whitetip shark	300 cm TL	2	2	1	Circumglobal, pelagic in tropical waters.	Occasionally held species. Species does well in multitaxa display. Few long-term successes with this species.
Carcharhinus melanopterus	blacktip reef shark	180 cm TL	1	2	4	Inshore waters of the Indo-Pacific.	Commonly held species. Species does well in multitaxa exhibit.
Carcharhinus perezi	Caribbean reef shark	295 cm TL	3	2	4	Tropical inshore waters of the Caribbean.	Difficult to transport.
Carcharhinus plumbeus	sandbar shark	239 cm TL	2	2	1	Circumglobal, in coastal and pelagic temperate and tropical waters.	Commonly held species. Species does well in multitaxa exhibit.
Carcharias taurus	sand tiger shark	318 cm TL	1	3	4	Circumglobal, in temperate and tropical waters.	Commonly held species. Becoming hard to obtain in certain regions.
Carcharodon carcharias	great white shark	640 cm TL	4	3	-	Circumglobal in coastal waters.	Very difficult specimen to keep. Longest captivity to date is 16 days.
Cephaloscyllium ventriosum	swellshark	100 cm TL	1	1	1	Eastern Pacific in temperate and subtropical waters.	Commonly held species. Frequently breeds in captivity.
Chiloscyllium plagiosum	whitespotted bamboo shark	95 cm TL	1	1	2	Inshore Indo-West Pacific.	Bottom-dwelling species. Readily breeds in captivity.
Chiloscyllium punctatum	brownbanded bamboo shark	104 cm TL	1	2	2	Inshore West-Pacific.	Bottom-dwelling species. Does well in smaller exhibits.
Dasyatis americana	southern stingray	180 cm DW	1	1	2	Western Atlantic.	Common captive species. Reproduces readily in captivity.
Dasyatis centroura	roughtail stingray	220 cm DW	2	2	2	Eastern and Western Atlantic.	Large ray species that does well in captivity.
Dasyatis sabina	Atlantic stingray	45 cm DW[4]	1	1	2	Northern West Atlantic.	Similar to southern stingray, but smaller.
Eucrossorhinus dasypogon	tasseled wobbegong	117 cm TL	3	2	4	Western South Pacific.	Will frequently prey on smaller exhibit inhabitants.
Galeocerdo cuvier	tiger shark	600 cm TL[2]	4	2	3	Circumglobal, in temperate and tropical waters.	Extremely difficult species to transport and keep in captivity.
Ginglymostoma cirratum	nurse shark	280 cm TL[3]	1	1	2	Western Atlantic.	Very common and hardy species in captivity.
Hemiscyllium ocellatum	epaulette shark	107 cm TL	1	1	2	Western South Pacific.	Bottom-dwelling reef species. Good for smaller exhibits.
Heterodontus francisci	horn shark	122 cm TL	3	2	1	Eastern Pacific in temperate and subtropical waters.	Will prey on invertebrates in captivity.

Table 2.2 (continued). A brief description of elasmobranchs commonly held in aquaria and some key signature species that have proven difficult to maintain. Species were selected using the American Elasmobranch Society (AES) captive census from 1997, 2000, and 2001. All biological data was taken from Compagno (1984), and Froese and Pauly (2000), for the sharks and batoids respectively, except: 1 Carlson et al., 1999; 2 Last and Stevens, 1994; 3 Castro, 2000; 4 Snelson et al., 1988; 5 Mollet et al., 2002. Hardiness: (1) adapts readily; (2) adapts well; (3) delicate; and (4) difficult. Availability: (1) easy; (2) average; and (3) difficult. Compatibility: (1) No compatibility problems; (2) sedentary, bottom dwelling; (3) timid, non-aggressive; (4) aggressive towards teleosts; (5) aggressive towards others; and (-) unknown. Please refer to body text for a more detailed description of the indices for hardiness, availability, and compatibility.

Species name	Common name	Maximum Size	Hardiness	Availability	Compatibility	Range	Description
Heterodontus portusjacksoni	Port Jackson shark	165 cm TL	3	2	1	West South Pacific around Australia.	Will prey on invertebrates in captivity.
Himantura uarnak	honeycomb stingray	200 cm DW	1	2	2	Indo-West Pacific.	Hardy display species which has bred in captivity.
Isurus oxyrinchus	shortfin mako	394 cm TL	4	3	-	Circumglobal in oceanic and coastal temperate and tropical waters.	Species has proven difficult to keep long-term.
Leucoraja erinacea	little skate	54 cm DW	3	1	2	Northern West Atlantic.	Hardy once the animal acclimates and begins eating.
Myliobatis aquila	common eagle ray	183 cm DW	3	2	1	Eastern Atlantic and Mediterranean.	Can be sensitive to temperature extremes within its range.
Myliobatis californica	bat eagle ray	180 cm DW	1	1	2	Eastern Pacific.	Commonly parasitized by flukes at time of collection.
Negaprion brevirostris	lemon shark	340 cm TL	1	2	5	Western Atlantic in tropical inshore waters.	Can be an aggressive shark in captivity. Will eat rays.
Notorynchus cepedianus	broadnose sevengill shark	290 cm TL	1	2	1	Wide-ranging in temperate seas.	Have a tendency to abrade their rostrum in captivity.
Orectolobus japonicus	Japanese wobbegong	103+ cm TL	3	2	4	Western North Pacific	Will frequently prey on smaller exhibit inhabitants.
Orectolobus maculatus	spotted wobbegong	320 cm TL	3	2	4	Western Pacific.	Will frequently prey on smaller exhibit inhabitants.
Orectolobus ornatus	ornate wobbegong	288 cm TL	3	2	4	Western Pacific.	Will frequently prey on smaller exhibit inhabitants.
Potamotrygon henlei	bigtooth river stingray	35 cm DW	3	2	1	South America in freshwater rivers, mainly in Brazil.	Similar to *P. leopoldi* , but has spots on ventral edges of disc. Hardy display specimen.
Potamotrygon leopoldi	white-blotched river stingray	50 cm DW	3	2	1	South America in freshwater rivers and specifically from the Rio Xingu basin.	Very similar to *P. henlei* , but lacking ventral spots.
Potamotrygon motoro	ocellate river stingray	100 cm DW	1	1	1	South America in freshwater rivers.	A common and hardy species that has bred in captivity.
Potamotrygon reticulatus	spotted freshwater ray	32 cm DW	3	2	1	South America in freshwater rivers.	A fairly common captive species with much variation in color and pattern.
Prionace glauca	blue shark	383 cm TL	4	2	3	Oceanic and circumglobal in temperate and tropical waters.	Very delicate species. Do not last long in captive environment. Tend to abrade fins and rostrum on perimeter of exhibit.
Pristis pectinata	smalltooth sawfish	550 cm TL[2]	3	3	4	Circumglobal in inshore and intertidal waters.	Protected species which is very difficult to obtain.
Pteroplatytrygon violacea	pelagic stingray	80 cm TL[5]	1	2	1	Circumglobal, pelagic in tropical and temperate waters.	Swims constantly. The only truly pelagic dasyatid.
Raja binoculata	big skate	244 cm DW	3	1	2	Northern Pacific.	Very large skate. Hardy once the animal acclimates and begins eating.
Raja eglanteria	clearnose skate	65 cm DW	3	1	2	Northern West Atlantic.	Hardy once the animal acclimates and begins eating.
Raja rhina	longnose skate	140 cm DW	3	1	2	Eastern Pacific.	Hardy once the animal acclimates and begins eating.

Table 2.2 (continued). A brief description of elasmobranchs commonly held in aquaria and some key signature species that have proven difficult to maintain. Species were selected using the American Elasmobranch Society (AES) captive census from 1997, 2000, and 2001. All biological data was taken from Compagno (1984), and Froese and Pauly (2000), for the sharks and batoids respectively, except: 1 Carlson et al., 1999; 2 Last and Stevens, 1994; 3 Castro, 2000; 4 Snelson et al., 1988; 5 Mollet et al., 2002. Hardiness: (1) adapts readily; (2) adapts well; (3) delicate; and (4) difficult. Availability: (1) easy; (2) average; and (3) difficult. Compatibility: (1) No compatibility problems; (2) sedentary, bottom dwelling; (3) timid, non-aggressive; (4) aggressive towards teleosts; (5) aggressive towards others; and (-) unknown. Please refer to body text for a more detailed description of the indices for hardiness, availability, and compatibility.

Species name	Common name	Maximum Size	Hardiness	Availability	Compatibility	Range	Description
Rhina ancylostoma	bowmouth guitarfish	270 cm TL	1	2	2	Indian Ocean and Western Pacific in tropical waters.	Common and hardy species.
Rhincodon typus	whale shark	1200 cm TL	4	3	1	Circumglobal in tropical oceanic and coastal waters.	Very difficult species to keep due to large size and feeding methods.
Rhinobatos lentiginosus	Atlantic guitarfish	75 cm TL	3	2	2	Western Atlantic.	Possible heavy parasite problems at time of collection.
Rhinobatos productus	shovelnose guitarfish	170 cm TL	3	1	2	Eastern Pacific.	Can be difficult to get through quarantine.
Rhinoptera bonasus	cownose ray	105 cm DW	3	1	1	Eastern and Western Atlantic.	Good multitaxa species. Males can be extremely aggressive towards females at mating time.
Scyliorhinus retifer	chain dogfish	47cm TL	2	2	1	Western North Atlantic.	A good species for smaller exhibits. Will breed in captivity.
Scyliorhinus stellaris	nursehound	162 cm TL	1	1	1	Eastern North Atlantic.	Tendency to swim along perimeter of exhibit.
Sphyrna lewini	scalloped hammerhead	370 cm TL	4	3	3	Circumglobal in temperate and tropical waters.	Can be a delicate species to keep and transport. Tendency to abrade head.
Sphyrna mokarran	great hammerhead	600 cm TL	4	3	3	Circumglobal in tropical inshore and pelagic habitats.	Can be a delicate species to keep and transport. Tendency to abrade head.
Sphyrna tiburo	bonnethead	150 cm TL	3	2	1	Western Atlantic and Eastern Pacific.	Delicate through quarantine, but hardy once acclimated.
Squalus acanthias	spiny dogfish	160 cm TL	3	2	1	Circumglobal in antetropical regions.	Frequently swim around perimeter of display with rostrum out of water.
Stegostoma fasciatum	zebra shark	354 cm TL	1	1	2	Indo-West Pacific in tropical inshore waters.	Excellent exhibit species. Can suffer eye damage from certain teleost species.
Taeniura lymma	bluespotted ribbontail ray	30 cm DW	4	1	2	Indo-West Pacific.	Difficult to feed in captivity. Long-term survival is rare.
Triaenodon obesus	whitetip reef shark	160 cm TL	1	1	4	Indo-Pacific in tropical inshore waters.	Can be aggressive towards rays and teleosts.
Triakis semifasciata	leopard shark	180 cm TL	2	1	1	Eastern North Pacific in temperate waters.	Delicate through quarantine, but hardy once acclimated.
Urolophus halleri	Haller's round ray	56 cm DW	1	2	2	Eastern Pacific.	Hardy species.
Urobatis jamaicensis	yellow stingray	36 cm DW	1	1	2	Western Atlantic in tropical waters.	Very common captive species. Good for smaller displays.

The Elasmobranch Husbandry Manual: Captive Care of Sharks, Rays and their Relatives, pages 25-41.
© 2004 Ohio Biological Survey

Chapter 3
Collecting Elasmobranchs: Legislation, Permitting, Ethics, and Commercial Collectors

JOSEPH M. CHOROMANSKI

Ripley Aquariums
Ripley Entertainment, Inc.
7576 Kingspointe Parkway, Suite 188,
Orlando, FL 32819, USA.
E-mail: jchoromanski@ripleys.com

Abstract: A number of international and national organizations, both governmental and non-governmental, have jurisdiction or influence over the management of marine fisheries, and hence, over the legal collection of elasmobranchs. It is the responsibility of aquarium staff to understand and adhere to any legislation, both international and regional, relevant to their elasmobranch collections. In addition, it is imperative that public aquariums and commercial collectors work closely with regulatory agencies to help educate them about the unique nature of our business. Regulatory agencies should be regarded as partners and not adversaries. Information learned through collection activities should be shared with regulatory agencies, whether required by law or not, to help build healthy relationships, dispel misconceptions, and improve a mutual understanding of the species in question. Zoos and aquariums justify the collection and display of wild animals by the educational, research, and conservation goals achieved. A frequently asked and basic ethical question is as follows: Do the benefits of a quality display of elasmobranchs at a professionally-operated public aquarium, having a strong educational, research, and conservation mission, outweigh the cost to individual animal welfare? We, as an industry, believe that they do. In addition to this basic question, other, more specific ethical concerns should be considered when formulating an elasmobranch collection for an aquarium. Is the species difficult to keep? Is it appropriate and permissible to release the species should it outgrow an exhibit? Is the species at threat of extinction in the wild and therefore protected? In seeking to better understand and meet the aforementioned ethical considerations, the public aquarium community has recourse to many professional zoo and aquarium associations.

Sharks, skates, rays (the elasmobranchs), and chimeras together comprise the class Chondrichthyes, or the cartilaginous fishes, a group of over 1,000 species of mostly marine fishes. Much of the legislation (e.g., commercial fishery regulations, etc.) that regulates the harvest of elasmobranchs encompasses a far greater number of individuals and species than the international aquarium community would ever conceivably display. Legislative information specific to the commercial fishery can be found elsewhere (Camhi, 1998; Camhi et al., 1998; Camhi, 1999; Anon., 2001a).

This chapter focuses on aspects of legislation and permitting, for as many countries as possible, as it pertains to elasmobranch species that are commonly collected and displayed by public aquariums. Due to space limitations, the chapter centers on legislation and permitting for collecting elasmobranchs. It does not address legislation and permitting, where required, for the possession or importation of elasmobranch species, as this information is readily available from governmental agencies. Likewise, the chapter does not detail fisheries management regulations (i.e., regulations to govern the commercial take of elasmobranchs for consumptive purposes), but rather addresses those regulations that may potentially affect the future collection of a species for public display. The chapter concludes by briefly discussing ethical considerations related to the collection and display of elasmobranchs, and the use of commercial collectors.

LEGISLATION AND PERMITTING

Many readers of this chapter will only want to know what paperwork is required to collect the species they desire and how to go about getting the proper permits. Before this can be addressed, it must be understood that the information provided in this chapter is current as of mid-2003 and is unavoidably a snapshot in time. Only a few countries (e.g., Australia, Canada, New Zealand, South Africa, and the United States) have fishery management plans for specific shark fisheries. As such, specific legislation and permitting regulations for only a few countries are detailed in this chapter. Fishery regulations often change, and curators and commercial collectors must remain informed and up-to-date about this rapidly changing arena. The information provided herein serves as a starting point for researching legislative and permitting changes that will no doubt occur over time.

International regulations

Elasmobranch collection is regulated to varying extremes throughout the world, ranging from outright prohibition, to taking only certain species, to no regulation whatsoever. At present, there are no international management programs or regulations that effectively address the capture of sharks (Anon., 2001a). Most sharks and many rays are highly migratory and routinely cross political boundaries (Camhi et al., 1998), making management challenging.

FAO

During 1999, the Food and Agriculture Organization of the United Nations (FAO), Committee on Fisheries (COFI), adopted the International Plan of Action for the Conservation and Management of Sharks (IPOA). The IPOA (Anon., 1999a), building on the FAO Code of Conduct for Responsible Fisheries, encompasses all elasmobranch fisheries and calls on member nations to develop National Plans of Action (NPOA) for the conservation and management of sharks. Although the IPOA applies to all States, entities, and fishers, participation is voluntary. As of late 2002, only two NPOAs have been completed (i.e., for the USA and Japan) out of 87 shark-fishing nations, 18 of which are considered major fishing nations (i.e., landing >10,000 metric tons year^{-1}). Several States have draft NPOAs (i.e., Australia and the EU) and several more are reported to be in preparation (e.g., South Africa) (Anon., 2001a; Anon., 2002a; Anon., 2002b; Anon., 2002c; Smale, pers. com.). Readers are urged to study detailed information about the IPOA, available at the FAO website (www3).

CITES

The Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES) is an agreement that provides for the protection of certain species against over-exploitation through international trade. Under CITES, species are listed in appendices according to their conservation status. Appendix I species are considered to be threatened with extinction, and international trade for commercial purposes is generally not permitted. Appendix II species are not necessarily now threatened with extinction, but may become so if trade is not strictly regulated. Appendix III includes species that any party (i.e., signatory country to CITES) has identified as being subject to regulation within its jurisdiction, to prevent or restrict exploitation, and is seeking cooperation in the control of the trade of that species. Species can only be added, removed, or transferred between Appendix I and II during regular (2-3 year) meetings of the Conference of Parties (COP) or by emergency postal procedures, whereas species can be added or removed from Appendix III by any party at any time (www4).

Prior to 2001, a number of elasmobranch species, including all of the sawfishes (Family: Pristidae), were proposed for listing on CITES Appendices I or II, but were not accepted (Anon., 2001a). In response, the basking (*Cetorhinus maximus*) and great white (*Carcharodon carcharias*) sharks were listed in Appendix III by the United Kingdom and Australia, respectively. During the 12th COP in 2002, Appendix II proposals were approved from India and the Philippines for the whale shark (*Rhincodon typus*), and from the United Kingdom for the basking shark (Table 3.1). Not only do these listings represent the first time elasmobranch species have been included in CITES Appendix II, they also represent the only international trade regulation affecting elasmobranchs. An Appendix II listing does not end or restrict trade as long as the exporting country can demonstrate that trade in a listed species, or its products, is not detrimental to the survival of that species. Appendix II listing requires data collection and reporting by any of the 160 member countries involved in the trade of listed species.

Table 3.1. Conservation and permitting status of elasmobranchs showing: Convention on the International Trade in Endangered Species (CITES) status; World Conservation Union (IUCN) Red List status; the American Fisheries Society (AFS) status list of Elasmobranch Species Distinct Population Segments; species regulated by the United States Federal Government National Marine Fisheries Service (NMFS); species regulated by the Shark Advisory Group (SAG) of the Australian Department of Agriculture, Fisheries and Forestry; and species regulated by the Marine Living Resources Act (MLRA) of the South Africa National Government.

Scientific Name	Common Name	CITES	IUCN Red List Status [a,b,c]	AFS (N. Am.) [d,e]	NMFS (USA) [f]	SAG (AUS) [g]	MLRA (SA)[h]
Aetobatus narinari	spotted eagle ray		DD			LR/lc	
Alopias superciliosus	bigeye thresher shark				P		
Alopias vulpinus	thintail thresher shark		DD			DD (A)	
Amblyraja radiata	thorny skate			VU (US) + NA (CA)			
Anoxypristis cuspidata	knifetooth sawfish		EN A1acde+2cde			VU (A)	
Bathyraja abyssicola	deepsea skate		DD				
Callorhinchus milii	ghost shark					(A)	
Carcharhinus altimus	bignose shark				P		
Carcharhinus amblyrhynchoides	graceful shark		LR/nt			LR/nt	
Carcharhinus amblyrhynchos	gray reef shark		LR/nt			LR/lc	
Carcharhinus amboinensis	pigeye shark		DD (LR/nt: SWI)			DD	
Carcharhinus borneensis	Borneo shark		EN C2b				
Carcharhinus brachyurus	copper shark				P	(A)	
Carcharhinus brevipinna	spinner shark		LR/nt (VU A1bd+2d: NWA)			LR/lc	
Carcharhinus galapagensis	Galapagos shark				P		
Carcharhinus hemiodon	Pondicherry shark		VU C2a				
Carcharhinus leiodon	smalltooth shark		VU B1+2c, C2b				
Carcharhinus leucas	bull shark		LR/nt			LR/lc	
Carcharhinus limbatus	blacktip shark		LR/nt (VU A1bcd+2cd: NWA)			DD	
Carcharhinus longimanus	oceanic whitetip shark		LR/nt			LR/nt	
Carcharhinus melanopterus	blacktip reef shark		LR/nt			LR/nt	
Carcharhinus obscurus	dusky shark		LR/nt (VU A1abd: NWA + GM)	VU (WA + EP)	P	LR/nt	
Carcharhinus perezi	Caribbean reef shark				P		
Carcharhinus plumbeus	sandbar shark		LR/nt (LR/cd: NWA)			LR/nt (A)	
Carcharhinus porosus	smalltail shark				P		
Carcharhinus signatus	night shark			VU (WA)	P		
Carcharias taurus	sand tiger shark		VU A1ab+2d	VU (WA)	P	EN (P)	AN 4
Carcharodon carcharias	great white shark	APP III	VU A1cd+2cd	CD (WA + EP)	P	VU (P)	AN 5
Centrophorus granulosus	gulper shark		VU A1abd+2d			DD	
Centrophorus harrissoni	dumb gulper shark					EN (EPBCA)	
Centrophorus uyato	little gulper shark					VU (EPBCA)	
Cetorhinus maximus	basking shark	APP II	VU A1ad+2d (EN A1d: NP + NEA)	VU (EP) + CD(WA)	P	DD (P)	
Dalatias licha	kitefin shark		DD (LR/nt: NEA)			DD (A)	
Dasyatis fluviorum	estuary stingray					LR/nt	
Dasyatis garouaensis	smooth freshwater stingray		VU B1+2cde, C2b				

a. 2000 IUCN Red List status categories: Critically Endangered (CR); Endangered (EN); Vulnerable (VU); Lower Risk (LR) where nt = near threatened, cd = conservation dependent and lc = least concern; and Data Deficient (DD).

b. 2000 IUCN Red List status criteria: Upper case letters, numbers and lower case letters adjacent to the category listings (e.g., A1abd+2d) refer to specific criteria defined for each red list category. The detailed descriptions of these criteria are available on the red lost web site (www 8).

c. 2000 IUCN Red List status regions: Australasian subpopulation (AU); Brazilian subpopulation (BR); Eastern Pacific subpopulation (EP); Gulf of Mexico (GM); Northeast Atlantic subpopulation (NEA); North Pacific subpopulation (NP); Northwest Atlantic subpopulation (NWA); Southwest Atlantic subpopulation SWA); Southwest Indian Ocean subpopulation (SWI); and Thailand subpopulation (TH).

d. AFS (North America) categories: Endangered (ED); Threatened (T); Vulnerable (VU); Conservation Dependant (CD); Not at Risk (NR); and not assessed (NA).

e. AFS (North America) regions: Canada (CA); Eastern Pacific (EP); Gulf of California (GC); Gulf of Mexico (GM); United States territorial waters (US); and Western Atlantic (WA).

f. NMFS (USA) categories: Endangered under the U.S. Endangered Species Act (E); Possession is prohibited in commercial and recreational fisheries (P).

g. SAG (Australia) categories: Categories are the same as used by the 2000 IUCN Red List (see footnote "a"). Parenthetical annotations: Protected in some state, territory, and/ or Commonwealth waters (P); Potentially of concern given consistent high catch rates in non-target fisheries (A); Being considered for listing as a threatened species under the Environment Protection and Biodiversity Conservation Act (EPBCA).

h. MLRA (South Africa) categories: Annexure 4 (non- saleable recreational list), fishers are allowed 10 in total from this list but no more than 5 of any one species (AN 4); Annexure 5 (specially protected list), no take allowed (AN 5).

Table 3.1 (continued). Conservation and permitting status of elasmobranchs showing: Convention on the International Trade in Endangered Species (CITES) status; World Conservation Union (IUCN) Red List status; the American Fisheries Society (AFS) status list of Elasmobranch Species Distinct Population Segments; species regulated by the United States Federal Government National Marine Fisheries Service (NMFS); species regulated by the Shark Advisory Group (SAG) of the Australian Department of Agriculture, Fisheries and Forestry; and species regulated by the Marine Living Resources Act (MLRA) of the South Africa National Government.

Scientific Name	Common Name	CITES	IUCN Red List Status [a,b,c]	AFS (N. Am.) [d,e]	NMFS (USA) [f]	SAG (AUS) [g]	MLRA (SA) [h]
Dasyatis laosensis	Mekong stingray		EN A1cde+2cde, B1+2ce				
Dipturus batis	skate		EN A1abcd+2bcd				
Dipturus laevis	barndoor skate		VU A1bcd	VU (CA + WA)			
Furgaleus macki	whiskery shark		LR/cd			LR/cd	
Galeocerdo cuvier	tiger shark		LR/nt			LR/lc	
Galeorhinus galeus	tope shark		VU A1bd (LR/cd: AU)			LR/cd (A)	
Glyphis gangeticus	Ganges shark		CR A1cde+2cde, C2b				
Glyphis glyphis (species A)	speartooth shark		EN C2a			CR (P)	
Glyphis sp. (species C)	northern river shark					EN (P)	
Haploblepharus edwardsii	puffadder shyshark		LR/nt				
Haploblepharus fuscus	brown shyshark		LR/nt				
Hemitriakis leucoperiptera	whitefin topeshark		EN B1+2ce, C2b				
Heteroscyllium colcloughi	bluegray carpetshark		VU C2b			VU (EPBCA)	
Hexanchus griseus	bluntnose sixgill shark		LR/nt		P	DD	
Hexanchus nakamurai	bigeye sixgill shark				P		
Himantura chaophraya	freshwater stingray		VU A1bcde+2ce (CR A1bcde+2ce: TH)			VU	
Himantura fluviatilis	Ganges stingray		EN A1cde+2cde, B1+2c				
Himantura oxyrhynchus	marbled whipray		EN B1+2c				
Himantura signifer	white-rimmed whipray		EN B1+2c				
Hydrolagus ogilbyi	Ogilby's ghostshark					(A)	
Hypogaleus hyugaensis	blacktip topeshark		LR/nt			LR/lc	
Isurus oxyrinchus	shortfin mako		LR/nt			LR/lc (A)	
Isurus paucus	longfin mako				P		
Lamna ditropis	salmon shark		DD				
Lamna nasus	porbeagle		LR/nt (VU A1bd: NEA) (LR/cd: NWA)			LR/lc (A)	
Leptocharias smithii	barbeled houndshark		LR/nt				
Manta birostris	giant manta					LR/lc	
Megachasma pelagios	megamouth shark		DD			DD (P)	
Mobula mobular	devil fish		VU A1cd				
Mustelus antarcticus	gummy shark		LR/cd			LR/lc (A)	
Mustelus canis	dusky smooth-hound		LR/nt				
Mustelus lenticulatus	spotted estuary smooth-hound		LR/cd				
Negaprion brevirostris	lemon shark		LR/nt				
Notorynchus cepedianus	broadnose sevengill shark		DD (LR/nt: EP)		P	DD (A)	
Odontaspis ferox	smalltooth sand tiger shark					LR/nt (P)	
Odontaspis noronhai	bigeye sand tiger shark		DD		P		
Orectolobus maculatus	spotted wobbegong					DD	
Orectolobus ornatus	ornate wobbegong					DD	
Poroderma africanum	striped catshark		LR/nt				AN 4
Poroderma pantherinum	leopard catshark						AN 4
Potamotrygon brachyura	short-tailed river stingray		DD				
Potamotrygon henlei	bigtooth river stingray		DD				

Table 3.1 (continued). Conservation and permitting status of elasmobranchs showing: Convention on the International Trade in Endangered Species (CITES) status; World Conservation Union (IUCN) Red List status; the American Fisheries Society (AFS) status list of Elasmobranch Species Distinct Population Segments; species regulated by the United States Federal Government National Marine Fisheries Service (NMFS); species regulated by the Shark Advisory Group (SAG) of the Australian Department of Agriculture, Fisheries and Forestry; and species regulated by the Marine Living Resources Act (MLRA) of the South Africa National Government.

Scientific Name	Common Name	CITES	IUCN Red List Status [a,b,c]	AFS (N. Am.) [d,e]	NMFS (USA) [f]	SAG (AUS) [g]	MLRA (SA) [h]
Potamotrygon leopoldi	white-blotched river stingray		DD				
Potamotrygon motoro	ocellate river stingray		DD				
Prionace glauca	blue shark		LR/nt			LR/lc (A)	
Pristidae	sawfishes (all species)						AN 5
Pristiophorus cirratus	longnose sawshark		LR/nt			LR/cd	
Pristis clavata	dwarf sawfish		EN A1acd+2cd			EN	
Pristis microdon	largetooth sawfish		EN A1bcde+2bcde (CR A1abc+2cd: SEA)			CR (P)	
Pristis pectinata	smalltooth sawfish		EN A1bcd+2cd (CR A1abc+2cd: NWA + SWA)	ED (US + GM)	E	DD	
Pristis perotteti	large-tooth sawfish		CR A1abc+2cd	ED (US + GM + GC)			
Pristis pristis	common sawfish		CR A1abc+2cd				
Pristis zijsron	longcomb sawfish		EN A1bcd+2cd			EN (A)	
Pseudocarcharias kamoharai	crocodile shark		LR/nt			LC/lc	
Raja binoculata	big skate		LR/nt	VU (EP)			
Raja clavata	Thornback ray		LR/nt				
Raja microocellata	Small-eyed ray		LR/nt				
Raja sp. L	Maugaen skate		EN B1+2c			EN (EPBCA)	
Rhincodon typus	whale shark	APP II	VU A1bd+2d	CD (USA + AT + GM); NR (GC)	P	DD (P)	
Rhinobatos horkeli	Brazilian guitarfish		CR A1bd+2bd				
Rhizoprionodon porosus	Caribbean sharpnose shark				P		
Rhynchobatus djiddensis	giant guitarfish		VU A1bd+2d			LR/lc	
Schroederichthys bivius	narrowmouthed catshark		DD				
Scoliodon laticaudus	spadenose catshark		LR/nt				
Scyliorhinus capensis	yellowspotted catshark		LR/nt				
Scylliogaleus quecketti	flapnose houndshark		VU B1+2c, C2b				
Sphyrna lewini	scalloped hammerhead		LR/nt			LR/lc	
Sphyrna mokarran	great hammerhead		DD			LR/lc	
Sphyrna zygaena	smooth hammerhead		LR/nt			LR/lc	
Squalus acanthias	spiny dogfish		LR/nt			LR/lc	
Squatina argentina	Argentine angelshark		DD				
Squatina californica	Pacific angelshark		LR/nt				
Squatina dumeril	sand devil				P		
Squatina guggenheim	angular angelshark		VU A1bd+A2d (EN A1bd+2d: BR)				
Squatina occulta	hidden angelshark		EN A1abd+A2d				
Squatina squatina	angelshark		VU A1abcd+A2d				
Taeniura lymma	bluespotted ribbontail ray		LR/nt			LR/lc	
Triaenodon obesus	whitetip reef shark		LR/nt			LR/lc	
Triakis acutipinna	sharpfin houndshark		VU C2b				
Triakis megalopterus	sharptooth houndshark		LR/nt				AN 4
Triakis semifasciata	leopard shark		LR/cd				
Urogymnus asperrimus	porcupine ray		VU A1bd, B1+2bcd			LR/nt	
Urogymnus ukpam	thorny freshwater stingray		EN B1+2abcd				

Conservation assessment lists

The conservation status of many elasmobranch species has been assessed by a variety of non-governmental (NGO) conservation agencies, in the form of classification lists. These lists have no governmental or regulatory authority per se, however, they often form the basis of existing or future fishery regulations.

IUCN Red list of Threatened Species™

The IUCN (World Conservation Union) brings together states, government agencies, and a diverse range of NGOs, in a unique world partnership with over 980 members in some 140 countries. The IUCN's mission is "…to influence, encourage, and assist societies throughout the world to conserve the integrity and diversity of nature and to ensure that any use of natural resources is equitable and ecologically sustainable…" (www5). Although the IUCN has no regulatory power, it does seek to influence the implementation of international conservation conventions such as CITES, World Heritage, and the Convention on Biological Diversity.

The IUCN Red List of Threatened Species™ is now widely recognized as the most comprehensive, apolitical global system for evaluating the conservation status of plant and animal species. From small beginnings, almost 30 years ago, the IUCN Red List has grown in size and complexity. The IUCN's scientifically rigorous approach to determining risk of extinction, introduced in 1994 and applicable to all species and infra-specific taxa, has virtually become a world standard (Anon., 1994). These criteria were updated in 2001 (Anon., 2001b), in part to address concerns over the application of earlier criteria to commercially exploited marine fishes, although most elasmobranch evaluations are still based on the criteria established in 1994. The last major printed publication of the IUCN Red List was in 2000 (Hilton-Taylor, 2000). Since 2000, the IUCN Red List has been updated annually on their official web site (www8). The next printed update of the IUCN Red List is planned for 2004.

The main purpose of the IUCN Red List is to catalogue and highlight those taxa that are at risk of global extinction (i.e., "critically endangered", "endangered", and "vulnerable"). The IUCN Red List includes information on taxa that are categorized as "extinct or extinct in the wild"; "data deficient" (i.e., taxa that cannot be evaluated because of insufficient information); and "near threatened" (i.e., taxa that are close to threatened thresholds). The IUCN Red List's regular program of updates and publications provides a means of monitoring changes in the status of listed species.

Between 1996 and 2000, the number of fish species on the IUCN Red List increased dramatically, largely as a result of an improved coverage of the sharks and rays. The 1996 IUCN Red List (Baille and Groombride, 1996) included 32 species of elasmobranchs, while the 2000 IUCN Red List (Hilton-Taylor, 2000) included 95 species (Table 3.1). A review of the IUCN Red List assessments for all chondrichthyan fishes is scheduled for 2004. [Author's Note (September, 2004): The current web-based IUCN Red List now contains 185 species of elasmobranchs (www 8).]

AFS

Musick et al. (2000), under the auspices of the American Fisheries Society (AFS), published the first recognized list of marine fish species and marine fish stocks at risk of extinction (MSRE). The AFS list identified 82 species or populations categorized as "vulnerable", "threatened", or "endangered" in North American waters, 22 of which may be "vulnerable" to global extinction. The status of these organisms was determined by applying risk criteria (i.e., rarity, small range limits and endemicity, specialized habitat requirements, population resilience to decline, and fecundity) developed from peer-reviewed knowledge and expert scientific opinion. Most stocks faced more than one risk factor, but life history limitations (e.g., low or very low reproductive capacity) were considered particularly important.

A fish stock refers to a group of fish that can be treated as a single unit for management purposes. In identifying which units were at risk, Musick et al. (2000) employed the concept of distinct population segments (DPSs). DPSs were defined as populations markedly separated from other populations of the same organism, as a consequence of significant physical, physiological, ecological, or behavioral factors (Anon., 1996).

Fisheries scientists believe it is important to recognize threatened fish populations early in their decline and implement conservation measures that will preclude further population reduction or extinction. AFS categories deal with

extinction risk, and not growth or recruitment, except where over-fishing threatens recruitment and thus a DPS with extinction. AFS recognizes the following categories of risk: (1) "endangered", i.e., high risk of extinction in the wild in the immediate future (years); (2) "threatened", i.e., not endangered but facing risk of extinction in the near future (decades); (3) "vulnerable" (special concern), i.e., not endangered or threatened severely, but at possible risk of falling into one of these categories in the near future; (4) "conservation dependent", i.e., reduced but stabilized or recovering under a continuing conservation plan; and (5) "not at risk", i.e., not at apparent risk of extinction. Of the 82 species listed in the AFS publication (Musick et al., 2000), 11 are elasmobranch species (Table 3.1).

The constraints of lists

Although conservation assessment lists are intended to help protect and conserve elasmobranch species, and represent considerable effort and research, they present a risk to public aquariums.

Firstly, there is the issue of non-standardized, if not confusing, nomenclature. For example, the IUCN Red List classes a species as "threatened" if it falls into any of the "critically endangered", "endangered", or "vulnerable" categories. Similarly, the U.S. Endangered Species Act (ESA) classifies species as either "threatened" or "endangered", based on population status, but it is common for ESA-assessed animals to be referred to in general as simply "endangered". In addition, the AFS list has adopted similar, but not identical, classifications as the IUCN Red List.

Secondly, there is the issue of confusing management units when distinguishing between a species, a distinct population, DPSs, or stocks. Most non-scientific individuals do not differentiate between the various forms of "endangered" and/or "threatened", nor between DPS's and species. This confusion can lead to bad legislation and especially confusing law enforcement. Aquariums have already observed this problem with the green sea turtle (*Chelonia mydas*), listed by ESA as "endangered" but having a Caribbean population classified under the less restrictive "threatened".

Thirdly, well intended fishery regulators may adopt conservation recommendations and incorporate assessment lists verbatim, creating blanket legislation that has no exemption for the collection and live display of elasmobranchs. Sweeping interpretations of this nature can preclude the opportunity of presenting important conservation messages to the public, through engaging and educational live displays.

It is essential that aquarists and fishery managers familiarize themselves with the different definitions used for, and the rationale behind, all conservation assessment listings. In addition, it is important to understand the difference between advisory, non-statutory lists (e.g., the IUCN Red List, the AFS MSRE, etc.) and lists enacted through legislation (see below).

National regulations: USA

Atlantic FMP, Shark FMP, and EFPs

The Magnuson-Stevens Fishery Conservation and Management Act (M-S Act) of 1976, is the primary legislation governing the conservation and management of marine fisheries within the U.S. Exclusive Economic Zone (EEZ). The M-S Act requires the National Marine Fisheries Service (NMFS), and eight regional fishery management councils (i.e., New England, Mid-Atlantic, South Atlantic, Gulf of Mexico, Caribbean, Pacific, North Pacific, and Western Pacific), to analyze fisheries under their jurisdiction and develop Fishery Management Plans (FMPs). In addition, NMFS works with three interstate marine fisheries commissions (i.e., the Atlantic States, Gulf States, and Pacific States) to monitor fisheries management at the state level, and to coordinate fishery issues that cross over state and federal boundaries. In general, waters under the jurisdiction of individual coastal states extend from the shoreline to a limit of three nautical miles (nine nautical miles in the case of Texas, the west coast of Florida, and Puerto Rico). Federally managed waters continue offshore from state waters to a 200 nautical mile limit (except where intercepted by the EEZ of another country). Management of elasmobranchs in state waters falls under the control of that state's regulatory authority; usually the marine division of the respective fish and wildlife department (Anon., 2001a).

In the early 1980's, directed Atlantic shark fisheries expanded rapidly when shark meat was marketed as an acceptable alternative to tuna and swordfish. Shark landings increased by almost 300% between 1985 and 1994. This trend was identified by the early 1990's and the first federal

shark fishery management plan was developed by NMFS in 1993. The 1993 Fishery Management Plan for Sharks of the Atlantic Ocean (Shark FMP) separated 39 species of sharks into three groups (i.e., large coastal sharks or LCS, small coastal sharks or SCS, and pelagic sharks or PS) and catch limits were imposed (Anon., 1993; Anon., 2001a). The three categories were based on the fishery in which the sharks were caught, rather than biological factors. LCS consisted of targeted commercial and sport fished species; SCS consisted of largely near-shore species, caught primarily by sport fishers and as by-catch of shrimp, long-line, and gillnet fisheries; and PS, offshore and deepwater species, were harvested primarily as by-catch of the tuna and swordfish long-line fisheries, and were also targeted by sport fishers (www9).

In 1997, NMFS prohibited the possession of five species of shark, the great white, whale, basking, sand tiger (*Carcharias taurus*), and bigeye sand tiger (*Odontaspis noronhai*) sharks. These species were identified as highly susceptible to overexploitation and prohibition was a precautionary measure to ensure a directed fishery did not develop (Anon., 2001a). From this point forward, an Exempted Fishing Permit (EFP) was required to collect sand tiger sharks, the only species of the five prohibited species to be routinely displayed by aquariums. During the same year (1997), NMFS added dusky (*Carcharhinus obscurus*), night (*Carcharhinus signatus*), and sand tiger sharks to the candidate species list for possible inclusion under the Endangered Species Act (see ESA below).

In 1999, NMFS added two categories (i.e., Prohibited Species, and Deepwater and Other Sharks) to the Shark FMP (Anon., 1999b; Anon., 2001a). NMFS then issued the Final Fishery Management Plan for Atlantic Tunas, Swordfish, and Sharks (Atlantic FMP). The retention of an additional 14 shark species was prohibited, bringing the total to 19 protected species (Table 3.1). In addition, the new Atlantic FMP imposed an annual catch quota of 60 metric tons whole weight (43 metric tons dressed weight) on sharks intended for display in public aquariums. This figure represents a tiny fraction of the annual commercial fishery catch quota of 2,028 metric tons dressed weight, broken down as follows:

1. Large coastal sharks (LCS), including (a) ridgeback species, i.e., the sandbar (*Carcharhinus plumbeus*), silky (*Carcharhinus falciformis*), and tiger (*Galeocerdo cuvier*) sharks: 620 metric tons; and (b) non-ridgeback species, i.e., the blacktip (*Carcharhinus limbatus*), spinner (*Carcharhinus brevipinna*), lemon (*Negaprion brevirostris*), bull (*Carcharhinus leucas*), and nurse (*Ginglymostoma cirratum*) sharks, and smooth (*Sphyrna zygaena*), scalloped (*Sphyrna lewini*), and great (*Sphyrna mokarran*) hammerhead sharks: 196 metric tons.

2. Small coastal sharks (SCS), including the Atlantic sharpnose (*Rhizoprionodon terraenovae*), blacknose (*Carcharhinus acronotus*), finetooth (*Carcharhinus isodon*), and bonnethead (*Sphyrna tiburo*) sharks: 359 metric tons.

3. Pelagic Sharks (PS), including (a) shortfin mako (*Isurus oxyrinchus*), thintail thresher (*Alopias vulpinus*), and oceanic whitetip (*Carcharhinus longimanus*) sharks: 488 metric tons; (b) porbeagle sharks (*Lamna nasus*): 92 metric tons; and (c) blue sharks (*Prionace glauca*): 273 metric tons.

Once shark catch quotas were established in 1993, it immediately became necessary to apply for EFPs when annual catch quotas were exceeded and corresponding fisheries closed for the season. This had a particular impact on LCS species, i.e., there was a demand for LCS species during periods when the fishery had already been closed. It is unclear when the first EFP was issued, but many requests were made between 1993 and 1998. The evolving EFP process, along with a growing list of prohibited species, led to the proposal for a dedicated public display quota in 1999, and a one-time quota of 75 sand tiger sharks was established for that year. Data provided by NMFS (Stirratt, pers. com.) indicated that 28 EFPs were requested and issued between 2000 and 2002. A total of 2,793 sharks were requested for public display and 10,577 were authorized (including sharks for research purposes), representing <50% of the annual display quota. The number of sharks actually collected in those same years was 144, representing <1% of the 60 metric ton display quota. NMFS is revising the requirements for EFPs and should release this update in 2004.

Although the status of most shark species in the Pacific Ocean is unknown, NMFS is developing a Pacific Highly Migratory Species FMP which will include certain shark species in California, Oregon, and Washington. Existing FMPs cover certain shark species in Hawaii, Guam, and American Samoa (i.e., the Western Pacific Pelagic Fisheries FMP), and Alaska (i.e., the North Pacific FMP).

ESA

The U.S. Endangered Species Act of 1973 (ESA) provides for the conservation and protection of species which have clear potential for endangerment or extinction throughout all, or a significant portion of, their range, and the conservation of the ecosystems on which they depend. There are two classifications under which a species may be listed. Species determined to be in imminent danger of extinction throughout all of a significant portion of their range are listed as "endangered". Species determined likely to become endangered in the foreseeable future are listed as "threatened". A "species" is defined by ESA to mean a species, a subspecies, or, for vertebrates only, a distinct population.

ESA authorizes the following: (1) the determination and listing of species as "endangered" and "threatened"; (2) the prohibition of unauthorized taking, possession, sale, and transport of "endangered" species (Note: The term "take" is defined by ESA to mean harass, harm, pursue, hunt, shoot, wound, kill, trap, capture or collect, or attempt to engage in any such conduct. The term "harm" is further defined to mean an act which actually kills or injures wildlife. Such act may include significant habitat modification or degradation where it actually kills or injures wildlife by significantly impairing essential behavioral patterns, including breeding, feeding, or sheltering.); (3) the acquisition of land for the conservation of listed species, using land and water conservation funds; (4) the establishment of cooperative agreements and grants-in-aid to states that establish and maintain active and adequate programs for endangered and threatened wildlife and plants; (5) the assessment of civil and criminal penalties for violating the Act or regulations; and (6) the payment of rewards to anyone furnishing information leading to the arrest and conviction for any violation of the Act or any regulation issued thereunder.

In general, the U.S. Fish and Wildlife Service (FWS) coordinates ESA activities for terrestrial and freshwater species, while NMFS is responsible for marine and anadromous species. After a listing petition is filed (i.e., to classify a species as "endangered" or "threatened"), it is decided whether the petition presented substantial information to warrant listing. If so, NMFS conducts a status review of the species, initiated by a public solicitation for information, and data relevant to population size and life history of the species are considered (Anon., 2002d). A final decision must be made within one year of issuance of the proposal. NMFS (or FWS) can initiate a status review without a petition.

A species is listed if it is "threatened" or "endangered" due to any of the following five factors: (1) present or threatened destruction, modification, or curtailment of its habitat or range; (2) overuse for commercial, recreational, scientific, or educational purposes; (3) disease or predation; (4) inadequacy of existing regulatory mechanisms; and (5) other natural or man-made factors affecting its continued existence. After a species has been listed, a recovery plan is prepared which identifies conservation measures to help the species recover. In addition, ESA requires that all federal agencies use their authorities to conduct conservation programs and to consult with NMFS (or FWS) concerning the potential effects of their actions on any species listed under the act.

Barndoor skates (*Dipturus laevis*), common (*Pristis pristis*) and smalltooth (*Pristis pectinata*) sawfishes, and sand tiger, dusky, and night sharks have all been added to the NMFS candidate list for threatened and endangered species, due principally to large documented declines caused by over-fishing (Diaz-Soltera, 1999). A candidate species is, as the name implies, a candidate for listing under the Endangered Species Act (ESA). More specifically, a candidate species is a species or vertebrate population for which reliable information is available that suggests a listing under the ESA may be warranted. There is no mandatory federal protection required under ESA for a candidate species, however NMFS urges voluntary protection for such species. [Author's note (September, 2004): To better reflect the purpose of the NMFS candidate list, candidate species are now considered "Species of Concern" (64 Federal Register 19975 - April 15, 2004). Only those species under active consideration for ESA listing are referred to as "Candidate Species." Neither status carries procedural or substantive protection under the ESA.]

The smalltooth sawfish, a popular aquarium species, was added to the ESA candidate species list in 1991, removed in 1997, and reinstated in 1999. In November of 1999, NMFS received a petition from the Center for Marine Conservation requesting that the smalltooth sawfish be listed as endangered under ESA. NMFS completed a status review of the smalltooth sawfish in December 2000 and published a proposed rule to list the U.S. population of this species as endangered under ESA on 16 April 2001

(www10). On 1 April 2003, the smalltooth sawfish was finally listed as an endangered species under ESA, the first elasmobranch species to be so listed (Anon., 2003). In September 2002, a separate petition to list the barndoor skate was ruled as "…not warranted at this time…" (Anon., 2002d). The barndoor skate remains on the ESA candidate species list.

State legislation and the ASMFC

Every coastal state in the USA has some form of marine fisheries unit within the state agency responsible for fish and wildlife management. Each state has different regulations and permitting requirements governing the collection or fishing of elasmobranch species, however, many states are beginning to follow federal regulations (i.e., NMFS), especially with regard to prohibited species. Some states even require collectors to obtain a NMFS EFP as a prerequisite to applying for a permit within their state.

In the past few years, some state agencies have become concerned about the collection activities of public aquariums and commercial collectors. In particular, state agencies have been concerned that some organizations have acquired permits from more than one state, as well as the federal government, and viewed this activity as double dipping, i.e., the potential to collect greater numbers of animals by requesting permits from more than one jurisdiction. In reality, aquariums have applied for permits from different geographical regions to provide collection flexibility (i.e., allowing for collection during convenient times where animals might best be found). Regardless, agencies in some states, unconvinced of the best intentions of public aquariums, requested that shark collection permitting be coordinated by the interstate Atlantic States Marine Fisheries Commission (ASMFC).

The ASMFC was formed by the 15 Atlantic coastal states (Maine to Florida) in 1942 to assist the management and conservation of shared coastal fishery resources under an interstate compact. In 1998, the policy board requested that the ASMFC investigate and consider options for enhancing the management of sharks in state waters. In 1999, workshops (technical and policy) were held to collect state-by-state information on shark fisheries, review the federal FMP, and develop options for possible shark management in state waters by the ASMFC. Although no consensus was reached, attendees agreed that the Commission should move forward with the development of a shark FMP. In 2001, the management and science committee of the ASMFC met with NMFS to review the database detailing permits issued for scientific and display purposes, and to discuss the effects of removing permitted animals from wild populations.

At the time of writing, NMFS would like to pursue some sort of umbrella exempted fishing permit between NMFS and the ASMFC states, to facilitate enforcement (White, pers. com.). The exact mechanism is unclear, but if a single permit were valid for both state and federal waters, it would help assuage fears of double dipping. In addition, the ASMFC has invited two aquarium representatives, via the American Zoo & Aquarium Association (AZA), to sit on their newly formed shark permit workgroup. As yet, there have not been permitting problems or concerns with states along the Gulf of Mexico or the Pacific coastal states. No regional shark management plans are in effect for these areas.

National regulations: Australia

Management responsibility and jurisdiction for Australian marine resources, including sharks, are shared between the six states, the Northern Territory, and the Australian Federal Government (Commonwealth). The states and territories of Australia have jurisdiction over waters out to 3 nautical miles, and the Commonwealth has jurisdiction for waters outside these limits to the edge of a 200 nautical mile Australian Fishing Zone (AFZ). This system presented challenges to the management of stocks occurring in both inshore and offshore waters, and was resolved by establishing offshore constitutional settlement (OCS) arrangements. Under OCSs, fish stocks can be managed through either a Joint Authority of State and Commonwealth bodies, or under the management of a single jurisdiction throughout a species' range. (Anon., 2001c; Anon., 2002c).

AFMA and SAG

The Australian Fisheries Management Authority (AFMA) is the Commonwealth statutory authority responsible for the sustainable use and efficient management of fishery resources on behalf of the Australian community and key stakeholders. The AFMA manages fisheries within the 200 nautical mile AFZ and, in some cases, by agreement with the Australian states, in state waters. The AFMA

provides fisheries management, and advisory, compliance, and licensing services (www11). AFMA was established in 1992 following the passage through Australian Parliament of the Fisheries Administration and Fisheries Management Acts in 1991. These two pieces of legislation created statutory authority for the day-to-day management of fisheries, vested in the AFMA, and for broader fisheries policies, international negotiations, and strategic issues, administered by the former Department of Primary Industries and Energy, now called the Department of Agriculture, Fisheries, and Forestry Australia (AFFA). AFFA established a Shark Advisory Group (SAG) and together developed Australia's draft NPOA-Sharks, published as a public consultation document in July of 2002 (Anon., 2002c).

While Australia's contribution to the global shark catch is relatively small (<1.5%), sharks are a significant part (~5%) of the total quantity of Australia's wild fish production. Of the over 1,000 species of chondrichthyans identified worldwide nearly 300 species are found in Australian waters and more than half of these are endemic (Anon., 2002c). The Australian shark assessment report identified 178 shark species as caught from Australian waters (Anon., 2001c). Of these sharks, 60 species and five families have been identified as species "of concern" (Table 3.1), including those on the 2000 IUCN Red List, those assessed against IUCN criteria by Pogonoski et al. (2002), and those identified as potentially of concern on the basis of consistently high catch rates recorded in Commonwealth fishing records. Two-thirds of the landings for the 1998-1999 season fell into 15 of the 178 shark species or groups.

Of the 95 chondrichthyan species listed in the 2000 IUCN Red List, 47 occur in Australian waters, with 14 categorized as "threatened" and the remainder listed as "lower risk" (26 species) or "data deficient" (7 species). Of the "threatened" species, five (i.e., of the family Pristidae) are considered "endangered" and nine are considered "vulnerable". An IUCN Red List assessment workshop was held in Australia in March of 2003 in order to review all Australian species of chondrichthyan fishes. The results of this workshop will be incorporated into the IUCN Red List to be published in 2004.

As more information on elasmobranch species becomes available, and more comprehensive risk assessments become possible, the conservation status assigned to each species will be updated on the IUCN Red List website (www8). There has been some concern that the criteria used for IUCN Red List assessments are not directly applicable to marine species. Although the criteria and categories have been recently updated (Anon., 2001b), most of the current elasmobranch Red List assessments (www8) are still based on criteria from 1994 (Anon., 1994). It is hoped that these assessments will soon be revised using IUCN Red List criteria from 2001 (Anon., 2001b). The conservation status of Australian shark species (Table 3.1) should therefore be regarded as the best currently available, rather than a definitive statement (Anon., 2002c).

EPBCA

There has been a recent boost to the environmental oversight of fisheries management in Australia, primarily by the Commonwealth Department of Environment and Heritage, or Environment Australia (EA). Under the Environment Protection and Biodiversity Conservation Act of 1999 (EPBCA), all Commonwealth-managed fisheries are subject to strategic assessments, while those fisheries managed by states or territories, which impact protected species, may also be assessed (Anon., 2001c). EPBCA strategic assessments are made against the Commonwealth Guidelines for the Ecologically Sustainable Management of Fisheries. The EPBCA came into effect on 16 July 2000, replacing five Commonwealth environment statutes from the 1970's and 1980's, including the Endangered Species Protection Act of 1992. Some states apply additional environmental assessments to fisheries under their jurisdiction, independent of species protected and fisheries assessed under the EPBCA.

At the time of writing, the following elasmobranch species are protected under the EPBCA (www13): (1) the East Coast population of sand tiger or grey nurse sharks and the speartooth shark (*Glyphis* sp. "A"), considered to be "critically endangered"; (2) the northern river shark (*Glyphis* sp. "C"), considered to be "endangered"; and (3) the West coast population of sand tiger or grey nurse sharks, the largetooth sawfish (*Pristis microdon*), the whale shark, and the great white shark, considered to be "vulnerable". It is a requirement of the EPBCA to prepare recovery plans for all "endangered" and "vulnerable" species that occur within Commonwealth jurisdiction. The recovery plan must include research and management

actions necessary to stop the decline of a target species so its chances of long-term survival in the wild are maximized. Of the species currently protected, detailed recovery plans have been prepared for the sand tiger and great white sharks (Anon., 2002e; Anon., 2002f).

State legislation

For species not covered by the EPBCA, state fishery regulations apply. For example, the bigeye sand tiger shark is protected in New South Wales, and the basking and megamouth (*Megachasma pelagios*) sharks are protected in Tasmania. In total, nine elasmobranch species have some form of protection at either the Commonwealth and/or state level where, in general, their collection is prohibited (Table 3.1).

Public aquariums are required to apply for permits, through their respective state fisheries management agency, to collect, hold, and display marine life. Many aquariums therefore obtain animals through licensed commercial collectors. As long as the aquarium has relevant state fisheries permits to hold and display marine life, the commercial collector is responsible for meeting permit requirements to collect the animals from a specific region.

In the event that a public aquarium intends to collect an elasmobranch species directly (e.g., for a species requiring specialized capture or transport techniques) they may be issued a special collection permit. Special collection permits allow for a restricted number of individuals, for the elected species, to be collected and held each year. Permit titles vary between states but in each case there is a permit issued to collect, hold, and display marine life, and a special permit awarded, on application, for the display of protected species (Thorburn, pers. com.).

While most commercial fishing activities are not directed at providing live specimens, there is a growing number of fishermen in Australia who have a real passion and concern for marine life, and are interested in learning how to minimize damage to both fishes and fisheries. Cooperation with these fishermen provides an excellent opportunity to collect smaller, robust species, such as smooth-hounds (*Mustelus* spp.), wobbegong sharks (*Orectolobus* spp.), etc. Of course, collected species must be covered by the fishermen's license and must not be protected under the EPBCA or state Acts.

Aquariums in Australia are unable to purchase specimens from members of the public or amateur fishermen, although they may accept specimens as a donation.

National regulations: South Africa

The coastal environs of South Africa are subject to legislation administered by local, provincial, and federal authorities. Responsibility for coordinating policy specific to the coast and its resources has been delegated to the Department of Environmental Affairs and Tourism, Branch of Marine and Coastal Management (MCM).

The Marine Living Resources Act (MLRA—Act 18 of 1998) was introduced during September of 1998. The MLRA consolidated the Sea Fisheries Act of 1988 and provincial nature conservation ordinances, both of which had previously regulated marine resources. The MLRA was an overdue revision of the Sea Fisheries Act, which benefited some sectors of society and stopped others from gaining access to marine resources. The MLRA allowed previously excluded communities full access to the fishing industry, and prepared the country for free trade and deregulated markets. The guiding principle of the MLRA stresses that the natural marine living resources of South Africa, as well as the environment in which they exist, are a national asset and the heritage of all South African people.

The main thrust of the MLRA and regulation gazette 6284, detailing specific regulations under the MLRA, is that anyone desiring to take a living organism from the marine environment is required to purchase a permit to do so. The user-pays principle generates income which goes toward the research, management, and control of resources. Recreational fishing is regulated via a fee-based permit system and permits may be obtained at any post office. Small-scale and commercial fishing activities are regulated by either the office of the Minister of Environmental Affairs and Tourism, or the Fisheries Transformation Council.

Part 3, Chapter 5 of Regulation Gazette Number 6284 details fishing regulations pertaining to sharks. Protected species are categorized as either annexure 4 ("non-saleable recreational") or annexure 5 ("specially protected") (Table 3.1). Another category, annexure 8 ("exploitable"), refers to species not covered by annexure 4 or 5 whereby a total of 10 elasmobranchs, of no size limit, may be taken.

Public aquariums are required to obtain exemptions to the MLRA through an annual application (or renewal) of permits to the MCM. MLRA exemptions allow aquarium staff to collect and hold more than the normally permitted number of marine taxa. Such exemptions specify that animals shall be used for research or display only, and may not be sold.

In April of 1991, South Africa became the first country to completely protect the great white shark. During 2000, the whale shark was given limited protection status in specific marine protected areas. Any sharks, including those not specifically listed, are afforded protection should they occur within a marine protected area that is closed to fishing or collecting for aquariums.

ETHICS

There are many ethical considerations associated with the maintenance of animals in a captive environment. For an excellent overview, critical analysis, and detailed essays examining both sides of the issue, albeit with a terrestrial zoo perspective, the reader is directed to Norton et al. (1995) and Hutchins et al. (2003).

Aquariums should ensure that all animal exhibits and husbandry procedures are ethically sound, not only because it is appropriate, but also because of a growing public awareness and concern for all animals in captive environments. The powerful public sentiment provoked by captive marine mammals today did not exist 40-50 years ago. As well-intended conservation groups raise the public consciousness about other animal taxa, such as sharks, the public will become increasingly critical of the standards employed in displaying those taxa. Although sharks and stingrays are still feared by the general public in many areas of the world, animal activist groups are becoming increasingly interested in their plight. It is only a matter of time before protesters actively and consistently campaign for improved conditions on behalf of captive elasmobranchs. However, this change in public opinion should not be viewed as a negative influence. Rather, it should be viewed as a testament to the success of aquariums as educational tools. In addition, protestations directed at improving captive conditions should be embraced. We, as an industry, can always improve our exhibits and husbandry techniques, and should capitalize on the opportunity to do so.

Justification

Unlike terrestrial zoos, aquariums must meticulously recreate the marine environment within a very restricted area, providing not only physical space and nutrition, but a slew of carefully controlled chemical and physical parameters. Elasmobranchs are especially difficult to maintain in aquariums, for a variety of reasons (i.e., large size, relatively poorly understood physiology, etc.). As such, our successes at maintaining elasmobranchs, although steadily improving, have been somewhat limited. For the same reasons, captive elasmobranchs have not been studied as extensively, nor maintained for as long, as most terrestrial vertebrates. Thus, alternatives to wild collection, such as captive breeding, have not been widely accomplished.

Zoos and aquariums justify the collection and display of wild animals through the educational, research, and conservation goals achieved. The following ethical question has often been posed: Do the benefits of a quality display of elasmobranchs, at a professionally operated public aquarium, having a strong educational, research, and conservation mission, outweigh the cost to individual animal welfare? We, as an industry, believe that they do. Aquariums benefit society through the presentation of live animal displays and associated public education programs. Aquariums contribute directly to the conservation of aquatic species and their habitat by raising public awareness, by raising funds, and through a direct participation in research activities. Aquariums further advance conservation research by testing new technologies (e.g., satellite pop-off tags, etc.), advancing aquatic animal medicine, developing animal handling techniques, and publishing results stemming from same. By studying elasmobranchs in captivity, and applying that knowledge to their husbandry, aquariums provide valuable and practical information that would be difficult or impossible to gather in the wild (Hutchins et. al, 2003).

Protected species

When collecting elasmobranchs, consideration should always be given to the conservation status of a chosen species and requisite permits obtained through appropriate channels. As caretakers of this important taxonomic group, we must, and must be seen to, uphold species management programs and work with

environmental, conservation, and regulatory agencies to police our own industry. Careful ethical consideration should be given to the collection and maintenance of threatened species, in particular where wild populations may suffer as a result of collection activities. Education and conservation benefits must be carefully weighed against risks to wild populations before threatened species are considered for display in an aquarium.

Difficult species

Ethical principals should be applied to the collection and maintenance of shark species that, historically, have not done well in captivity (e.g., species easily damaged in aquariums such as shortfin mako sharks, and those that do not feed readily and can perish after a short period, such as great white sharks). Although it may appear to be ethically unacceptable to collect a species that has not been successfully maintained in an aquarium, to learn more about these difficult species it is necessary to challenge what is known about their husbandry, and this can only be achieved through trial and error. Indeed, as advances have been made in both husbandry and facility design, species previously thought to be problematic or impossible to keep (e.g., tiger sharks) are now being maintained successfully (Marin-Osorno, pers. com.; www14). Experimentation with difficult species should only be considered acceptable if attempts are well researched and planned, experienced personnel are used, and appropriate institutional resources are made available. Specific pre-planned parameters (e.g., minimum space requirements) and clear milestones enabling trials to be aborted before animal health is compromised should be established. In general, an aquarium should never acquire a species that will outgrow their facilities. However, in some rare cases, the risks of acquiring excessively large species may be offset by knowledge gained (e.g., Hewitt, 1984; Ellis and McCosker, 1991).

Releases

Releasing an elasmobranch into the wild, after holding it in captivity, presents a risk to wild populations (i.e., through the introduction of exotic diseases, exotic genetic material, etc.). However, if releases are undertaken correctly (i.e., within the elasmobranchs native range and near the point of collection), with appropriate precautions (i.e., pre-release isolation, medical evaluations, etc.), and with appropriate governmental approval, such releases can provide valuable scientific information (see Van Dykhuizen et al., 1998). Under the IUCN Species Survival Commission (SSC) Guidelines for Re-Introductions (Anon., 1995; www12), this type of release is defined as a “reinforcement” or “supplementation” (i.e., the “...addition of individuals to an existing population of conspecifics...”), as opposed to a “re-introduction” (i.e., “...an attempt to establish a species in an area which was once part of its historical range, but from which it has been extirpated or become extinct...”). Re-introductions are beyond the scope of this chapter, but if considered they should adhere to IUCN SSC guidelines and those of your regional zoological association (see below).

Professional affiliations

Ethical considerations are not normally legislated by governments. Rather, ethics are developed within the culture of the countries in which we live, and often by the professional organizations to which we belong. Most public aquariums belong to a regional zoo and aquarium association, the majority of which abide by a code of professional ethics. These codes address, amongst other issues, the ethical considerations of animal acquisition and disposition through wild collection, commercial suppliers, and trade. A breach of these professional codes can result in some form of penalty to institutions or individuals.

Readers are urged to familiarize themselves with their regional zoo and aquarium association, and correspondingly, their code of ethics. Regional associations include: (1) the American Zoo & Aquarium Association (AZA); (2) the African Association of Zoos and Aquaria (PAAZAB); (3) the Australasian Regional Association of Zoological Parks and Aquariums (ARAZPA); (4) the Canadian Association of Zoos and Aquariums (CAZA); (5) the European Union of Aquarium Curators (EUAC) and the European Association of Zoos and Aquaria (EAZA); (6) the South East Asian Zoos Association (SEAZA); and (7) globally, the World Association of Zoos and Aquariums (WAZA).

Ethical constraints vary between countries and cultures, but presumably we share the same basic goals, i.e., to provide excellent live animal displays for public education and broader conservation purposes, and to breed

endangered or threatened species. By breeding endangered or threatened species we strive to provide self-sustaining captive populations and thereby minimize or eliminate take from the wild, improve knowledge of basic biological parameters, and in some specific cases reinforce wild populations.

COMMERCIAL COLLECTORS

Commercial collectors are defined in this chapter as individuals or companies that collect marine organisms directly from the wild for the purposes of sale to public aquariums. Collection may be done at the specific request of a public aquarium or, in case of non-restricted species, collection may be made in advance with a view to selling the specimens at a later date (i.e., "on speculation").

Commercial collectors can provide specimen collection, quarantine, and transportation services to those aquariums unable to perform these tasks themselves (i.e., aquariums whose geographic location, lack of expertise or resources, or daily operations and busy programs prevent them from undertaking collecting expeditions). These services are especially important for new aquariums, where staff are preoccupied with other husbandry challenges imposed by unpredictable construction schedules and maturing life support systems. Under such conditions, it becomes difficult for aquarium staff to concentrate on large-scale elasmobranch collection expeditions. Established aquariums may consciously leave the collection of elasmobranchs to commercial collectors, opting to avoid high costs associated with buying and maintaining boats and other equipment. Commercial collectors may provide another service to established aquariums by rapidly replacing animals that have suffered unexpected mortality.

Many reputable and professional commercial collectors have served public aquariums in a laudable manner. Unfortunately, a few commercial collectors have not always subscribed to the same high standards set by their colleagues. At times, these unscrupulous individuals have caused the aquarium community significant difficulty and damage. A few unprincipled collectors have been investigated for illegal activities and public aquariums have been tainted by association. Recent plans to overhaul NMFS's shark collection permit procedures has been a direct result of such transgressions (Rogers, pers. com.).

Commercial collectors, working in U.S. waters, obtain permits from relevant state and/or federal government agencies, and then collect elasmobranchs as requested by specific aquariums or "on speculation" for future sale. Some commercial collectors have requested permits for a high number of elasmobranchs and subsequently reported collecting far fewer animals. Although innocent, this behavior confuses permitting agencies and creates suspicion; agencies believing that collectors and aquariums are not being entirely honest. In addition, commercial collectors (and public aquariums) have applied for multiple permits covering different jurisdictions, enabling the collection of a given species within limited time frames despite seasonal variations of availability, etc. Again, permitting agencies can be confused by this practice believing that collectors are "double dipping", i.e., applying for permits from different jurisdictions in order to collect a higher number of animals than legally allowed.

It is imperative that public aquariums and commercial collectors work together with regulatory agencies to educate them about the unique nature of our business. Regulatory agencies should be regarded as partners and not adversaries. Information learned through collection activities should be shared with regulatory agencies, whether required by law or not, to help build healthy relationships, dispel misconceptions, and improve a mutual understanding of regulated species.

It is incumbent upon all aquariums to thoroughly research the reputation and legal status of any commercial collector they choose to contract. Many regulatory agencies are moving toward the practice of issuing collection permits directly to aquariums, and not commercial collectors, especially for restricted or protected species. Aquariums must then contract a commercial collector to obtain the species desired. This practice will make aquariums directly accountable for the activities of contracted commercial collectors. Some U.S. state agencies may go even further, requiring federal permits, and in some cases AZA accreditation, before state permits will be granted to an aquarium.

ACKNOWLEDGEMENTS

I would like to acknowledge the assistance of Craig Thorburn and Malcolm Smale who made valuable contributions toward this chapter.

REFERENCES

Anon. 1993. Fishery management plan for the sharks of the Atlantic Ocean. NOAA/NMFS, U.S. Department of Commerce, 25 February 1993. 272 p.

Anon. 1994. The IUCN Red List of Threatened Species™: categories and criteria (version 2.3). International Union for the Conservation of Nature, Gland, Switzerland (www6).

Anon. 1995. IUCN/SSC Guidelines for re-introductions. International Union for the Conservation of Nature, Gland, Switzerland (www12).

Anon. 1996. Draft policy regarding controlled propagation of species listed under the Endangered Species Act. United States Fish and Wildlife Service and National Marine Fisheries Service, 7 February 1996. Federal Register 1(26): 4716-4721.

Anon. 1999a. International Plan of Action for Reducing Incidental Catch of Seabirds in Longline Fisheries. International Plan of Action for the Conservation and Management of Sharks. International Plan of Action for the conservation and management of sharks. Management of Fishing Capacity. Food and Agriculture Organization of the United Nations, Rome, Italy. 26 p.

Anon. 1999b. Final fishery management plan for Atlantic tuna, swordfish, and sharks. National Marine Fisheries Service, National Oceanic and Atmospheric Administration, U.S. Department of Commerce, April 1999. 1192 p.

Anon. 2001a. Final United States national plan of action for the conservation and management of sharks. National Marine Fisheries Service, National Oceanic and Atmospheric Administration, U.S. Department of Commerce, February 2001. 90 p.

Anon. 2001b. The IUCN Red List of Threatened Species™: categories and criteria (version 3.1). International Union for the Conservation of Nature, Gland, Switzerland (www7).

Anon. 2001c. Australian shark assessment report for the Australian national plan of action for the conservation and management of sharks. Shark Advisory Group, Canberra. 207 p.

Anon. 2002a. The World Conservation Union, Species Survival Commission, Shark Specialist Group and TRAFFIC. Report on implementation of the international plan of action for sharks (IPOA-Sharks): Paper submitted for discussion at the 18th CITES Animals Committee meeting, Costa Rica, 8-12 April, 2002. (www1)

Anon. 2002b. The World Conservation Union, Species Survival Commission, Shark Specialist Group and TRAFFIC The role of CITES in the conservation and management of sharks. June, 2002. (www2).

Anon. 2002c. The Australian national plan of action for the conservation and management of sharks - public consultation draft. Shark Advisory Group, Canberra. 59 p.

Anon. 2002d. Endangered and threatened wildlife and plants; 12–month finding for a petition to list barndoor skate (*Dipturus laevis*) as threatened or endangered. 27 September 2002. Federal Register 67(188): 61055-61061.

Anon. 2002e. Recovery plan for the Grey Nurse shark (*Carcharias taurus*) in Australia, June 2002. Environment Australia, Canberra. 45 p.

Anon. 2002f. White Shark (*Carcharodon carcharias*) Recovery Plan, July 2002. Environment Australia, Canberra. 43 p.

Anon. 2003. Endangered and threatened species; Final endangered status for a distinct population segment of smalltooth sawfish (*Pristis pectinata*) in the United States. 1 April 2003. Federal Register 68(62): 15674-15680.

Baille, J. and B. Groombride (eds.) 1996. The 1996 IUCN Red List of Threatened Animals. International Union for the Conservation of Nature Publications Service Unit, Cambridge, UK. 448 p.

Camhi, M. 1998. Sharks on the Line: A State-by-State Analysis of Sharks and their Fisheries. National Audubon Society, Living Oceans Program, Islip, New York, USA. 158 p.

Camhi, M. 1999. Sharks on the Line II: An Analysis of Pacific State Shark Fisheries. National Audubon Society, Living Oceans Program, Islip, New York, USA, 158 p.

Camhi, M., S. L. Fowler, J. A. Musick, A. Brautigam, and S. V. Fordham. 1998. Sharks and their Relatives: Ecology and Conservation. IUCN/SSC Shark Specialists Group, International Union for the Conservation of Nature, Gland, Switzerland. 39 p.

Diaz-Soltera, H. 1999. Endangered and threatened species; revision of candidate species list under the Endangered Species Act. Federal Register 64(120): 33166-33467.

Ellis, R. and J. E. McCosker. 1991. Great White Shark. Harper Collins Publishers, New York, USA. 270 p.

Hewitt, J. 1984. The great white shark in captivity: A history and prognosis. American Association of Zoological Parks and Aquariums annual conference proceedings, 9-13 September 1984, Miami, Florida, USA. 317-324 pp.

Hilton-Taylor, C. (ed.). 2000. The 2000 IUCN Red List of Threatened Species. International Union for the Conservation of Nature, Gland, Switzerland. 61 p.

Hutchins, M., B. Smith, and R. Allard. 2003. In defense of zoos and aquariums: The ethical basis for keeping wild animals in captivity. Journal of the American Veterinary Medical Association. 223(7): 958-966.

Musick, J. A., M. M. Harbin, S. A. Berkeley, G. H. Burgess, A. M. Eklund, L. Findley, R. G. Gilmore, J. T. Golden, D. S. Ha, G. R. Huntsman, J. C. McGovern, S. J Parker, S. G. Poss, E. Sala, T. W. Schmidt, G. R. Sedberry, H. Weeks, and S. G. Wright. 2000. Marine, estuarine, and diadromous fish stocks at risk of extinction in North America (exclusive of Pacific salmonids). Fisheries 25(11): 6-30.

Norton, B. G., M. Hutchins, E. F. Stevens, and T. L. Maple (eds.). 1995. Ethics on the ark: Zoos, animal welfare and wildlife conservation. Washington: Smithsonian Institution Press, USA. 330 p.

Pogonoski, J. J., D. A. Pollard, and J. R. Paxton. 2002. Conservation overview and action plan for Australian threatened and potentially threatened marine and estuarine fishes, February, 2002. Environment Australia, Sydney. 375 p.

Van Dykhuizen, G., H. F. Mollet, and J. M. Ezcurra. 1998. Homing behavior of a sevengill shark released from the Monterey Bay Aquarium. California Fish and Game 84(4): 180-181.

PERSONAL COMMUNICATIONS

Marin-Osorno, R. 2003. Acuario de Veracruz, Veracruz, CP 91170, Mexico.

Rogers, C. 2002. National Marine Fisheries Service, Highly Migratory Species Management Division, Silver Spring, MD 20910, USA.

Stirratt, H. 2003. National Marine Fisheries Service, Highly Migratory Species Management Division, Silver Spring, MD 20910, USA.

Thorburn, C. 2001. Thorburn Consultants Ltd., PO Box 99669, Auckland, New Zealand.

White, G. 2001. Atlantic States Marine Fisheries Commission, Washington, D. C. 20005, USA.

INTERNET RESOURCES

www1 www.traffic.org/news/ipoasharks.html

www2 www.traffic.org/cop12/esf042a.pdf

www3 www.fao.org/fi/ipa/manage.asp

www4 www.cites.org

www5 www.iucn.org

www6 www.redlist.org/info/categories_criteria1994.html

www7 www.redlist.org/info/categories_criteria2001.html

www8 www.redlist.org

www9 www.flmnh.ufl.edu/fish/organizations/ssg/sharkconswna.htm

www10 www.nmfs.noaa.gov

www11 www.afma.gov.au

www12 www.iunc.org/themes/ssc/pubs/policy/reinte.htm

www13 www.deh.gov.au

www14 www.galeocerdo.com

The Elasmobranch Husbandry Manual: Captive Care of Sharks, Rays and their Relatives, pages 43-51.
© 2004 Ohio Biological Survey

Chapter 4
Quarantine and Isolation Facilities for Elasmobranchs: Design and Construction

JOSEPH M. CHOROMANSKI

Ripley Aquariums
Ripley Entertainment, Inc.
7576 Kingspointe Parkway, Suite 188,
Orlando, FL 32819, USA.
E-mail: jchoromanski@ripleys.com

Abstract: When designing and constructing quarantine and isolation tanks for elasmobranchs, three key issues must be considered: the size of the tanks, the shape of the tanks, and the design of life support systems (LSS). Tanks must be sufficiently sized and shaped to cater for the swim-glide swimming pattern of the most sensitive or demanding species held. The design of LSSs should focus on maximizing biological carrying capacity, as stocking densities are frequently high in quarantine and isolation tanks. Effluent water treatment and disposal systems should be carefully considered during LSS design. Concrete is an excellent choice for constructing quarantine and isolation tanks, primarily because of its strength and relatively low cost for volume. Fiberglass reinforced plastic (FRP) offers an excellent alternative to concrete, having the advantage that no ferrous reinforcing is required. Buildings housing quarantine and isolation facilities should be constructed from non-ferrous materials. Quarantine and isolation facilities must be designed to allow unimpeded access for the staff (and husbandry equipment) to the tanks and the animals, and clear and easy access for trucks, trailers, and boats used to transport animals from collection sites. Quarantine and isolation facilities must incorporate husbandry support areas, including: (1) a food preparation area; (2) a water quality laboratory; (3) an office and record-keeping area; (4) a necropsy room; (5) a dive locker room; and (6) a storage area for husbandry equipment. Quarantine and isolation facilities should be air-conditioned and dehumidified, and provided with security systems to avoid fire, theft, vandalism, and power cuts.

The successful maintenance of captive elasmobranchs begins by providing the correct environment, designed with a careful consideration of the ecological, physiological, and behavioral requirements of the species held. Maintaining captive elasmobranchs is not a new enterprise. However, it has been historically difficult to maintain larger, pelagic, obligate ram-ventilating species (Clark, 1963; Gruber and Keyes, 1981; Murru, 1990). Advances in technology and an increase in available financial resources (from an increased public popularity of aquariums) has provided the means to construct larger exhibits and resulted in the successful display of larger and more challenging species (for an excellent review of the history of elasmobranch exhibits refer to Chapters 1 and 5 of this manual). Advances in aquarium design are frequently the result of trial and error, and important advances are rarely published in the literature. This chapter therefore relies heavily on the collective experiences of public aquarium biologists, with limited references to published literature. This chapter will focus on sharks, which are typically more challenging than skates, rays, and chimeras from the standpoint of aquarium design.

Although there are many similarities, the design of a quarantine and isolation facility for elasmobranchs differs in many ways from the design of an elasmobranch aquarium exhibit. For the purposes of this chapter the main functions of an elasmobranch quarantine and isolation facility include: (1) the acclimatization and

recovery of newly acquired sharks from the stress associated with capture, transport, handling, disease, or injury; (2) the quarantine of sharks prior to display; (3) the short-term isolation and treatment of sick or injured sharks; and (4) the long-term holding of sharks for some specific purpose (e.g., breeding, research, etc.).

With about 400 different described species, no shark can be considered as "typical" for the purposes of designing a quarantine and isolation facility. Each species has particular characteristics which impact husbandry requirements and facility design. Likewise, individual factors (e.g., size, age, behavior, susceptibility to stress, health status, etc.) vary and must be taken into consideration. Tanks should therefore be designed to accommodate the specific requirements, numbers, and maximum sizes of the most sensitive or demanding species to be held.

The construction of an adequate quarantine and isolation facility for elasmobranchs can be expensive, especially for larger, more sensitive species. Institutions or individuals with insufficient resources should not undertake the construction of such facilities or attempt to hold elasmobranchs.

TANK DESIGN

Tank size

The physical dimensions of quarantine and isolation tanks are of critical importance. The sheer size of adult sharks presents the first husbandry and, thus, design challenge. In general, large sharks require large tanks, and the larger the tank, the better it can accommodate a wide variety of elasmobranch species. Tank size requirements for large benthic sharks will be different from those required for large obligate ram-ventilating sharks (refer to Chapter 5 for a more detailed discussion). The key, once again, is to plan and design for the maximum sizes and numbers of the most sensitive or demanding species to be held.

Limited data exist on the spatial requirements for elasmobranchs in captivity. The only attempt to quantify the minimum dimensions of an elasmobranch enclosure was undertaken by Klay (1977) in an article examining shark dynamics and exhibit design. Klay claims to have studied the swimming behavior of 29 different species of sharks (although data for only seven species were reported in his article). Klay maintains that sharks over 1.8 m total length (TL), with the exception of bull (*Carcharhinus leucas*), lemon (*Negaprion brevirostris*), nurse (*Ginglymostoma cirratum*), and sand tiger (*Carcharias taurus*) sharks, require an introduction tank of dimensions 30.5 m long x 12 m wide in order to adopt normal swimming patterns and exhibit normal behavior (Klay, 1977).

Although not published, Klay is credited with developing a proprietary formula to determine the minimum tank dimensions for what he described as average sharks. Average sharks in this context referred to species he normally encountered as a commercial collector (i.e., bull, lemon, nurse, and sandbar (*Carcharhinus plumbeus*) sharks), while non-average sharks referred to more demanding species (i.e., tiger (*Galeocerdo cuvier*), hammerhead (*Sphyrna* spp.), and blacktip (*Carcharhinus limbatus*) sharks) (Hewitt, pers. com.). Klay's proprietary formula states that the tank dimensions for most average sharks should be as follows (where Z refers to the maximum expected TL of the largest species to be held):

12(Z) long x 5(Z) wide x 2.5(Z) deep

For example, if the largest shark is, or will be, 1.5 m TL, then the tank should be 18 m long x 7.5 m wide x 3.75 m deep (Hewitt, pers. com.). Klay (1977) further believed that obligate ram-ventilating species (e.g., mako (*Isurus oxyrinchus*), great white (*Carcharodon carcharias*), tiger, and blue (*Prionace glauca*) sharks) had more demanding biological requirements and therefore required much larger enclosures. Although Klay's studies are relatively unscientific, and the conclusions generalized, they do represent a potential starting point for the designer of tanks for elasmobranchs. In addition, Klay's article was one of the first to present the swim-glide hypothesis (see below), a behavioral characteristic of elasmobranchs that has implications for tank design and was thus reflected in Klay's formula.

Klay's formula appears to be overgenerous for some elasmobranch species, exaggerates depth requirements (i.e., depth does not need to increase in a linear relationship to increasing horizontal dimension), fails to address differences in tank geometry (e.g., rectangular vs. circular, etc.), and does not account for a disruption of the swim-glide swimming pattern by other animals or obstructions within the tank. As a general rule, horizontal tank dimensions are more important than vertical depth, if a normal swimming pattern is to be maintained (Murru, 1990). In addition, it

is possible to maintain hardier species in smaller tanks than Klay's formula would suggest. For example, Ripley's Aquarium of the Smokies, Gatlinburg, Tennessee, USA, successfully maintained eight adult sand tiger sharks, two adult sandbar sharks, two medium-sized common sawfish (*Pristis pristis*), and two adult roughtail stingrays (*Dasyatis centroura*), for almost two years, in a tank measuring 12.2 m in diameter x 1.7 m depth. The tank contained ~195.7 m^3 of water and the life support system included an oversized biological filter.

Indirect evidence suggests that for the long-term, larger tanks are more suitable for holding elasmobranchs. For example, Gruber and Keyes (1981) reported a significant decrease in food consumption by lemon sharks when the animals were moved to a larger pool, indicating a decreased metabolic demand.

Tank shape

Consideration of size alone does not guarantee a successful tank design for elasmobranchs; the shape of a tank can be of equal importance. In general, the swimming pattern of sharks comprises a number of discrete stages: (1) a forward power component, either cruising or bursts of high speed; (2) a rest/glide phase; and (3) a recovery phase (Klay, 1977). This generalized swimming pattern, referred to as the swim-glide hypothesis, enables sharks to conserve valuable energy reserves. As sharks lack a swim bladder for buoyancy control, most species use forward motion, in combination with their rigid pectoral fins, to generate lift. If a tank has restrictive horizontal dimensions, sharks will struggle to maintain their position within the water column, will be unable to complete the swim-glide sequence, and will consume excess energy reserves. If this situation persists, exhaustion and ultimately death can result.

Historically, many different tank shapes have been used for elasmobranchs. Rectangular tanks are common and inexpensive to build, but they can present problems. The right-angle corners of rectangular tanks represent wasted space for most shark species and can exacerbate the acclimatization of new sharks as they expend excessive energy attempting to navigate out of, or recover from entrapment within, corners (Murru, 1990). Rectangular tanks can be improved by chamfering the corners or rounding the corners to large-radius bends.

Cylindrical tanks have been used successfully with many species; however, problems can arise if the tank is not large enough to allow animals to complete species-specific, and/or swim-glide, swimming sequences. If a shark is subjected to these unsuitable conditions it will initially hit the walls, and then swim close to the tank perimeter hugging the wall surface. This behavior disrupts the shark's normal swimming pattern, causes the animal to make constant small turning adjustments, increases metabolic demand, and consumes excess energy reserves. It has been further suggested that wall-hugging may create inefficiencies in oxygen transfer across the gills (Klay, 1977). Hugging the walls can result in external abrasions to the shark's skin, with an associated risk of infection.

Variations on the cylindrical tank include roundabouts, racetracks, or doughnuts, whereby the center of the tank is filled with a structure designed to prescribe a circular path for the animals. The center structure can be exhibit décor (hiding LSS components, holding areas, etc.) or even serve as a visitor's viewing area. The roundabout tank theoretically provides an endless column of water for swimming sharks. However, despite much experimentation, these tanks rarely perform as desired, resulting in the sharks failing to constantly swim in the direction intended. Experience demonstrates that sharks will swim until they encounter an object, whereupon they will turn and swim until they encounter another object (i.e., their swimming patterns are modified by obstacles, as and when they are encountered, rather than by pre-planned routes). This turn-and-go behavior means that prescribed circular paths are less than optimal.

Modern tank designs frequently include figure-eight or dumbbell shapes, allowing for swim-glide swimming patterns. First developed by SeaWorld, San Diego, USA (Keyes, 1979), this design has been used successfully by many other aquariums (e.g., the Pacific shark exhibit at the John G. Shedd Aquarium, Chicago, USA). Other modern tank designs include free-form shapes, most of which are acceptable as long as they have sufficiently large horizontal dimensions and corners greater than right-angles.

Stocking density and life support systems

The design of a life support system (LSS) for elasmobranch quarantine and isolation tanks should focus on maximizing biological carrying

capacity (i.e., the capacity of the system to remove biological waste products, ammonia and nitrite) rather than spending valuable resources on optimizing water clarity. Although water clarity is a necessary and important consideration for quarantine and isolation facilities, the LSS does not need to provide the +30 m visibility required for most large elasmobranch exhibits. In order to maximize the functionality of holding and quarantine tanks, LSS designers should focus resources on enhancing or increasing biological filtration and oxygenation rather than fine-particle mechanical filtration. After tank size and tank shape, biological carrying capacity is the most important design consideration for quarantine and isolation tanks. In combination, these three factors determine the overall holding capacity of the quarantine and isolation facility. The biological carrying capacity of an LSS is measured by its maximum allowable bio-load or stocking density (measured in kilograms of animal per cubic meter of water). A typical public display may be bio-loaded at a stocking density of ~1.0 kg m^{3-1} (Garibaldi, 1982). Intensive aquaculture systems may be stocked at 50 kg m^{3-1} or even higher. As quarantine and isolation facilities are expensive to build, and provide a vital support to public exhibits, they should be prepared for the highest practicable bio-loading, well in excess of the typical elasmobranch exhibit. In general, it is usually less expensive to add surface area to the biological filters, than to add more volume to the tank itself. LSS design considerations for maximizing allowable bio-load can be found in aquaculture literature (e.g., Wheaton, 1977; Huguenin and Colt, 1989).

The higher bio-loading of quarantine and holding systems (and systems with high-metabolism elasmobranchs) necessitates an enhanced mass-transfer removal of carbon dioxide (CO_2). If not effectively addressed, excess CO_2 accumulation will cause a decline in the pH of the water with negative physiological ramifications for the animals (refer to Chapter 8 of this manual). Excess CO_2 can be removed via counter-current exchange in foam fractionators, de-gassing towers, and wet-dry biological filters. Designers of LSSs should therefore consider the inclusion of additional foam fractionators, air supplies to de-gassing towers and biological filters, and a back-up aeration/oxygenation system to promote gas exchange and maintain dissolved oxygen levels.

Seawater sourcing (i.e., acquisition or manufacture), pre-treatment (i.e., mechanical filtration, sterilization, etc.), and storage represents another important aspect of LSS design for quarantine and isolation tanks. The relative advantages and disadvantages of natural seawater (NSW) and artificial seawater (ASW) are discussed elsewhere (refer to Chapter 6 of this manual). Considerable cost savings can be achieved if the same raw water pre-treatment and storage systems are employed for both quarantine and holding, and exhibit LSSs. Water storage tanks can be located aboveground, but it is often desirable and more practical to place them underground if they can be installed during building construction. Storage tanks can be made from concrete or fiberglass-reinforced plastic (FRP). The addition of an aeration system will assist the mixing of salts (in the case of ASW) and keep the water well oxygenated for immediate use. A re-circulation pump should be attached to storage tanks, allowing easy transfer of raw water to destination tanks. If freshwater is used (i.e., for manufacturing ASW or for use in exhibits containing freshwater species—e.g., *Potamotrygon* spp.) it should be carefully analyzed for heavy metals and other contaminants, and pre-filtered with activated carbon.

The location and design of tank drainage lines (i.e., surface skimmers and bottom drains) and seawater supply lines (i.e., inlets returning water to the tank) should be carefully considered. Drains and surface skimmers should be carefully screened or protected to prevent animal entrapment. Seawater supply lines should be designed and located to create a slight current for obligate ram-ventilating species.

Effluent water treatment and disposal systems should be carefully considered during LSS design. Effluent water from water exchanges, filter backwashes, foam fractionator overflows, and chemical treatments should all be considered. Local regulations should be carefully reviewed as many municipalities—particularly those that recycle sewerage—do not allow the discharge of seawater into municipal sewer systems. Likewise, the discharge of chemical treatments may be subject to regulation. LSS designers must understand local restrictions on the quality of discharged water and consider the addition of pressurized ozone reactors and/or other similar effluent water treatment systems as required. Effluent treatment is critically important for flow-through systems that discharge water directly to the natural environment (Garibaldi, 1982).

One way to conserve costs (both capital and operational) during LSS design is to divide each

LSS component into smaller additive pieces and only operate those pieces required (i.e., as a function of stocking density). For example, the LSS could be operated with a number of smaller pumps rather than a single large pump, each of the smaller pumps engaged as required. This modular approach to LSS design has the added benefit of built-in equipment redundancy. LSS design considerations are addressed in more detail in Chapter 6 of this manual.

TANK CONSTRUCTION

Quarantine and holding tanks must be built with suitable materials to provide strength (e.g., resistance to head pressure, water surges, impacts from animal collisions), long-term durability (and thus investment protection), water-tightness, non-toxicity (to the animals), and resistance to corrosion.

Recommended construction materials

Concrete

Concrete is an excellent choice for large aquarium tanks, primarily because of its strength (dependent upon mix recipe and steel reinforcing or rebar) and relatively low cost for volume. Concrete relies on internal steel reinforcing, or rebar, to resist tensile stress. Rebar can create problems if not installed correctly (see Hawkins and Lloyd (1981) and Chapter 5 of this manual for a more detailed review of concrete tank construction).

The construction of concrete seawater aquariums is not in the domain of ordinary structural design. Not only should strength be designed into concrete tanks, but a careful selection of reinforcing materials and concrete ingredients should be considered. Particular attention should be paid to joints, intersecting structural elements, reinforcing patterns, secondary stresses, flow of stress within the reinforcing patterns, and penetration details. Above all, tank designers must educate the contractor, since the quality of finished products depends a great deal on construction techniques.

Polyvinyl pipe (PVC) penetrations through cast concrete tank walls can cause leakages at the interface between the different materials, so some form of mechanical water-stop should be incorporated into pipe stubs prior to pouring the concrete. Although holes can be drilled through the concrete after it has cured, and pipe penetrations sealed with mechanical seals (e.g., Link-Seal, PSI-ThunderLine Link-Seal, USA), this should be avoided where possible as it can expose steel rebar to tank water and thus corrosion. Concrete tanks are often limited by cost and logistics to relatively simple shapes (e.g., rectangular), as they must be formed and cast in place.

Fiberglass reinforced plastic (FRP)

Fiberglass reinforced plastic (FRP) is an excellent choice for tank construction, having several advantages over concrete. FRP is inherently strong when molded into the shape of a tank, especially when the tank is cylindrical and/or incorporates a flange at the top, and usually requires no other reinforcing other than the incorporated woven fiberglass mesh or chopped matting. Odd-shaped, tall, or long tanks may require additional structural support, provided by a steel skeleton wrapped within the FRP or, alternatively, structurally robust pultruded FRP shapes may be employed.

PVC pipe penetrations through FRP tank walls are facilitated by FRP pipe fittings, and present little risk of leakage. Depending on size, FRP tanks are usually less expensive than concrete tanks. FRP tanks can be partially buried to improve structural strength and provide easier staff access to the interior of the tank.

FRP tanks can be cast in one piece from a mold or assembled from pre-fabricated panels. If they are pre-fabricated off-site, consider access into the quarantine and holding facility for their final installation. Pre-fabricated panels require bolting and then sealing with either fiberglass resin or silicone. Some pre-fabricated panel tanks are effectively expandable (i.e., by adding straight wall sections between rounded end sections to form a large oval, or by adding additional sections to the top of the walls). Pre-fabricated panel tanks can be pulled apart relatively easily and relocated and assembled for use elsewhere.

Waterproofing and tank coatings

Concrete tanks can be designed and constructed to be completely watertight, although it is recommended that an additional waterproofing material (e.g., Vandex, Vandex International, Ltd., Switzerland) or a post-cure internal tank coating

(e.g., Polibrid, Polibrid Coatings, Inc., Brownsville, Texas, USA) be applied. The effectiveness of waterproofing treatments relies on a high-quality design and construction of concrete substrates.

Because FRP is a dense plastic, it is inherently inert, non-toxic, and watertight upon curing. In addition, FRP can be readily painted with epoxy paints or molded with a colored gel-coat. In some cases it may be desirable to coat the interior walls of the tank to produce a smoother, longer-lasting, and non-toxic interior finish, or to color the walls to assist with animal acclimatization. An all-white tank can be disorienting for newly-acquired elasmobranchs, so blue colors which better mimic the oceanic environment, or vertical lines of contrasting colors, which denote the walls, might be preferred for quarantine and holding tanks. If internal coatings are used, they should be completely non-toxic upon curing and compatible with the tank construction materials. In the past, epoxy-based paints (e.g., Sta-Crete, Epmar Corporation, Santa Fe Springs, California, USA) have worked well for this purpose, as have some newer polymer coatings (e.g., Polibrid, Polibrid Coatings, Inc., Brownsville, Texas, USA). The addition of a soft vinyl boundary wall (or a curtain of air bubbles), suspended inside the tank wall, may also be used to denote the outer wall for disorientated animals (Farwell, 2001; Choromanski and Hamilton, 1997). The use of substrate on tank bottoms may be warranted for some batoids, but is generally not recommended in quarantine or holding tanks.

Less-desirable construction materials

Prior to the availability of FRP (or other similar polymer materials) many tanks were constructed from ferrous metals, including galvanized iron, etc. Some of these tanks survive to this day, usually in facilities with flow-through seawater supplies that continuously dilute the toxic metals (e.g., zinc, chromium, etc.) leached from the tank walls. With the availability of modern, non-toxic construction materials, metals of any kind should not be used when constructing quarantine and holding tanks.

The construction of tanks using fiberglass wrapped around a wooden skeleton and wooden sheeting is not recommended. Although inexpensive, the wood has a tendency to rot and the tank to fail structurally. Rigid PVC foam (e.g., Divinycell, American Foam Group, Chambers-burg, Pennsylvania, USA) is a good alternative to wood, providing shape, insulation, and impermeability.

Pond liners, laid on top of open-earth excavations, have been used successfully for open-air elasmobranch exhibits (e.g., Discovery Cove, Orlando, Florida, USA). However, this construction technique is not recommended for quarantine and isolation facilities.

BUILDING CONSTRUCTION

In addition to the tanks themselves, the choice of construction materials has a tremendous bearing on the building that houses the quarantine and isolation facilities. Construction costs increase with the use of non-corrosive materials, but as the saying goes, you get what you pay for. Although there are many brands of pre-engineered, metal building systems available (e.g., Butler Manufacturing Company, Kansas City, Missouri, USA), offering both inexpensive and easily-constructed enclosures, they require additional insulation materials for climate control and special coatings to prevent corrosion in the salty environment. A superior method of construction uses pre-cast concrete or cinder block walls and wooden trusses for the roofing system. This technique reduces the concerns of insulation and corrosion, although such custom designs are generally more expensive. Temporary fabric buildings (e.g., canvas attached to a strong, rigid frame, etc.) have been used for animal holding facilities, but these have poor climate control and an associated high energy cost to operate.

The floor of the quarantine and holding building should be designed and engineered to accommodate the weight of the tanks, water, and associated LSS equipment. Despite all efforts to the contrary, water will leak and spill onto the floor, so careful consideration should be given to an extensive drainage system throughout the building. Drains should be provided for both LSS equipment (e.g., foam fractionator effluent, etc.) and general work spaces, to contain spilled water. Trench drains, although expensive, are ideal for effectively draining large spaces. Drains and piping should be constructed from PVC, acrylo-nitrile butadiene styrene (ABS), or some other saltwater-resistant material, and drains should be fitted with screens to trap debris. Concrete floors should be sloped toward floor drains and coated with a saltwater-resistant non-skid coating (e.g., Terralite, Marbelite International Corp, Sarasota, Florida, USA; or Silikal, Specialty Resin Systems, Waterbury, Connecticut, USA).

Construction specifications should be carefully developed by designers so that inert, non-toxic materials are always selected. Non-toxicity is important not only for materials that will come into direct contact with aquarium water (e.g., PVC pipes, titanium plate heat exchangers, etc.), but also materials that will be located anywhere near the quarantine and holding tanks. There are too many examples of tanks and LSSs built using appropriate materials that are surrounded by buildings and infrastructure made of potentially toxic materials (e.g., PVC piping suspended over a tank using FRP hangers, adjacent to an anti-fire sprinkler system constructed of iron or copper pipe and suspended over the tank using ferrous hangers). The corrosion of inappropriate toxic construction materials and their potential introduction into system water should always be avoided. Many excellent inert construction materials are available, including PVC electrical conduit (e.g., Carlon-Lamson and Sessions Company, Cleveland, Ohio, USA) and FRP pipe hangers and structural members (e.g., Aickinstrut-Tyco International, Portsmouth, New Hampshire, USA). For additional information about aquarium construction materials refer to Garibaldi (1982), and Hawkins and Lloyd (1981).

ACCESS

A quarantine and isolation facility must have unimpeded access for the staff (and husbandry equipment) to the tanks and the animals. It must be possible for animals to be moved easily to any part of the facility (e.g., from a community tank to an isolation tank, etc.). Although it is tempting to fill available space with additional tanks, ample access must be provided for observation, husbandry, cleaning, feeding, LSSs, etc. (Garibaldi, 1982).

The quarantine and isolation facility should be designed to provide clear and easy access for the trucks, trailers, and boats (i.e., an adjacent berth) used to transport animals from collection sites. Loading bays should be adjacent to the quarantine and holding tanks. Vehicle access can be in the form of an internal driveway with doors on either end of the building (especially useful in extreme climates) and access for forklifts or an overhead crane rail should be incorporated into the building design. This equipment is especially useful when handling large animals and heavy transport tanks. Ample space should be available between tanks for the movement of transport and lifting equipment.

All tanks must have windows enabling a clear view of the animals. Windows need to be sufficiently large and strategically located so that all parts of the quarantine and isolation tanks can be seen. It is imperative that sick or injured animals do not go unnoticed. Acrylic is the most desirable material for tank windows because it is optically and structurally superior to glass, it can be polished if scratched, and it can be readily made into any shape, including curves for cylindrical tanks.

Raised catwalks, around the perimeter of quarantine and holding tanks, will provide access for cleaning the tanks and feeding the animals. The floor of the catwalk should be located 0.9 m below the top of the tank and guard rails should be fitted to the outside of the catwalk if it is higher than 0.76 m above the base of the floor. Water levels should be maintained at 0.15-0.30 m below the top of the tank (species-dependent) to prevent animals from escaping, but not so low that it causes access problems for staff. Removable anti-jump guards (e.g., plastic mesh stretched over a PVC pipe frame) can be added to the tank perimeter for additional security.

Similar to large elasmobranch exhibits, large quarantine tanks should have an attached acclimatization and isolation pool for husbandry procedures. This pool should be able to accommodate the largest anticipated species (as per the criteria discussed above) and husbandry staff should be able to enter and exit the pool easily when burdened with husbandry equipment (e.g., SCUBA, shark stretchers, etc.). The acclimatization pool should be shallow (i.e., ~0.9 m) to allow the performance of husbandry procedures. It is particularly useful to have a false floor, capable of withstanding the weight of both sharks and husbandry personnel, which can be raised clear of the water (as per marine mammal husbandry and weighing apparati). It should be possible to completely isolate the acclimatization and isolation pool (using water-tight doors and independent LSSs) from the adjacent quarantine tank. This precaution will enable the true isolation of animals for disease treatment and control, or for the application of specialized environmental parameters (e.g., alternative temperatures, salinities, etc.). Sharks should be able to access the isolation pool with ease (i.e., the pool should have wide entrances, etc.) and the doors should be strong and easy to operate rapidly. If a separate acclimatization and isolation pool is not available, staff may need to capture target animals using surface-deployed nets (e.g., the moveable

gantry and net system employed by SeaWorld's Shark Encounter, Orlando, USA), or by draining the tank to workable water levels. In the latter case, it may be desirable to have a system for saving and re-using drained water, such as pre-installed underground tanks, portable pillow-style storage tanks (e.g., Interstate Products, Sarasota, USA), pumping systems, etc.

OTHER CONSIDERATIONS

Husbandry support areas

The design of an elasmobranch quarantine and isolation facility would not be complete without the provision of a husbandry support area. This area should include: (1) a fully-equipped food preparation area with a walk-in freezer, high-capacity refrigerator, and stainless steel sinks and tables that meet National Sanitation Foundation certification (or equivalent) for food safety (www1); (2) a fully-equipped water quality laboratory; (3) an office for animal record-keeping and fire-protected file storage; (4) a necropsy room; (5) a dive locker room with showers and equipment storage; and (6) storage rooms for husbandry equipment and seasonal collecting equipment. Provision should be made for enclosed truck, trailer, and boat storage areas, especially in colder climates. If the quarantine and isolation facility is adjacent to the exhibit building, some or all of these areas may be shared.

Lighting

Natural lighting is ideal as it provides a natural photoperiod and seasonal cues. Skylights can be designed into most buildings and should be considered, especially over tanks. Skylights are a potential site for heating or cooling losses. However, they can save on electrical consumption for artificial lighting. Skylights should be controllable, via louvers or shade cloth. Additional work lighting will always be required.

An advantage of artificial lighting over natural lighting is that it can be finely adjusted, controlled, and manipulated for husbandry purposes (e.g., altering photoperiods to stimulate captive breeding, etc.). Artificial light fixtures should be designed to allow easy and safe access for lamp replacement (e.g., movable lighting systems on tracks, etc.). Artificial lighting should be designed to simulate sunrise and sunset (so that animals are not startled), by slowly ramping illumination up and down, and should be adjustable to replicate season-dependent photoperiods.

There is no published literature on the spectral lighting requirements of elasmobranchs and, with the exception of some deeper-water species, they seem to tolerate a broad range of lighting wavelengths and intensities. Fluorescent, incandescent, and metal vapor-arc lamps have all been used successfully. Combinations of lamp types can be used to address the slow warm-up times of some lamps (e.g., the use of incandescent or fluorescent lights, in lieu of metal halide high-intensity discharge lamps). High-intensity lighting is unnecessary, can lead to excessive algae growth, and create maintenance issues. For additional information on lighting see Hawkins and Anthony (1981) and Spotte (1979).

Heating, ventilation, and air conditioning

Because water temperatures are normally controlled independently by the heat exchanger systems of the LSS, budget-driven designers might be tempted to eliminate the air conditioning of building air spaces. However, in most climates, air conditioning (i.e., heating, cooling, and importantly, dehumidification) will be required. This precaution is especially valuable if tanks are maintained at temperatures below ambient. Not only will husbandry staff be safer, comfortable, and more productive, it will save tremendous wear-and-tear on the building and equipment from condensation. In addition, dry areas must be provided for record-keeping, necropsy, and laboratory areas.

Security

Security systems should be implemented to avoid fire, theft, vandalism, etc. A perimeter fence around the entire quarantine and isolation facility is highly recommended. Electronic surveillance systems are readily available and can be modified to include simple LSS alarms (e.g., water flow switches, water level switches, electrical power status, etc.), as well as fire and burglar alarms. Camera systems can be installed and monitored remotely via the Internet. An emergency electricity generator, with an automatic power transfer switch and adequate fuel supply, is highly recommended, especially in areas where power is frequently interrupted by severe weather.

ACKNOWLEDGEMENTS

The author wishes to thank John Hewitt, Aquarium of the Americas, New Orleans, Louisiana, USA for his significant assistance in the preparation and review of this paper. Dave Powell (Monterey Bay Aquarium, Monterey, California, USA - retired) and Mik Peller (Peller & Associates, Inc. Structural Engineering, Cleveland, Ohio, USA) provided valuable comments, and C. J. Slager (Monterey Bay Aquarium) provided a valuable final review.

REFERENCES

Choromanski, J. M. and R. M. Hamilton. 1997. Fatal attraction: solving the tuna collision problem at the Monterey Bay Aquarium. *In:* Annual Conference Proceedings, 14-18 September 1997, Albuquerque, New Mexico, p. 142-145. American Zoo and Aquarium Association, Silver Spring, Maryland, USA.

Clark, E. 1963. The maintenance of sharks in captivity, with a report on their instrumental conditioning. *In*: Sharks and Survival, p. 115-149. P. W. Gilbert (ed.). D.C. Heath and Company, Lexington (now: Houghton Mifflin Company, Boston, Massachusetts, USA).

Farwell, C. F. 2001. Tunas in captivity. *In:* Tuna: Physiological Ecology and Evolution, p. 391-412. B. A. Block and E. D. Stevens (eds.). Academic Press, New York, USA.

Garibaldi, L. E. 1982. Design features of fish and marine invertebrate exhibits. *In:* Zoological Park and Aquarium Fundamentals, p. 105-118. K. Sausman (ed.). American Association of Zoological Parks and Aquariums (American Zoo and Aquarium Association, Silver Spring, Maryland, USA).

Gruber, S. H. and R. A. Keyes, 1981. Keeping sharks for research. *In:* Aquarium Systems, p. 373-402. A. D. Hawkins (ed.). Academic Press, New York, USA.

Hawkins A. D. and Anthony, P. D. 1981. Aquarium design. *In:* Aquarium Systems, p. 1-46. A. D. Hawkins (ed.). Academic Press, New York, USA.

Hawkins, A. D. and Lloyd, R. 1981. Materials for the aquarium. *In:* Aquarium Systems, p. 171-196. A. D. Hawkins (ed.). Academic Press, New York, USA.

Huguenin, J. E. and J. Colt. 1989. Design and Operating Guide for Aquaculture Seawater Systems. Elsevier, New York, USA. 264 p.

Keyes, R. S. 1979. Description of live shark exhibit and research center, Sea World San Diego. San Diego Research Center Report 79(1). 48 p.

Klay, G. 1977. Shark dynamics and aquarium design. Drum and Croaker 17: 29-32.

Murru, F. L. 1990. The care and maintenance of elasmobranchs in controlled environments. *In:* Elasmobranchs as Living Resources: Advances in Biology, Ecology, Systematics and the Status of Fisheries, p. 203-209. H. R. Pratt, S. H. Gruber, and T. Taniuchi (eds.). U. S. Department of Commerce, NOAA Technical Report NMFS 90.

Spotte, S. 1979. Seawater Aquariums. John Wiley and Sons, New York, USA. 413 p.

Wheaton, F. W. 1977. Aquaculture Engineering. Robert E. Kreiger Publishing, Malabar, Florida, USA. 708 p.

PERSONAL COMMUNICATIONS

Hewitt, J. D. 2001. Aquarium of the Americas, New Orleans, LA 70130, USA.

INTERNET RESOURCES

www1: www.nsf.org

The Elasmobranch Husbandry Manual: Captive Care of Sharks, Rays and their Relatives, pages 53-67.
© 2004 Ohio Biological Survey

Chapter 5
Design and Construction of Exhibits for Elasmobranchs

DAVID C. POWELL

Monterey Bay Aquarium (emeritus)
651 Sinex Avenue,
Pacific Grove, CA 93950, USA.
E-mail: tiburon@infostations.com

MARTY WISNER

Mauna Lani Resort
68-1400 Mauna Lani Drive,
Kohala Coast, HI 96743, USA.
E-mail: mwisner@webtv.net

JOHN RUPP

Point Defiance Zoo & Aquarium
5400 Pearl Street,
Tacoma, WA 98407, USA.
E-mail: jrupp@pdza.org

Abstract: Early attempts to keep elasmobranchs successfully were limited to small and hardy species. A breakthrough occurred during the late 1970's when the energy-efficient swimming pattern of pelagic elasmobranchs was recognized and exhibits were designed to accommodate the requirements of these more delicate and demanding species. Sharks and rays can be divided into four basic groups (i.e., benthic, semi-pelagic, pelagic non-obligate ram ventilators, and pelagic obligate ram ventilators) each of which has specialized husbandry requirements. The successful design of an elasmobranch exhibit should take into account the specific needs of species considered for display, viewing opportunities, safety for the visitor, and access requirements for husbandry and maintenance staff. The design should ensure that animal exposure to electromagnetic fields is minimized, metallic products are not used (if at all possible), sudden changes in lighting intensity are avoided, and adequate facilities for specimen introduction and isolation are included.

THE RECENT HISTORY OF SHARK EXHIBITS

Prior to the late 1930's, the successful display of elasmobranchs was limited by small tank sizes and was confined to small, benthic species of sharks and rays, with the exception of nurse sharks (*Ginglymostoma cirratum*) and sand tiger sharks (*Carcharias taurus*). Taronga Zoo and Aquarium, Sydney, Australia opened an aquarium complex in 1927 with three floors of small exhibits and one 20 m long x 15 m wide irregular-shaped shark pool. The pool contained several sand tiger sharks and other elasmobranch species including wobbegong sharks (*Orectolobus* spp.), Port Jackson sharks (*Heterodontus portusjacksoni*), and several large rays of the family Dasyatidae. One of the sand tiger sharks was reported to live for three years and another for over seven years (Whitley, 1940). After 1930 a number of small shark displays comprising basic swimming pools were constructed in and around Sydney. Captured sharks, mostly sand tiger sharks, were introduced into these exhibits and frequently swam around until they died. Jack Evans's Pet Porpoise Pool opened during the 1950's at Coolangatta, Queensland, Australia. This display had a small shark pool with sand tiger, tiger

(*Galeocerdo cuvier*), and zebra (*Stegostoma fasciatum*) sharks, and a variety of shark species from the family Carcharhinidae. Only the hardiest of species survived for long in these basic exhibits.

The development of marine parks that had large exhibits with underwater viewing marked the beginning of a new era in the display of marine animals, including elasmobranchs. In June 1938, Marine Studios opened in St. Augustine, Florida, USA. It was originally built as a facility for filming sea creatures for the movie industry, but their trained dolphins soon became a popular attraction for visitors. Renamed Marineland, it became the first true oceanarium and featured two large exhibits, one for marine mammals and the other for fishes, including sharks and rays. Marineland of the Pacific in Los Angeles County, California, USA, opened in 1954 following the overwhelming success of Marineland, Florida. Like Marineland of Florida its primary focus was marine mammals. However, another feature was a large 30 m long x 15 m wide oval tank containing an extensive collection of temperate water fishes from California and Mexico. It was open to sunlight, and copper sulfate was used to control algae growth and maintain water clarity. Two large-tooth sawfishes (*Pristis perotteti*) were successfully maintained in the exhibit, but pelagic shark species fared less well.

Marineland of the Pacific's popularity led to the opening of Seaquarium in Miami, USA during 1955. Seaquarium was the first organization to design a major exhibit specifically for sharks. It consisted of a circular channel (228 m outside diameter x 7.3 m wide x 2 m deep) with viewing from above (Figure 5.1). Little was known about the physiological needs of sharks and only the hardiest of species survived for any length of time. The collection was primarily limited to four species: lemon sharks (*Negaprion brevirostris*), nurse sharks, bull sharks (*Carcharhinus leucas*), and tiger sharks. To quote William Gray (1960), Director of Collections at Seaquarium: "…There are about 350 kinds of sharks in the world, nearly fifty of which may be found off the coast of Florida. Only a few of these types are commonly met with, however, and fewer still will live very long in captivity. On our twenty-five hooks we could expect to find about six or eight sharks. Of these, one or two would die on the way to the Seaquarium or shortly after arrival, and at the end of a month maybe two would still be alive. Because of this high mortality rate, we have to go out hunting about every six weeks if we wish to keep the Seaquarium stocked with the usual fifty to one hundred sharks…". The high mortality rate of delicate shark species at Seaquarium and other facilities was due to a combination of poor capture and transportation methods, and the periodic use of copper sulfate to control algae growth in exhibits exposed to direct sunlight.

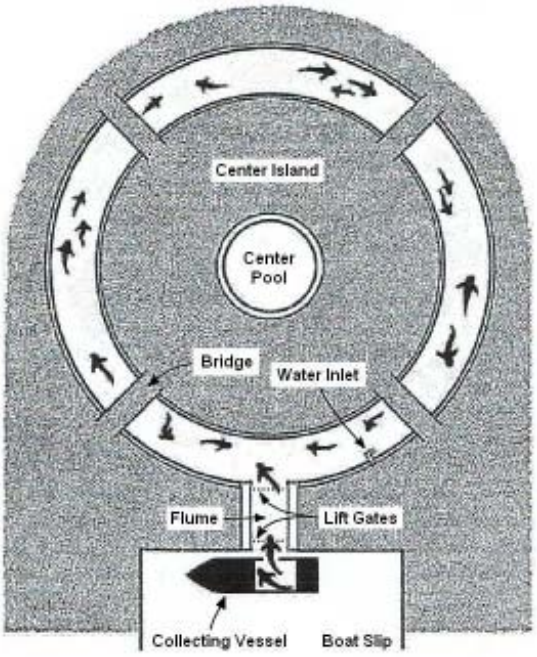


Figure 5.1. Plan view of the Shark Channel exhibit (228 m outside diameter x 7.3 m wide x 2 m deep): Miami Seaquarium, USA (Phillips, 1964).

The popularity and financial success of these aquariums led to the construction of a number of similar facilities in the USA and around the world. These included Searama in Galveston, Texas, USA, the Durban Aquarium, South Africa (both of which successfully displayed bull sharks), and Marineworld Africa-USA, San Francisco, USA. All of these institutions built shark aquariums that were circular or oval in shape and open to the sky.

In 1968, SeaWorld San Diego, California USA designed a 15 m diameter saucer-shaped experimental tank to investigate methods of keeping the pelagic blue (*Prionace glauca*) and mako (*Isurus oxyrinchus*) sharks (Figure 5.2). Much was learned from this process including successful techniques for collecting sharks, and the fact that a shallow circular tank is a poor design for open ocean sharks; forcing them to constantly turn in a circle and ultimately become exhausted. Marineland opened a similar saucer-shaped exhibit for blue sharks and these specimens did poorly, suffering abrasions from the tank walls and bottom.

In the 1970's, the sensitivity of pelagic sharks to poor water quality and toxic water treatments (e.g.,

copper sulfate, etc.) was recognized. To eliminate the need for algaecides and minimize the effects of seasonal changes on water quality, a number of new shark exhibits were covered and artificially illuminated. The Vancouver aquarium, Canada and the New York Aquarium, USA both constructed covered, environmentally controlled shark exhibits.

In 1976 Steinhart Aquarium, San Francisco, USA opened the Fish Roundabout (20 m outside diameter), modeled after the donut-shaped exhibit at Shima Marineland, Japan, where viewers were located within a "donut hole" surrounded by a circle of schooling fishes, sharks, and rays (McCosker, 1999). Although not specifically designed for elasmobranchs, this exhibit contained several species of sharks and rays including, for periods of a few days, great white sharks (*Carcharodon carcharias*). The roundabout concept was further developed at the National Aquarium in Baltimore, USA when they opened a long, oval exhibit in 1981 (33.5 m long x 18.5 m wide, including the void inside the oval raceway). The oval shape had the physiological advantage of minimizing energy-consuming turns.

A breakthrough in exhibit design for pelagic sharks came during the late 1970's with the discovery of their energy-efficient pattern of straight, active swimming followed by passive gliding (Klay, 1977). The first exhibit design to incorporate this "swim-glide" concept was SeaWorld San Diego's dumbbell-shaped Shark Encounter (30.5 m long x 12.2 m wide) (Figure 5.3). This successful design was later used in SeaWorld's other parks. The reef structure was low and the ends of the tank were wide and unobstructed to give the sharks an easy, relaxed turning radius. A traveling gantry spanned the tank and allowed part of the exhibit to be netted off so divers could service the shark-free section in safety. It was a highly successful exhibit both from the standpoint of shark health and as a dramatic exhibition of the animals and their behavior. A similar shape was used in 1984 for the multi-species Monterey Bay Habitats exhibit (27 m maximum horizontal dimension) at the Monterey Bay Aquarium, Monterey, USA. This exhibit included sharks, teleosts, invertebrates, diving birds, and live algae (Figure 5.4).

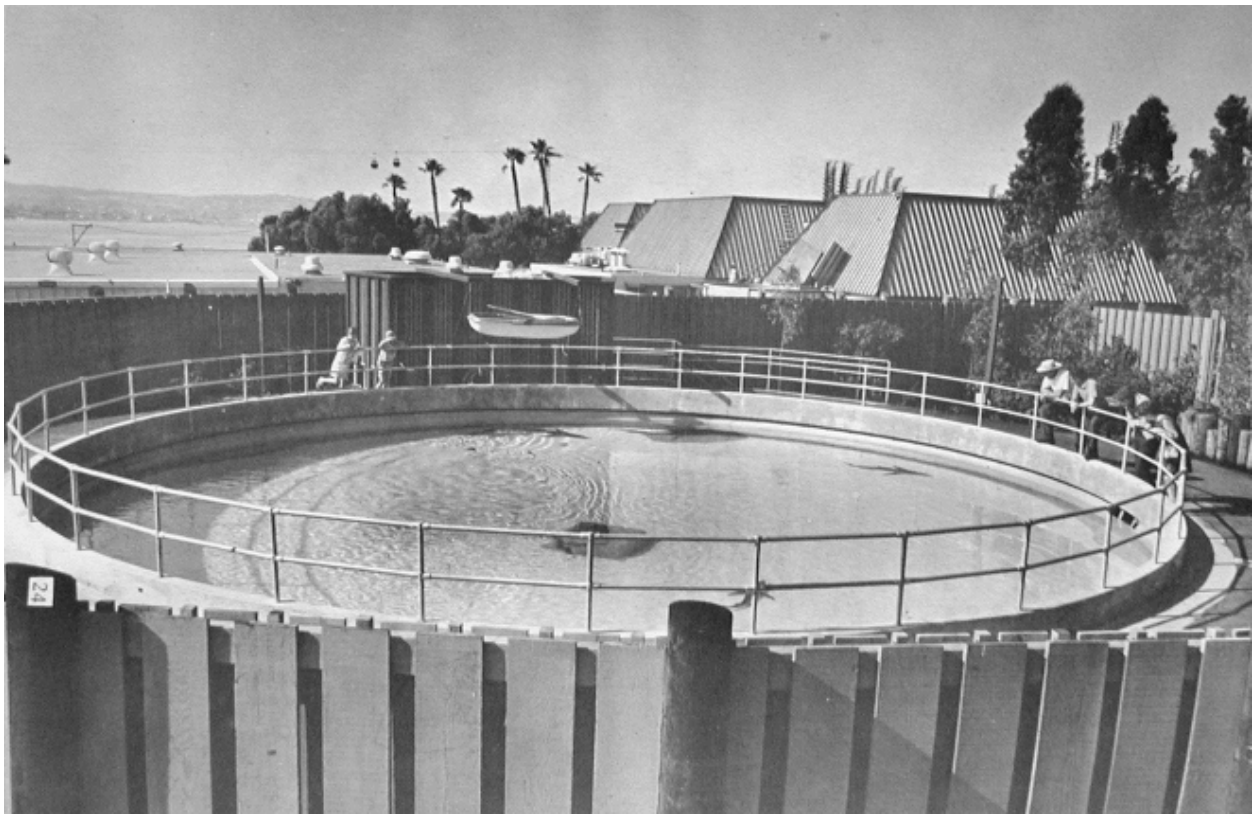


Figure 5.2. Experimental saucer-shaped tank (15 m diameter) for pelagic sharks: SeaWorld, San Diego, USA (Powell, 1968).

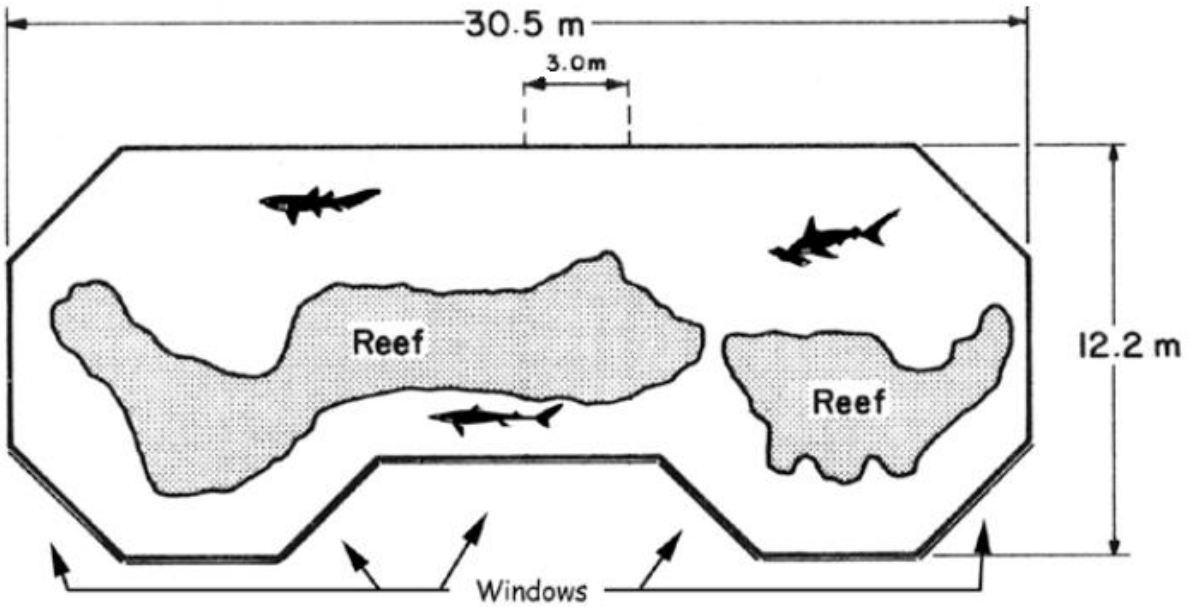


Figure 5.3. Plan view of the Shark Encounter exhibit, incorporating "dumbbell" shape designed to accommodate the natural swim-glide behavior of pelagic sharks: SeaWorld, San Diego, USA (Weihs et al., 1981).

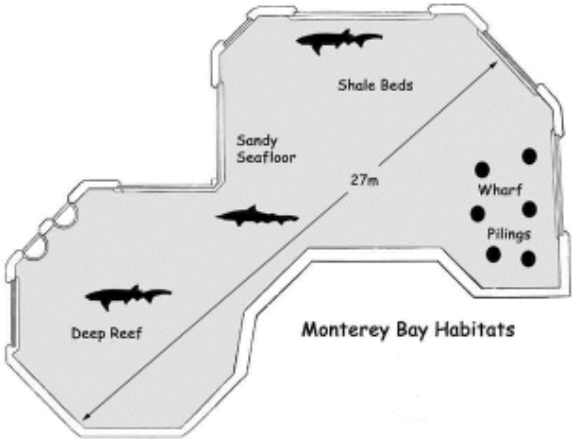


Figure 5.4. Plan view of the Monterey Habitats exhibit, incorporating the modified "dumbbell" shape: Monterey Bay Aquarium, Monterey, USA.

The display of whale sharks (*Rhincodon typus*) from 1980 up to the present day has been the outstanding achievement of the Okinawa Expo Aquarium, Japan. The exhibit tank is not exceptionally large (27 m long x 12 m wide x 3.5 m deep), nor was it designed with whale sharks in mind, yet it has proven to be successful for a wide variety of elasmobranchs. The Okinawa Expo Aquarium opened a much larger exhibit (7,500 m^3 total volume), intended specifically for whale sharks, during late 2002 (Figure 5.5).

CHOICE OF DISPLAY SPECIES

An aquarium containing elasmobranchs can have a variety of educational goals. An anatomical adaptation such as camouflage can be demonstrated in an exhibit focusing on wobbegong sharks. Taxonomic relationships, and behavioral and anatomical differences between sharks, can be shown in a dedicated "shark tank" containing a variety of species. An entire habitat, such as a coral reef or a kelp forest, can show how elasmobranchs are a small part of the broad spectrum of representative organisms that make up complex ecosystems. Elasmobranch development can be graphically demonstrated using exhibits of living eggs, embryos, and hatchlings.

Ideally, the exhibit design process begins with the selection of a species list, including all species that one might possibly acquire in the future. Regardless of the primary goal of an exhibit the first criterion in its design is to satisfy the needs of the animals, including species with the most stringent requirements. This criterion, of course, applies not just to elasmobranchs, but to all taxa. Although most new exhibits start out with juvenile or sub-adult elasmobranchs, the design of the exhibit must be suitable for the size that specimens will ultimately reach. If it is known that a shark or ray could eventually become too large for an exhibit then provisions should be made either for its release to a suitable environment, if that is possible, or for

Figure 5.5. The Whale Shark exhibit (7,500 m^3 total volume) incorporating the world's largest acrylic window (22.5 m long x 8.2 m high x 0.6 m thick): Okinawa Expo Aquarium, Japan.

transfer to a larger facility. In the past there have been too many cases of public aquariums and hobbyists attempting to keep sharks that outgrew their exhibits. The ultimate goal public aquariums should strive to attain is to provide adequate space and conditions for the successful reproduction of the elasmobranchs in their care.

ELASMOBRANCH LIFESTYLES AND EXHIBIT FORM

Elasmobranchs come in a wide variety of sizes, body plans, and life-styles. These factors must be carefully considered during exhibit design as they will influence the suitability of a display for specific species and hence their chances of survival. Table 5.1 presents four basic groups of sharks and rays, showing representative species. Each group has been organized by the unique requirements of their anatomy, physiology, and behavior.

Benthic

For small, reef-dwelling, benthic sharks one can simulate a natural environment by incorporating real or artificial rock, and compatible fishes, invertebrates, and algae, and not compromise the physiological needs of the sharks. For some sharks (e.g., the whitetip reef shark, *Triaenodon obesus*) one can create an overhanging ledge adjacent to a window where they will be in full view, exhibiting their characteristic and natural "resting" behavior.

Benthic rays and angel sharks (*Squatina* spp.) require ample areas of sand for resting. Such areas should be located adjacent to windows to optimize viewing of these cryptic species. Many species thrive when they are able to periodically bury themselves. Suitable substrate depths and grain sizes should be researched and incorporated into the exhibit.

Although benthic sharks spend a great deal of time resting on the bottom, care must be taken in the design of the reef structure to allow them ample room for when they are up and swimming. Narrow, dead-end, cul-de-sacs may trap or restrict a large animal or cause it to have difficulty turning around. This design flaw could result in damage to the animals or to the rockwork and corals.

Semi-pelagic

Although rocks and reef structures are appropriate for semi-pelagic species such as lemon sharks and tope (*Galeorhinus galeus*), they must also be provided with open, sandy areas for "resting" periods. These sharks must have adequate space to easily resume swimming without being confined by the close proximity of rockwork.

Care must be taken to provide ample water circulation to those areas where sharks may rest on the bottom. Non-swimming sharks need to actively respire and it is important to maintain an adequate oxygen concentration in all areas of the exhibit. In a multi-species exhibit containing both predators and prey, the habitat can be designed to include areas that physically exclude larger predators thereby providing a safe haven for potential prey species.

Pelagic (non-obligate ram ventilators)

The sand tiger shark will swallow air at the water surface and store it in its stomach to approximate neutral buoyancy (Hussain, 1989). This behavior allows the shark to hang almost motionless in the gentle currents that sweep the rocky gutters of their preferred natural habitat. Sand tiger sharks are able to actively ventilate but can be observed ram ventilating. A suitable exhibit for this species would include a gentle current and long stretches of open sandy areas, between rocky outcrops.

Pelagic (obligate ram ventilators)

Obligate ram ventilators have evolved in a world with few, if any, physical obstructions. For this reason they need considerably more open space

Table 5.1. The four basic categories of elasmobranchs showing representative species of sharks and rays.

Category	Representative shark species	Representative ray species
1. Benthic Sedentary species with low metabolism. Spend majority of time on bottom. Able to actively ventilate.	Bamboo sharks (Hemiscylliidae) Horned sharks (Heterodontidae) Wobbegong sharks (Orectolobidae) Cat sharks (Scyliorhinidae) Angel sharks (Squatinidae)	Stingrays (Dasyatidae) Sawfishes (Pristidae) Guitarfishes (Rhinobatidae) Electric rays (Torpedinidae) Round rays (Urolophidae)
2. Semi-pelagic Free-swimming species. Periodically rest on bottom. Able to actively ventilate.	Smooth-hound (*Mustelus mustelus*) Lemon shark (*Negaprion brevirostris*) Spiny dogfish (*Squalus acanthias*) Whitetip reef shark (*Triaenodon obesus*) Leopard shark (*Triakis semifasciata*)	Spotted eagle ray (*Aetobatus narinari*) Common eagle ray (*Myliobatis aquila*) Cownose ray (*Rhinoptera bonasus*)
3. Pelagic (non-obligate ram ventilator) Regulates buoyancy by swallowing air. Able to hang suspended, almost motionless, in gentle water currents.	Sand tiger shark (*Carcharias taurus*)	
4. Pelagic (obligate ram ventilator) Swims constantly to create hydrodynamic lift, aid respiration, and circulate body fluids.	Blacktip shark (*Carcharhinus limbatus*) Caribbean reef shark (*Carcharhinus perezi*) Sandbar shark (*Carcharhinus plumbeus*) Great white shark (*Carcharodon carcharias*) Sevengill shark (*Notorynchus cepedianus*) Blue shark (*Prionace glauca*) Whale shark (*Rhincodon typus*) Scalloped hammerhead shark (*Sphyrna lewini*)	Giant manta (*Manta birostris*) Devil ray (*Mobula diabola*) Pelagic stingray (*Pteroplatytrygon violacea*)

than benthic or semi-pelagic species and more attention needs to be given to the design of their exhibit. Although ram ventilators need to swim continuously, space requirements vary considerably for each species. For example, the blacktip reef (*Carcharhinus melanopterus*) and bull sharks both inhabit inshore waters with natural obstructions, and as long as there is sufficient space for these sharks to turn and reverse direction they survive well in an exhibit with some rockwork structures. At the opposite extreme are the truly pelagic blue and mako sharks which to date have not adapted to life in aquariums—even aquariums without physical obstructions (i.e., aside from the walls and windows). It is a general rule that bigger is better for pelagic sharks but a compromise always needs to be found between exhibit size and budget. The requirements for pelagic rays such as *Manta* spp. and *Mobula* spp. should be treated in a similar manner (i.e., ample open space with minimal obstructions).

In any exhibit designed for obligate ram ventilators it is wise to leave most of the upper portion of the exhibit open and unobstructed. This gives the sharks a choice of where to swim. At times the sharks may choose to maneuver close to the rocky or coral reef, and at other times cruise the open water above. An artificial reef can be designed to give the sharks several possible swimming routes. Subtle intra- and inter-specific interactions can occur between sharks, and giving the collection multiple swimming routes helps minimize social stress. A large, open area may be especially important for courtship and mating, although to date there is little data to indicate how much space is required by each species.

Considerations for all species

Underwater structures within the tank need to be carefully evaluated from the standpoint of potential interference with swimming patterns. The placement of objects slightly above the water should be carefully examined. Overhanging ledges, ladders, light fixtures, and pipes need to be located well above the height that the dorsal fin or wingtip of a specimen may extend out of the exhibit.

Care should be taken during the initial design of an exhibit to consider the requirements of species that an aquarium might obtain at some time in the future. There are many examples of exhibits that were designed with little or no thought for the needs of species that were later displayed. For example, problems developed in a display at Atlantis, Bahamas when a growing tiger shark began to scrape its dorsal fin on an overhanging walkway located close to the exhibit water surface. Similarly, the design of the Ruins Lagoon at Atlantis did not anticipate the later acquisition of a giant manta (*Manta birostris*). As the giant manta grew it began abrading its wingtips on the exhibit décor when passing through confined parts of the exhibit. The Kaiyukan Aquarium, Osaka, Japan was designed with little or no consideration for the spatial requirements of the whale sharks that were later added to its main display. As the whale sharks grew it became clear that the exhibit was of insufficient dimensions, resulting in the death of the first animal and the eventual release of subsequent specimens.

DESIGNING THE ELASMOBRANCH EXHIBIT

There is practically an unlimited number and variety of options available for the design of elasmobranch exhibits. It is paramount that as the design process evolves, the requirements of the visitor, animals, and staff who will work on the exhibit be kept firmly in mind. The designer must approach the process through the eyes of the visitor. What is it you want the visitor to see and experience as he or she approaches each view into the exhibit? However, the requirements of the animals cannot be ignored. Enthusiasm for a particularly exciting design can easily cause planners to overlook other needs. While husbandry staff, designers, engineers, and marketing personnel all need to participate in the development of an exhibit, the husbandry staff must check each version of the plans to make sure it remains fully compatible with the needs of the animals.

Elasmobranch exhibits have steadily improved over the decades, primarily through a process of trial and error. It is therefore critical that exhibit designers take advantage of the experience of others. Visit other facilities and ask the husbandry staff what has worked and what has not. Is the exhibit effective for the public? Is animal health good and survivability high? Have elasmobranchs reproduced in the exhibit? Such research will result in a far superior exhibit.

There are a number of features that can be incorporated into an aquarium design regardless of the species to be displayed. If the primary focus is an entire ecosystem, a large floor-to-ceiling viewing panel could be the most effective way to visually immerse the viewer in the exhibit and get the educational message across. On the other hand, if the exhibit is to show a wide variety of species and

individuals, as well as several sub-habitats, it may be best to highlight each of these features by designing a number of different viewing experiences. This goal can be achieved by creating variety and complexity in the environment within the tank, and by including a variety of viewing window shapes, sizes, and placements. Tall, low, curved, irregularly shaped, or hemispherical windows can all be used to achieve specific visual effects. Maximum impact and interest will be achieved by carefully planning the view a visitor will see from each type of window.

Examples of possible effects and design features that can be considered include the following:

1. Unexpected views create an element of surprise. For example, an unexciting corridor followed by a corner that suddenly reveals a striking view of the exhibit and animals. This effect was successfully used at SeaWorld San Diego's Shark Encounter. Another surprising effect is to have a shark suddenly appearing on the left, swimming across the window at eye level, and disappearing on the right.

2. Familiar subjects can sometimes be presented in an innovative way. For example, rays can be viewed directly from below (e.g., Ripley's Aquarium of the Smokies, Gatlinburg, USA) or directly from above through walkover glass floor panels (e.g., Colorado's Ocean Journey, Denver, USA).

3. A single exhibit window strategically positioned so a glimpse of it can be seen from a distance is an effective means of moving people in a desired direction. This technique was used effectively in The Dig exhibit at Atlantis.

4. Use window angles to direct visitor views towards or away from features in the exhibit. Monterey Bay Aquarium's Outer Bay exhibit features a window whose upper part slopes towards and over the heads of viewers, focusing their attention upwards and making the bottom inconspicuous or disappear. Conversely, by tilting the head of a window away from the viewer, it is possible to direct attention on what is below and away from the surface.

5. Coordinate known behavior of animals with window placement and tank environment to orchestrate the viewer's experience. For example, it is possible to have a shark or ray swim directly toward the visitor and then turn away at the last moment. Careful placement of acrylic tunnels and half-tunnels can give the viewer a feeling of being inside an exhibit, with sharks directed to swim over or under the visitor.

6. Take advantage of the angle of light refraction through air, acrylic, and water to make drains, pipes, surface skimmers, and other windows invisible to the viewer. It is important to ensure these objects are located within the 45° critical angle (from the plane of the window) so that they disappear from view. Successful aquarium exhibits are like theatre, and obvious drains, pipes, surface skimmers, etc. will detract from the intended experience for the viewer.

7. It is possible to create the illusion of vastness by focusing lights on foreground features, by using appropriate colors for the floors and distant walls, and by strategic placement of reef edges to conceal the far wall. The South Pacific exhibit at the Point Defiance Zoo and Aquarium, Tacoma, USA is a good example of this type of design and lighting regime (Figures 5.6 and 5.7). SeaWorld San Diego's Shark Encounter successfully minimized the visibility of a darkly painted back wall by directing lighting to the foreground of the exhibit.

8. Public access to an open shark exhibit should be carefully controlled if it contains animals that could mistake a visitor's fingers for food.

Prior to final approval of the exhibit design a scale model of the tank should be constructed, complete with viewing windows and simulated habitat. The model should be filled with water, and then studied and photographed from each viewing window.

Growth and development exhibits

An exhibit of developing elasmobranch embryos, along with some newly hatched juveniles, makes an eye-catching and informative exhibit. Depending on species and water temperature, elasmobranch embryos can remain within their egg case for 6-24 months before hatching. Highly effective exhibits have been created at the Monterey Bay Aquarium by backlighting the semi-transparent egg cases of the swellshark (*Cephaloscyllium ventriosum*). The big skate (*Raja binoculata*) produces a large, 20-30 cm egg case containing three to seven embryos. A striking exhibit of their eight-month development was created at the Monterey Bay Aquarium by cutting away a section of the egg case and carefully attaching a clear acrylic window with cyanoacrylate

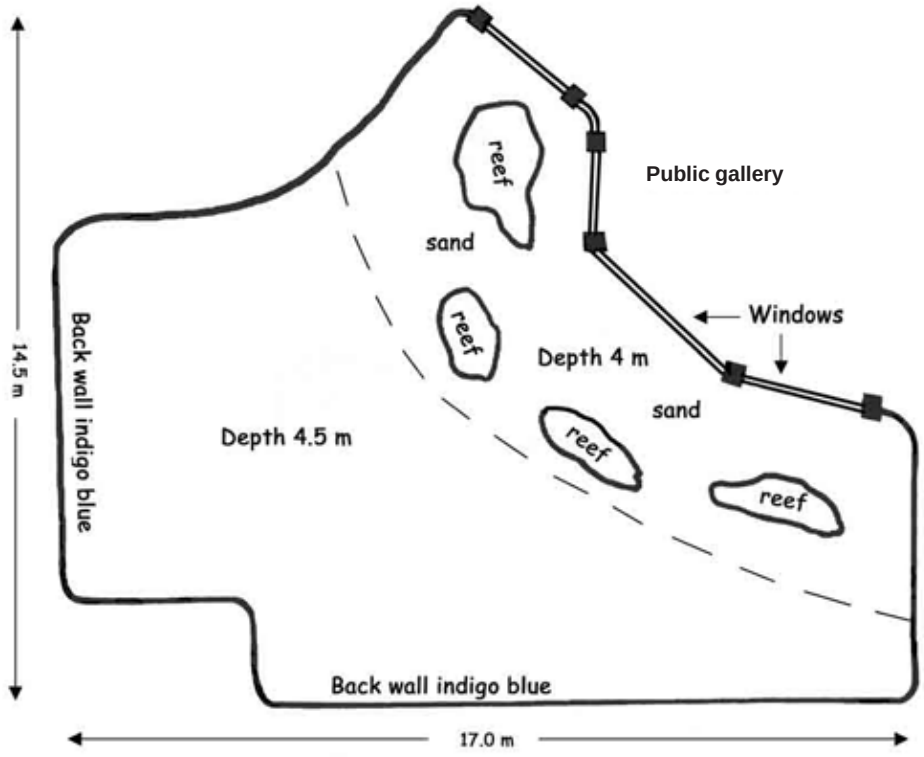


Figure 5.6. Plan view of the South Pacific Ocean exhibit, an innovative design with well-illuminated reefs and sandy flats in the foreground promoting the illusion of limitless water beyond: Point Defiance Zoo and Aquarium, Tacoma, Washington, USA.

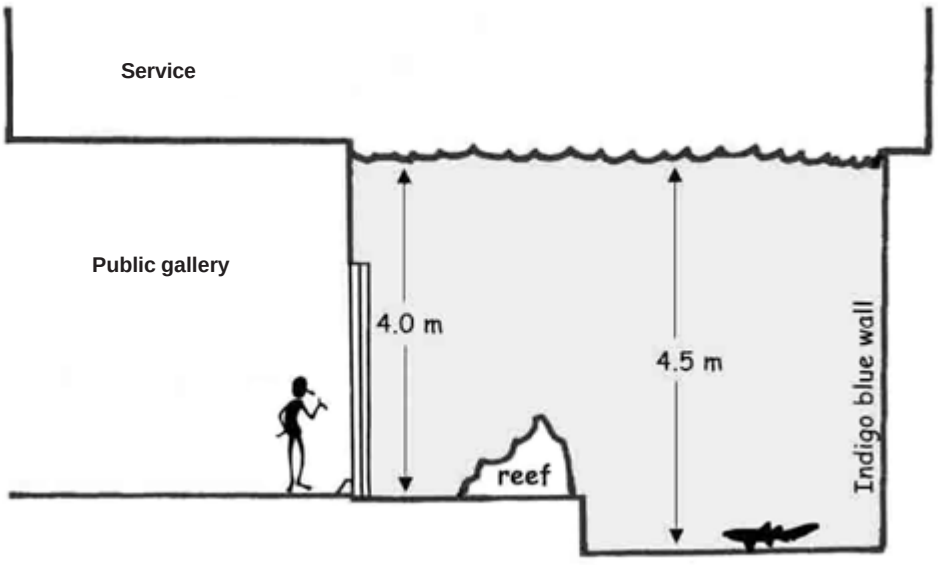


Figure 5.7. Elevation view of the South Pacific Ocean exhibit showing the reef drop-off that helps create the illusion of limitless water beyond: Point Defiance Zoo and Aquarium, Tacoma, Washington, USA.

glue (e.g., Zap CA, Recon Products Corporation, USA). Other elasmobranch species produce egg cases suitable for such exhibit techniques (West and Carter, 1990; Croft, 1997).

Interactive exhibits

Interactive elasmobranch exhibits are becoming increasingly popular. Most concerns associated with these exhibits are husbandry related, but a few other design considerations need to be addressed. The size of interactive elasmobranch exhibits needs to be matched to an accurate projection of visitor use, with ample space provided for the animals, visitors, and husbandry staff. A good example of a successful interactive exhibit is Discovery Cove of SeaWorld Orlando, USA.

Interactive exhibits that involve visitors wading, snorkeling, or scuba diving with the animals should have safe entries and exits—a sloping sandy beach has proven to be successful. Adequate changing rooms, showers, dive equipment sanitation facilities, and medical care staff should all be provided. It is important to ensure that hygiene protocols and water treatment systems are designed to minimize toxic chemicals that may be introduced by the visitor (e.g., sunscreen, etc.), and conversely, to protect the visitor from microbiological contaminants in the water.

For outdoor pools it is important to install a sufficient number of surface skimmers, in appropriate locations, to handle surface debris blown in by the vagaries of the wind. The pool perimeter needs to be protected from dirt, fertilizer, and pesticide runoff from surrounding landscaping and walkways.

MATERIALS AND CONSTRUCTION TECHNIQUES

Construction of the tank

Concrete is highly resistant to compression stress, but when not supported it lacks tensile strength and is susceptible to shear stress. This problem is normally overcome by imbedding a network of steel wire or bars inside the concrete, referred to as reinforcing bar or “re-bar”. Reinforcing bar is normally placed near the outer skin of the supportive structure to more effectively withstand applied loads. In general, up to 4% (by volume) of steel reinforcing bar can be added to a concrete structure to improve its structural integrity. The use of ferrous materials in the construction of a structure to hold water immediately presents the risk of corrosion. Under normal circumstances, steel reinforcing bar is corrosion-passivated by the high alkalinity of the surrounding concrete. However, any breach in the integrity of the concrete (e.g., micro-fractures, misaligned sutures or cold joints, unformed or dissolved aggregate matrices, etc.) will increase concrete permeability and allow the intrusion of corrosive agents such as chloride ions (Cl^-) (Hawkins and Lloyd, 1981). Should the concentration of chloride ions adjacent to reinforcing bar increase from 0.15% (recommended for new concrete) to 0.40% (by weight), corrosion will be accelerated by a process known as the Lorenz reaction (Christiansen and Yglesias, 1993). If concrete permeability increases to the point where both raw seawater and oxygen contact the reinforcing bar, a corrosive electro-chemical cell will be established between the exposed steel and the deeper passivated metal causing it to rapidly rust. As reinforcing bar rusts, the overlaying concrete becomes stained and then eventually splits as the increasing volume of ferrous oxide forces the concrete apart—a process known as spalling. Eventually, the corrosive process becomes self-perpetuating. Water is drawn between the reinforcing and the concrete by capillary action, and continues to corrode the steel and “explode” the concrete. The reinforcing bar is subsequently exposed to more water and oxygen which drives the corrosion reaction forward (Hawkins and Lloyd, 1981).

Aside from the obvious aesthetic problems of red oxide stains appearing on the walls of the aquarium, and colloids floating in the water, the ramifications of reinforcing bar corrosion are far-reaching. If left untreated, the destruction of the aquarium structure by spalling will eventually require extensive repair. Unfortunately, this process frequently demands the closure of a facility and an associated loss in time, resources, revenue, and public image. In many cases there is no way to adequately repair concrete tanks without first removing the animals. Aquariums rarely have off-exhibit holding facilities capable of accommodating even a small percentage of the animals in their major exhibits. Many sharks grow to a large size and their removal from exhibition and confinement in temporary holding, in order to perform tank repairs, can result in the loss of valuable specimens. It is therefore extremely important to construct the tank correctly at the outset, following the highest standards for marine aquariums.

It is possible to inhibit the initiation and progress of reinforcing bar corrosion in marine aquariums, by adopting the following practices:

1. Use of non-metallic reinforcing material (e.g., carbon fiber, glass fiber, polymer rods, Kevlar, etc.). Be aware that these materials can sometimes lack the required strength and can be prohibitively expensive. As such, the use of these materials in aquariums is generally limited to specialized applications.

2. Protection of steel reinforcing bar by coating with an epoxy resin or similar compound.

3. Protection of steel reinforcing bar by burying it deeper within the concrete structure. A layer of at least 75 mm of high density concrete must lie between the reinforcing bar and the surface. Some aquariums have purportedly used concrete skins as thick as 100 mm. It is important that a low shrinkage index is specified to minimize cracking.

4. Use of high density concrete. The water to cement ratio should never exceed 0.5 (by weight). The amount of cement within the mix should not be less than 350 kg m^{-3}. This precaution ensures the availability of adequate binder to help fill any voids between the aggregates and facilitates the formation of a tight matrix. This process will be enhanced by the use of fine additives (e.g., Fly ash type F).

5. Careful construction techniques. Vibration of the concrete during pouring will produce homogeneous slurry that is free of voids. Care must be taken to ensure that fine aggregates are not lost out of the formwork. Finally, if at all possible, the concrete should be poured in a single operation to avoid misaligned sutures or cold joints.

6. Employ a qualified, client-paid inspector to monitor every step of concrete fabrication (i.e., form design and construction, reinforcing installation, and concrete pouring, curing, and waterproofing).

Waterproofing

The final defense against reinforcing corrosion and leakages is the use of an impermeable membrane or “waterproofing”. Epoxy resins, polyester resins, butyl-rubber composites, multi-layer polymers, granulated desiccants, plastic liners, and other assorted membranes have all been employed in aquariums with varying degrees of success. Only through the use of a completely impermeable membrane, can we rest in the knowledge that the integrity of the aquarium structure will remain intact. Plastic liners such as those used in aquaculture facilities should only be considered if the entire liner is accessible should repairs become necessary. A plastic liner located beneath large, artificial rockwork structures should be avoided at all costs.

Construction of the habitat

One of the first design considerations, following the selection of exhibit inhabitants, is the decision to use living or simulated décor. The obvious appeal of living coral or living kelp must be weighed against their availability, water quality requirements, and difficulty of culture.

The goal of most modern aquariums is to present a realistic environment to the viewing public. The ultimate realization of this philosophy is the creation of a totally living habitat. Such exhibits may take years to mature, an option not open to new aquariums. There is, however, a way to gradually convert artificial kelp and coral exhibits to natural living habitats if plans are made during the design process. The reef environment is created using realistic, removable artificial corals or plants. Lighting, water movement devices, and water treatment systems should be designed at the outset to meet the needs of the fishes as well as the developing living corals or kelp. During construction an off-exhibit coral culture facility is built where corals can be grown under the same light and water flow conditions that will exist in the main exhibit. As the corals become large enough for display the artificial corals are gradually replaced with their live counterparts. It takes time, patience, and diligent husbandry, but the end result is far superior to an artificial environment. Living exhibits represent an ideal opportunity to communicate the complexity and interrelationships of the many inhabitants of intricate ecosystems.

Whether live or artificial décor is used, plants or corals must be attached to a solid substrate base. Common materials for the substrate include solid concrete, hollow concrete, and GFRC (glass fiber reinforced concrete) or FRP (glass fiber reinforced polymer resin) panels.

Solid concrete rockwork

Solid concrete rockwork makes up the bulk of underwater rock in most large elasmobranch displays. This rockwork consists of a base of hollow concrete blocks, a PVC (poly-vinyl chloride) armature framework and plastic netting, and a solid fill of

Figure 5.8. Construction of artificial rockwork armature using PVC piping, prior to attachment of plastic mesh: Oceanário de Lisboa, Lisbon, Portugal (Photograph courtesy of the David L. Manwarren Corp.).

concrete. This relatively simple, solid structure prevents the formation of stagnant water pockets between the rockwork and the tank wall or floor. The concrete blocks are laid out on the tank floor to form an outline of the rock footprint. These blocks have 30-cm-long studs of two centimeter diameter PVC pipe protruding from them. A PVC pipe framework is shaped, using heat guns, and assembled and attached to the studs making up the overall rock shape. All piping is connected and secured with glued PVC fittings or plastic cable ties. The finished piping framework is then covered with a polypropylene plastic netting (e.g., Naltex®, Delstar Technologies Incorporated, USA) secured with cable ties (Figure 5.8). A one-centimeter layer of cement is then sprayed over the framework and netting which creates a sturdy shell. The entire rock framework is then filled with concrete in stages, at a rate of about half a meter per day. When filling is complete, a 10-20 cm layer of concrete is sprayed over the entire structure. At this point, the surface of the rockwork can be textured by hand to resemble true rock. Texturing can be achieved by the application of latex molds taken from real rock in situ.

Once set, the alkaline character of new concrete needs to be neutralized with a weak acid. This curing process is especially important in closed or semi-closed systems where leaching may modify the pH of the system and affect the health of the animals and plants. Following construction, the exhibit should be filled with water and then muriatic acid or HCl added to lower the pH to 4.0. Filling the exhibit with freshwater is preferred as it has a lower buffering capacity and less acid will be required. The pH should be tested daily and as it rises, more acid should be added to bring it back down to 4.0. The leaching process can be considered sufficiently complete when the pH no longer increases. The tank can now be drained and the rockwork thoroughly pressure-washed to remove any loose surface debris.

Hollow concrete rockwork

A drawback to solid concrete rockwork is the weight. While not as strong, hollow concrete rockwork is considerably lighter and is desirable where the total weight of an exhibit may present a structural problem. Overhanging ledges and arches can be structurally supported by means of FRP I-beams or 20 cm diameter PVC pipes filled with concrete. While similar in construction to solid concrete rockwork, hollow concrete rockwork needs to be at least 10-20 cm thick, over the PVC and mesh armature, to ensure proper long-term strength. Wet concrete is added to the framework in layers and a permanent solid shell is formed. The final layer can be textured to resemble real rock.

Fiber-reinforced rockwork panels

FRP and GFRC rockwork panels are often used when the weight of solid concrete presents a structural problem. Although they can be fabricated in-house, custom made panels are available from exhibit supply companies. Both types of panels can have artificial coral or plants attached to them. FRP simulated rock panels have the advantage of being quite light in weight. Often referred to as tank “inserts,” these plastic panel sections have become common for backdrop and base rock in small- to medium-sized aquarium displays.

GFRC panels can be used to provide a quick finish layer over solid concrete structures, avoiding detailed sculpting or embossing with latex hand molds. These panels are normally less than seven centimeters thick and can be formed off-site and transported to the exhibit, an important consideration when construction time inside the display is limited. GFRC panels are often used where the strength of reinforced concrete is needed to support the weight of diver entry platforms, caves, and overhanging ledges. These panels are heavier than FRP but can support considerable weight. It is especially important that no steel wire or mesh be used in the

manufacture of underwater GFRC panels. A disadvantage of GFRC panels is their higher cost when compared to either solid or hollow concrete rockwork. Another disadvantage of using GFRC and FRP is the potential for water stagnation in "pockets" between the panels and the tank walls or floor. In these cases it is necessary to include ventilation screens and supply inlets to introduce oxygenated water into the spaces behind the panels.

Artificial decoration

Acid stains may be used to create dark areas in rock cavities and crevices. However, some acid stains may contain metals that are toxic to marine life (e.g., chromium). Most artificial corals produced for attachment to prefabricated panels and solid concrete structures are cast from polyurethane. The more recently developed urethanes allow for the fabrication of flexible stony corals, soft corals, sea fans, and gorgonians. These durable pieces resist collision breakage from large sharks or clumsy divers, and their flexibility adds to the realism of an exhibit if placed in areas of strong current or surge. Plastic corals are usually attached to rockwork with plastic pegs. By casting plastic all-thread pegs in the coral piece during manufacture a secure mounting device can be achieved. Plastic corals are attached by drilling into the concrete with pneumatic drills and filling the holes with epoxy to hold the mounting pegs. If removable coral pieces are to be used in the exhibit, effective mounting pegs can be made using rods of PVC or other plastic material. This process is then followed by the application of acrylic paints to create simulated coralline algae, sponges, and tunicates. Following a thorough rinse, live corals may be attached with underwater epoxy.

OTHER IMPORTANT CONSIDERATIONS

Electro-magnetic fields

Located around the front of a shark's head are bio-electrical sensors known as the ampullae of Lorenzini. These sensory pores detect extremely faint electric fields (i.e., an electrical potential as weak as 0.01 µV) produced by other living creatures and generated by the earth's magnetic field (Kalmijn, 2000). Electro-sensitivity performs a valuable function for sharks and rays in the wild, but it can become a problem in the artificial environment of an aquarium. The ampullae will detect a wide variety of electric fields that may be present in an aquarium but remain unknown to husbandry staff. These extraneous electric fields may interfere with the normal sensory system of the elasmobranch and affect their natural behavior.

Possible sources of electric fields include:

1. Electrolysis between dissimilar metals within the tank (e.g., sacrificial or impressed current anodes installed to protect metal filters, etc. from electrolysis).

2. Electrolysis produced by the corrosion of reinforcing steel or wire ties within the concrete tank structure.

3. Electrical equipment (e.g., pumps, etc.) located adjacent to the tank.

4. High amperage electric panels located near the tank.

5. Electrical conduit, cables, or light fixtures mounted in or on the outside wall of the tank.

Although it is known that sharks and rays will detect a variety of electric fields within an aquarium, it is not known quantitatively how they affect the animals (McCosker, 1999). In many cases animals acclimatize to a wide range of continuous stimuli (e.g., light, sound, etc.) and it is likely that elasmobranchs would adjust to constant electric fields (Kalmijn, pers. com.). However, it is prudent to eliminate or minimize all sources of electrical interference during the design and construction of a new aquarium. Eliminating electric fields is especially important if behavioral studies are planned. With these concerns in mind, Kalmijn (pers. com.) suggested the following design criteria prior to the construction of the Outer Bay exhibit at the Monterey Bay Aquarium:

1. Keep all electrical panels and pumps as far away from the tank as possible.

2. Avoid all forms of metal within the tank.

3. Keep all non-essential electrical conduits away from the outside wall of the tank.

4. If illuminated graphic panels must be attached to the outside wall of the tank, the wires should be tightly twisted within the plastic conduit. Twisting the wires cancels out the electro-magnetic field generated around the wires.

5. Avoid all underwater light fixtures and electrical cables.

6. Ideally, all reinforcing steel should be in electrical contact (i.e., welded together) and grounded prior to the pouring of structural concrete.

Lighting

Elasmobranchs seem to have no special requirements with regard to the spectral quality of light. Most sharks, with the exception of some deep-sea species, (e.g., the bluntnose sixgill shark, *Hexanchus griseus*, and the filetail catshark, *Parmaturus xaniurus*) appear to accept a broad range of light intensity. In the mornings and evenings lighting intensity should be increased and decreased through a series of stages to simulate dawn and dusk. Sudden changes in intensity from full illumination to total darkness or vice versa should be avoided as it will startle specimens.

A low intensity night-light should be installed above multi-species exhibits as this will help reduce nocturnal predation of teleosts or other sharks by larger sharks. This light should have an emergency power supply to prevent complete darkness during power interruptions.

Water inlets and outlets

Adequate overall water exchange must be sustained to ensure suitable water quality in all parts of an exhibit. Water inlets and outlets should be positioned to maximize the efficiency of water treatment systems and yet not conflict with the behavior of the animals. It is possible to optimize the display of benthic sharks (e.g., nurse sharks, etc.) by placing a controllable, concealed water inlet in a cave or beneath an overhanging ledge adjacent to a window whose height provides eye-to-eye viewing. The flow of water will induce sharks to use these areas, in full view of the public. Surface skimmers and bottom drains need to be well screened and large enough to prevent the trapping of rays.

Introduction and isolation pools

Any exhibit that displays large elasmobranchs should ideally have an introduction pool attached to the tank. This pool allows for the safe introduction and removal of sharks and can be used for the temporary isolation of pregnant, newborn, or injured animals. Usually this pool is open to the main exhibit but separated by a mesh gate when in use. However for medical treatments requiring the use of chemicals, the gate should be watertight and the pool plumbed to allow for variable water depth. Some additional suggested design criteria for the introduction and isolation pool include:

1. Pool dimensions should be three times the total length (TL) of the largest specimen held and at least 3 m long x 3 m wide (10 m long x 3 m wide for sand tiger sharks and Carcharhinidae).

2. Pool water depth should be 0.5-1.0 m to allow a safe working level for staff and sufficient water for swimming sharks.

3. The pool should have curved or 45° angle (to the plane of the wall) corners to accommodate shark swimming patterns.

Service areas

Some suggested design criteria for exhibit service areas include:

1. Steps inside the tank around the perimeter and recessed into the wall for quick entry and exit of staff.

2. A minimum workspace of one meter around the outside of the pool, with one side adjacent to an open service deck and the exterior of the building (Note: regulations in the USA require a minimum of 1.22 m workspace around a pool unless railings are included).

3. Facilities for the application of medicated baths, with appropriate space, drainage, etc.

4. An overhead crane rail stretching from truck access outside the building to an area above the exhibit and/or introduction pool. (The overhead rail will facilitate the addition and removal of larger animals—allow ample clearance from the bottom of the overhead rail to the deck and pool edge to accommodate animal containers and support cables.)

5. Ceiling height that is sufficient to allow for handling of long pole nets, etc. (For exhibits displaying large animals the roof may need to open to allow specimen removal by means of a large crane or helicopter.)

6. A gantry spanning the exhibit for deploying a net to isolate sharks in one part of the exhibit.

(This process eases exhibit cleaning and servicing, and facilitates specimen capture.)

7. Minimizing noise from pumps and blowers so that effective communication in the service area is possible during net deployment, maintenance, etc.

8. Removable safety railings in the exhibit service area, facilitating net deployment, moving animals, etc.

REFERENCES

Christiansen, J., and Yglesias, L. 1993. Concrete in marine aquariums: an analysis of concrete at the Monterey Bay Aquarium. Boston: Proceedings of the 3rd International Aquarium Congress, 177-182.

Croft, G. S. 1997. Maintenance of pre-term embryos of the lesser dogfish, *Scyliorhinus canicula*, in artificial egg cases. Drum & Croaker 28: 11-12.

Gray, W. B. 1960. Creatures of the Sea. New York: Wilfred Funk Inc., USA. 216 pp.

Hawkins, A. D., and Lloyd, R. 1981. Materials for Aquariums. In 'Aquarium Systems' (Eds. A. D. Hawkins) New York: Academic Press, USA, 171-196pp.

Hussain, S. M. 1989. Buoyancy mechanism, and density of sand tiger shark *Eugomphodus taurus*. Indian Journal of Fisheries 36: 266-268.

Kalmijn, A. D. 2000. The Detection and Processing of Electromagnetic and Near Field Acoustic Signals in Elasmobranch Fishes. Philosophical Transactions of the Royal Society 355: 1135-1141.

Klay, G. 1977. Shark dynamics and aquarium design. Drum and Croaker 17: 29-32.

McCosker, J. E. 1999. The history of Steinhart Aquarium: A very fishy tale. Virginia: The Donning Company, USA. 158 pp.

Phillips, C. 1964. The Captive Sea. Philadelphia: Chilton Books, 284 pp.

Powell, D. C. 1968. An experimental tank for pelagic sharks. Drum & Croaker 13: 25-26.

Weihs, D. K., Keyes, R. S., and Stalls, D. 1981. Voluntary swimming speeds of two species of large carcharhinid sharks. Copeia 1981(1): 219-222.

West, J. G. and Carter, S. 1990. Observations on the development and growth of the epaulette shark *Hemiscyllium ocellatum* (Bonnaterre) in captivity. Journal of Aquariculture and Aquatic Sciences 5: 111-117.

Whitley, G. P. 1940. The Fishes of Australia (Part 1): The Sharks, Devil-fish and other Primitive Fishes of Australia and New Zealand. Sydney: Royal Zoological Society of New South Wales, Australia, 50-51 pp.

PERSONAL COMMUNICATIONS

Kalmijn, A. D. 1995. Scripps Institution of Oceanography, La Jolla, CA 92093, USA.

The Elasmobranch Husbandry Manual: Captive Care of Sharks, Rays and their Relatives, pages 69-88.
© 2004 Ohio Biological Survey

Chapter 6
Water Quality and Life Support Systems for Large Elasmobranch Exhibits

PETER J. MOHAN

5802 Thorndale Drive
Kent, OH 44240, USA.
E-mail: petemohan@aol.com

ANDREW AIKEN

National Aquarium In Baltimore
501 E. Pratt St.
Baltimore, MD 21202, USA.
E-mail: aaiken@aqua.org

Abstract: High water quality and effective water treatment are an essential part of any successful husbandry program for elasmobranchs. Water sources, salt constituents, and contaminants can all contribute to problems encountered during the mixing of artificial seawaters, preparation of natural seawater, system start-up for elasmobranch LSSs, and daily LSS operation. Critical water quality concerns such as dissolved oxygen concentrations and gas supersaturation must be understood so that potential disasters are considered and prevented during LSS design. LSS objectives such as particulate removal, dissolved organic removal, biological filtration, chemical filtration, and sterilization are essential for optimum water quality and a variety of system designs accommodate these demands. Rapid sand filters, foam fractionators, algal turf scrubbers, biological filters, ozone contact systems, and degassing systems possess overlapping attributes, and various combinations of these LSS elements will achieve effective water treatment. While system designers frequently disagree about how these elements should be arranged, and which elements provide the best result, all concur that effective systems should provide acceptable water clarity, biological filtration, removal of dissolved organics, minimization of bacterial pathogens, and effective gas balance.

High water quality and effective water treatment techniques are an essential part of any successful husbandry program for elasmobranchs. If water quality is high many possible husbandry challenges are eased. It is therefore incumbent upon elasmobranch husbandry personnel to strive for optimal water conditions.

This chapter has been divided into three sections. The first section reviews general water quality issues as they pertain to elasmobranchs. The second section gives an overview of generic LSS (life support system) components. The third section describes different LSS philosophies used for elasmobranch exhibits resulting in diverse arrangements of the components described in section two.

WATER QUALITY

Water quality parameters for elasmobranch systems do not differ significantly from those considered desirable for bony fishes. Maintaining water quality parameters for elasmobranchs is generally more challenging because large volumes of water must be processed. With notable exceptions, cartilaginous fishes are sensitive to therapeutic copper, organophosphates, and low salinity. A thorough review of general water quality issues can be found in Spotte (1992). Suggested water quality parameter limits for elasmobranch exhibits have been summarized in Table 6.1.

Table 6.1. Recommended basic water parameter limits for elasmobranch exhibits. Where possible water parameters, in particular temperature, should be restricted to natural ranges encountered by wild conspecifics. Major elements (i.e., Na, Cl, Mg, K, SO_4, and Ca) should be maintained at levels ±15% of natural values. The ORP range given is suitable if other parameters that affect ORP (e.g., total residual oxidants, ozone concentrations, etc.) are within safe limits. The value quoted for nitrate should be considered an operational goal rather than an absolute.

Parameter	Range	Units
Salinity	25.0-35.0	g l^{-1} (ppt)
pH	8.0-8.4	
DO (dissolved oxygen)	85-98	% saturation
Turbidity	<0.15	NTU
ORP	250-380	mV
Ammonia, unionized (at 10°C)	<0.1	mg l^{-1}
Ammonia, unionized (at 17°C)	<0.2	mg l^{-1}
Ammonia, unionized (at 28°C)	<0.3	mg l^{-1}
Nitrites	<0.1	mg l^{-1}
Nitrate (as nitrate nitrogen)	<70.0	mg l^{-1}
Total coliform	<1000	ufc 100 ml^{-1}
Copper	<0.01	mg l^{-1}
Zinc	<0.01	mg l^{-1}
Nickel	<0.01	mg l^{-1}
Iron	<0.03	mg l^{-1}

Temperature

The water quality parameters of an animal's natural habitat should be used to determine temperature limits for a display. Typically, non-biologist exhibit designers create specimen "wish lists" based on physical appearances rather than water quality and husbandry requirements. It is unwise to mix animals having a purely temperate distribution with those that require high tropical temperatures. For example, keeping zebra sharks (*Stegostoma fasciatum*) and leopard sharks (*Triakis semifasciata*) in the same exhibit is a risky long-term arrangement (geographic confusion aside) because overlap in their natural temperature preferences is minimal at best. The health of one or both species will be compromised.

Nitrogenous wastes

Ammonia (NH_3/NH_4^+) and nitrite (NO_2^-) concentrations considered safe for fishes are quite low and are often derived from studies based on salmonids, fishes particularly sensitive to these compounds. Most marine fishes, including sharks, tolerate somewhat higher concentrations of these nitrogen compounds. Regardless, there is no logical reason to expose elasmobranchs to water parameters more extreme than those considered safe for salmonids and other teleosts.

Project managers must allow sufficient time for biofilter stabilization and a subsequent gradual stocking period when developing time lines for opening new exhibits. Use of biofilter seeding techniques and a 4-5 week maturation period will allow workers to avoid the accumulation of dangerous levels of ammonia and nitrite. Since nitrite levels will often increase near the end of biofilter maturation, this waste product must be monitored closely.

We recommend nitrite levels $\leq$ 0.10 mg l^{-1} (=ppm). Properly designed and operated LSSs will rarely allow nitrite to exceed this value. Based on

observations by one of the authors (Mohan) short-term (i.e., 3-5 days) exposure to nitrite levels as high as 0.50 mg l^{-1} seems to be relatively safe for a number of species (e.g., California bat rays, *Myliobatis californica*; sand tiger sharks, *Carcharias taurus*; sandbar sharks, *Carcharhinus plumbeus*; lemon sharks, *Negaprion brevirostris*; blacktip reef sharks, *Carcharhinus melanopterus*; and whitetip reef sharks, *Triaenodon obesus*). Nitrite reacts with ozone almost immediately and is unlikely to be of great concern in ozonated systems. However, attempts to compensate for poor nitrification in new systems by increasing ozone dosing may lead to disaster once nitrite is depleted and excess ozone enters the exhibit (Johnson, 2001).

Alkalinity and pH

Alkalinity and pH tend to be low in systems that have high animal loads or poor facilities for the removal of organics. Sodium bicarbonate ($NaHCO_3$) and sodium carbonate (Na_2CO_3), or soda ash, can be used to make adjustments in systems where pH and alkalinity are already relatively stable. Some facilities experiencing chronically low pH use dosing pumps to inject sodium hydroxide (NaOH), or caustic soda, on a continuous basis. These chemicals represent a temporary solution, requiring constant monitoring and dosing, as they modify pH but do not act as true buffers. Systems challenged by decreasing pH may benefit from the addition of foam fractionation and better gas balance facilities. Foam fractionation and improved gas balance enabled SeaWorld Ohio, USA to maintain a pH of 8.0 in a 1,500 m^3 system. The system contained 25 large sharks, two adult sawfish, two sea turtles, and several hundred teleosts. Additions of sodium carbonate were required for this system, although they were infrequent (11.5-23 kg $month^{-1}$). In general, a pH range of 8.0-8.4 is recommended for elasmobranch exhibits.

Artificial seawater and salinity

It can be economical to make artificial seawater from scratch, but only if large quantities are needed. The reagent and analytical grade chemicals used to provide trace elements require an initial outlay of funds, but some of these small quantities will last for many years. Bulk quantities of major salts such as food grade sodium chloride, magnesium sulfate, magnesium chloride, calcium chloride, and potassium chloride are readily available.

Over the years the number of trace element components used in artificial seawater formulas has dwindled. Nickel, vanadium, titanium, rubidium, iron, boron, and other elements formerly added to general-use artificial seawater are now often omitted. Many of these elements are already present as contaminants in the major ingredients of artificial seawater. Infinitesimal quantities are probably present in tap water, or may enter systems via food.

Many facilities tailor their institutional salt formulas to meet special requirements. For example, phosphate (HPO_4^{2-}) need not be added to established aquarium systems because it typically accumulates to unnaturally high levels through input via food. However, low levels are thought to be required by nitrifying bacteria during biofilter maturation and should always be included when an exhibit is initially filled.

Many aquariums have stopped adding bromide to their artificial seawater mixes because it reacts during aggressive ozonation (i.e., when ORP is >400 mV) to form long-term residual oxidants that are harmful to fishes. Although this is one of the most abundant minor elements, it does not appear to be required by marine fishes at the levels present in natural seawater.

The expected composition of seawater in a closed system can change as a result of interactions with LSS components or exhibit decoration. When a standard artificial seawater formula is mixed using hard tap water, the resulting calcium and magnesium levels may be higher than desired or found in natural seawaters. Institutional salt formulas can be easily adjusted to include less calcium and magnesium. Periodic testing of the artificial seawater in established systems may indicate an accumulation or depletion of some elements through salt contamination, product variation, mixing errors, biological activity, or the composition of local tap water. Special batches of artificial seawater tailored to correct these imbalances can be formulated and used during subsequent water changes.

The leaching of un-reacted construction materials can affect the composition of artificial seawater. Concrete rockwork is known to leach calcium into the water, while simultaneously causing the precipitation of both calcium and magnesium (Grguric et al., 1999). This precipitation is typically easy to correct, through minor adjustments to artificial seawater, if major seawater elements are checked periodically. Severe imbalances can

occur if concrete mixes are used that are incompatible with seawater. Despite assurances from the manufacturer that a product was suitable for marine applications, SeaWorld (Busch Entertainment Corporation, St. Louis, USA) discovered that one commercial concrete mix rapidly crumbled when exposed to seawater. One of the authors (Mohan) observed that calcium concentrations in system water increased to several times their normal value (i.e., >1,000 mg l^{-1}) during experimental trials with the concrete.

Contaminated raw materials can sometimes cause problems in artificial seawater mixes. Unexpectedly high levels of manganese, nickel, ammonia, or cyanide compounds have been detected in low quality components supplied to aquariums. This problem can occur if: low-grade materials are purchased in error because of lack of research, orders are substituted by purchasing agents attempting to save money, or shippers accidentally send the wrong material. The use of specific raw materials, whose safety record has been proven by other aquariums, is a wise precaution.

While salinity tolerances for elasmobranchs are not well documented, examples of broad euryhaline activity in wild sharks have been observed. The bull shark (*Carcharhinus leucas*) is found at salinities of 0-53 g l^{-1} (=ppt), while blacktip reef sharks have been found in brackish estuaries and freshwater lakes (Compagno, 1984). Both sandbar sharks and blacktip sharks (*Carcharhinus limbatus*) are common at the entrance of rivers, where they experience lower salinities, but neither species is known to ascend rivers into freshwater (Compagno, 1984). Merson and Pratt (2001) routinely collected young sandbar sharks in Delaware Bay at salinities as low as 22.8 g l^{-1}. Similarly, cownose rays (*Rhinoptera bonasus*) have been found in lower salinity waters of the Chesapeake Bay. The species mentioned above can be maintained long-term at salinities of >25-26 g l^{-1} (Thoney, pers. com.).

While freshwater dips have been routinely used for parasite removal on a variety of elasmobranchs (Henningsen, pers. com.), the New England Aquarium, Boston, USA, reported that yellow stingrays (*Urobatis jamaicensis*) and bamboo sharks (*Chiloscyllium* spp.) were extremely intolerant of freshwater; although it has been observed that the latter can be kept safely at 15 g l^{-1} for extended periods (Bailey, pers. com.).

The Aquarium of the Pacific, Long Beach, USA, successfully acclimated sandbar sharks, blacktip reef sharks, whitetip reef sharks, and zebra sharks to salinities as low as 14 g l^{-1} (Clarkson, pers. com.). Most sharks exhibited a reduced interest in food during a slow acclimation over a one-week period. After one month, salinities were increased rapidly to normal levels. Two mortalities were observed; a juvenile sandbar shark and an adult whitetip reef shark. These unexpected deaths could have resulted from the rapid change in salinity, or the combined effects of the rapid change and long-term maintenance at a lower salinity.

Low salinities are not tolerated well by sand tiger sharks for extended periods of time. During aquarium startup, Moody Gardens, Galveston, USA, lowered shark exhibit salinity to 15 g l^{-1} for 1.5 months and further reduced it to 11 g l^{-1} for one week. Behavioral side-effects, including inappetence, lethargy, changes in ventilation, and a tendency to remain near the pool bottom indicated that long-term brackish conditions were unsuitable for sand tiger sharks. These signs disappeared when salt concentration was raised to >22 g l^{-1} (Zoller, pers. com.). Introduction of freshly transported specimens to low salinity conditions appeared to be contraindicated. Sand tiger sharks are often collected at 25 g l^{-1} in the Delaware Bay and routinely displayed in the same salinity at the National Aquarium in Baltimore (USA) and other facilities (Henningsen, pers. com.). Every attempt should be made to maintain elasmobranchs within the salinity ranges at which they naturally occur.

Gas balance

The proportions of dissolved gases in exhibit water should approach normal atmospheric values. Respiration and photosynthesis within a LSS and display may impact oxygen (O_2) and carbon dioxide (CO_2) concentrations as well as their ratio to one another.

Dissolved oxygen

Daily testing of dissolved oxygen (DO) levels is recommended for all systems. Under-oxygenation can occur, for a variety of reasons, in systems that otherwise appear to function normally. Where ozone contact chambers or degas towers are the primary sources of aeration, plugged or defective diffuser systems or venturis (=eductors) can lower oxygen concentrations. Reduced flow through these towers, as a result of a faulty or a stopped pump, can lower oxygen concentrations. Where

foam fractionators are an important source of aeration, pump or venturi failure can cause similar problems. This risk is reduced if multiple foam fractionators are used. Employing a biotower (i.e., a wet-dry trickle biofilter) as the primary source of aeration can reduce the risk of under-oxygenation. When water flowing through the biotower is mixed with ambient air, a constant source of oxygen and nitrogen is supplied at atmospheric pressures, excess carbon dioxide from fish and bacterial respiration exits the system, and gas levels in the water can return to natural atmospheric partial pressures.

Some species tolerate low oxygen better than others. For example, the sand tiger shark is more tolerant of low oxygen concentrations than the sandbar shark. At many facilities (e.g., SeaWorld Ohio) oxygen saturation levels are maintained at 85-95%. Some facilities choose to maintain oxygen saturation levels >100% (i.e., oxygen supersaturation only; nitrogen saturation must be maintained at, or below, atmospheric concentrations). These levels can only be achieved through heavy ozonation or oxygen enrichment.

Supersaturation

Pressurized seawater can hold more dissolved gases than seawater at one atmosphere. When pressure is removed (e.g., as water moves from a pump into an exhibit), these gases become supersaturated and will gradually come out of solution. As supersaturated gases come out of solution in the blood-stream of fishes it causes emboli. The resulting condition, called gas bubble disease or the bends, causes tissue damage, organ failure, or death. Theoretically, oxygen and carbon dioxide can be supersaturated without harming fishes. However, emboli will develop when the sum of the partial pressures of all dissolved gases exceeds atmospheric pressure (www1). Nitrogen dissolves or leaves solution slowly by comparison to other gases. Although the total pressure of all dissolved gases determines whether supersaturation will occur, nitrogen is the most dangerous individual gas causing “bends” when it comes out of solution inside an animal. High concentrations of nitrogen (>110% saturation) should be avoided at all costs.

Various measurement devices can be used to evaluate total dissolved gas levels. Gasometers and saturometers measure total gas pressure (TGP; the sum of the partial pressures of atmospheric gases measured in mm Hg) or dissolved gas tension (△P; the difference between TGP and atmospheric pressure), representing the force available to inflate bubbles or emboli and determine if gas bubble disease will occur at a given depth (Bouck, 1980). Manometer-based devices measure △P directly, while those employing an electronic pressure transducer, calibrated to absolute pressure, measure TGP. Atmospheric pressure (pAtm) must be known to extract △P values from TGP measurements as follows (www1):

$$\mathbf{TGP = \triangle P + pAtm}$$

Most commercially produced gasometers are somewhat expensive. However, Bouck (1982) has described the construction of an inexpensive gasometer that will operate continuously and can be built from materials familiar to aquarists.

Data in the literature are often reported as percent total gas pressure (TGP%), or percent saturation. TGP% is calculated as follows:

$$\mathbf{TGP\% = (\triangle P + pAtm) \times (100 / pAtm)}$$

Saturation levels above 110% will usually cause gas bubble disease, while levels under 102% are typically safe for fish systems (Wedemeyer et al., 1976). Oxygen saturation values can be a predictor of nitrogen levels in systems where excess oxygen is not introduced (naturally or artificially), or where air entrainment is not occurring in oxygen-deficient parts of an LSS. The use of meters that measure total gas saturation is the only way to accurately determine whether nitrogen supersaturation is occurring (following a comparison with DO measurements). Independently acceptable TGP and oxygen saturation levels may hide nitrogen supersaturation capable of damaging fishes. Atlantic menhaden (*Brevoortia tyrannus*) are known to exhibit erratic swimming behavior at 95% TGP accompanied by 105% nitrogen saturation (Weitkamp and Katz, 1980).

Oxygen-specific supersaturation is relatively benign, but in outdoor ponds it can lead to hypercapnia and cause gill damage to fishes through the retention of excess carbon dioxide. This phenomenon is rare (as CO_2 diffuses rapidly) and is usually only observed in aquaculture ponds where algal photosynthesis dramatically increases dissolved oxygen levels (www2). Designers of outdoor elasmobranch systems should consider this problem when sizing gas-balance devices.

The symptoms of supersaturation may be subtle, or in serious cases, disturbingly obvious. Sand tiger sharks will develop rapidly spreading white patches on their skin as bubbles accumulate in their subcutaneous capillary beds. If caught immediately, this is a reversible condition. An effervescence at the surface of the water and unexplained bubble accumulation on algae-free walls or substrate are good indications that supersaturation is occurring.

Good system design and regular testing of dissolved gas levels is the best way to prevent nitrogen supersaturation events. The use of a biotower, with ambient air mixing, allows for the re-equilibration of any gas that may exceed its ambient partial pressure.

Water depth may offer some protection against supersaturation in closed systems. Weitkamp and Katz (1980) note that each meter of depth compensates for about 10% of near-surface saturation levels. Where 120% TGP is observed near the surface, the actual environment at two meters is closer to 100% saturation. This situation suggests that exhibit animals preferring upper regions of displays may become the first victims of supersaturation events, and in some cases seek refuge in lower parts of the exhibit.

Supersaturation can be caused by a number of equipment or design failures. A leak on the suction side of a pump is probably the most common scenario. Such leaks appear dry during system operation, but suck air into the system. These bubbles are then forced into solution by the high pressures within the pump. When the system is shut off, water often (but not always) leaks from the compromised piping.

Another common cause of supersaturation is entrained air bubbles that are forced into solution at a high-pressure area within LSS piping. Pumps, plumbing restrictions, and venturis are common problem areas. Improperly designed systems, where air bubbles can enter a water intake, can cause supersaturation events. Gravity sand filters are prone to this problem when float valves are employed to control water levels, as air may be sucked through the sand bed if the valve sticks open. Throttling valves placed between degas chambers and exhibits is another location where pressure drops occur and may cause supersaturation as a result of air entrainment through valve restrictions (Linn, pers. com.).

A less common source of supersaturation in closed systems is the introduction of bubbles at depth. Waterfalls that cascade into deep pools, and diffusers or venturis located at the bottom of deep tanks or foam fractionators, can all result in supersaturation. $\triangle$P increases of 73 mm Hg are added for every meter of depth in plunge pools beneath dams or waterfalls (Colt, 1986). In general, air-stones or venturis can have similar effects on gas pressure (www1) depending on the depth at which they are located in a pool, foam fractionator, or ozone contact chamber. LaBonne (pers. com.) suggests that adding air at a depth of 1.8-2.4 m, the maximum depth of many commercial foam fractionators, is probably safe. However, fine diffusers may produce supersaturation when placed deeper than a meter, while coarse and medium bubble diffusers could cause gas saturation problems at depths exceeding two meters (www2). Since it may not be practical to inject air at depths less than a meter in many foam fractionators, provision for post-treatment gas balance should be made. Placing a degas chamber or biotower at the end of the process stream is an excellent way to protect systems against supersaturation.

Facilities using well water should be especially mindful of dissolved gas issues. There have been many reports of wells producing water with dissolved nitrogen levels of up to 180% saturation (www1).

The addition of gas-saturated cold water to a warmer system can cause supersaturation (Powell, pers. com.), as can rapidly heating or adding salts to saturated water (www2). Rapid heating of saturated water, starting at 0°C, can produce a $\triangle$P increase of 20 mm Hg $°C^{-1}$ (Colt, 1984), while water initially held at 15°C experiences a $\triangle$P increase of 15 mm Hg $°C^{-1}$ with rising temperature (www1).

A number of freak conditions that can result in supersaturation are often overlooked. Johnson (pers. com.) warns that systems on the verge of supersaturation can be pushed over the edge by the passage of a severe low-pressure storm front. It has been suggested (www2) that this phenomenon played a role in the death of millions of marine fishes during a hurricane in 1992. The passage of a storm front typically causes changes in barometric pressure of +5 to -17 mm Hg (Craig and Weiss, 1971). A 17 mm Hg decrease in barometric pressure produces a 17 mm Hg increase in $\triangle$P.

Variations in atmospheric pressure may affect dissolved oxygen measurements. A table of $\triangle$P

vs. altitude (and associated atmospheric pressures) is available on the world wide web (www1). Rapid changes in altitude, pressure drops during take-off (Jewell, pers. com.), and re-pressurization after landing at a high altitude (Lerner, pers. com.) may all cause supersaturation in bodies of water carried aboard aircraft.

Toxicants

Harmful materials can enter an exhibit in a variety of unexpected ways. As noted above, a variety of harmful compounds have occasionally been found in low-grade chemicals used to make artificial seawater. Human error is implicated in virtually all other situations that result in the introduction of toxic materials to aquarium systems. The improper application of protective coatings, poor fabrication of exhibit decoration, LSS component materials, and general building maintenance activities are usually implicated as the source of toxicants.

Properly designed and maintained systems are less likely to experience contamination by toxicants. Should a proactive approach to the management of toxic chemicals fail, the United States Environmental Protection Agency's Ecotox searchable internet database (www3) will help identify harmful levels of various substances. The National Institute of Health's National Library of Medicine hosts a similar site (www4).

Volatile organic compounds (VOCs)

Volatile organic compounds have been implicated in the mortality of sharks in new exhibits (Rasmussen and Garner, 1999). Appropriate planning for any painting or coating activities in and around exhibits will minimize organic chemical contamination. Floor sealants, paints, and adhesives should always be evaluated before use. Every attempt should be made to use products that have been successfully applied elsewhere or tested on sample fishes. Ironically, materials suitable for drinking water may be unsuitable for aquarium use (Atz, 1970). For example, a polysulfide-based material, formulated as a liner for potable water tanks, was tested by one of the authors (Mohan) and found to be lethal to goldfish (*Carassius auratus*).

Epoxy compounds used to manufacture artificial coral and other tank décor can be toxic if the mix ratio is incorrect. Even fresh, correctly made corals can be expected to release volatiles. It may be wise to place recently manufactured corals in direct sunlight for days or weeks before use. Pre-filling new exhibits with freshwater, which is discarded before the final fill with seawater, is another worthwhile preventative measure. Filling, flushing, circulating, and dumping are advisable for any open or semi-open systems. Where natural seawater is abundant it makes sense to use this cheap natural resource for the initial fill. The precautionary use of activated carbon during initial operation of the LSS is recommended.

Concrete alkalis

New concrete, especially uncoated surfaces such as rockwork, will typically leach alkalis. There are many opinions as to how to neutralize these leachates (Choromanski, pers. com.). Some workers simply pre-fill the exhibit with domestic freshwater for 5-10 days, drain it, refill with seawater, and then add acid to bring pH into normally expected ranges (i.e., 8.0-8.4). Others reduce pH to 3.0-5.0 during the initial freshwater bath for 1-45 days. Muriatic acid is the most commonly used pH reducer. Concrete type and volume, as well as system type (i.e., open, closed, etc.) may determine which of these methods should be used.

Inventors of the reef ball (Reef Ball Development Group, USA), an artificial reef module that is widely used in natural waters, suggest a more proactive method of neutralizing alkalis. Micro-silicates added to a Portland II concrete mix reacts with excess calcium hydroxide and results in a finished product having a pH of 8.3 (Barber, pers. com.; www5). While the addition of micro-silicates increases concrete cost, it minimizes the need for post-construction pH adjustments. Its use should be considered for systems that incorporate large volumes of concrete rockwork.

Metals and metalloids

Harmful metal ions can be introduced into closed aquarium systems if equipment containing inappropriate materials is specified during LSS design. Antifouling paints intended for watercraft contain copper, other metals, or other poisons and should never be used in aquarium systems.

Metals such as lead, copper, zinc, and nickel are generally less toxic in marine systems than in freshwater, largely because of the protective effect

of high pH, hardness, and alkalinity (Sorensen, 1991). Although significant amounts of dissolved metals precipitate at the high pHs typical of marine systems, the corrosive effects of saltwater can make metal accumulation from LSS equipment or other hardware a serious problem. Any large metallic LSS components placed in direct contact with seawater should be made of titanium. Metal contamination of aquarium seawater is not a serious concern in systems where water is continuously replaced with raw seawater. Stainless steel pumps with magnesium cathodic protection have been used successfully in such situations (Powell, pers. com.).

Elevated levels of nickel have occasionally been attributed to the use of nickel-copper alloys (e.g., Monel®, Inco Corporation, USA) that may be used in valve components and pump shafts. While these alloys are recommended for marine applications they have been reported to cause elevated nickel concentrations in closed systems (Davis, pers. com.). Certain grades of stainless steel can be a source of nickel and other heavy metals (e.g., chromium, etc.). Davis (pers. com.) observed that threaded metal rods used to secure the lid of cartridge filters were a source of nickel contamination for small aquariums at SeaWorld Orlando. Nickel contamination was successfully removed using activated carbon.

High zinc levels in closed systems can usually be traced to the inappropriate use of sacrificial zinc anodes in rapid sand filters. Aluminum or magnesium-based anodes should be used in closed-system seawater LSSs. The immersion of any galvanized materials will lead to zinc contamination. Hughes (1968) observed that 100% of striped bass (*Morone saxitalis*) fingerlings survived 96 hours at 0.05 mg l^{-1} of zinc, but half died within 48 hours at 0.10 mg l^{-1} of zinc. Brungs (1969) reported that 0.18 mg l^{-1} of zinc inhibited reproduction in fathead minnows (*Pimphales promelas*) but did not affect growth or maturation. Gill damage occurred at 0.80 mg l^{-1} of zinc in rainbow trout, *Oncorhynchus mykiss* (Brown et al., 1968). There is evidence to suggest that gender may affect elasmobranch susceptibility to zinc poisoning. Crespo et al. (1979) reported that male smallspotted catsharks (*Scyliorhinus canicula*) accumulate up to three times more zinc in their gill arches than females. Zinc intoxication compromises equilibrium and damages gills, kidneys, liver, and muscle tissue (Sorensen, 1991). Magnesium competes with zinc and may be somewhat protective in hard water (Zitko and Carson, 1976), especially in marine systems.

Copper contamination typically results from errors in LSS design or repair. The use of copper or brass fittings in LSS piping can produce elevated copper levels in closed systems (Atz, 1970). The accidental use of pumps with bronze components can cause copper contamination. Coating copper LSS components will not dependably protect specimens from intoxication. Stonecypher et al. (1992) observed that vinyl-coated copper chiller coils leached dangerous levels of copper (i.e., exceeding therapeutic levels) in little more than a week. Copper contamination can come from unexpected sources. One of the authors (Mohan) has observed a cracked glass submersible heater continue to operate while flooded and found that it shed copper ions into the surrounding water. Some facilities (e.g., the Pittsburgh Zoo and Aquarium, Pittsburgh, USA) are supplied with domestic freshwater from reservoirs containing seasonally toxic levels of copper (Billin, pers. com.). New facilities should thoroughly test their water supply before it is used to make water exchanges in animal exhibits. Unlike exposure to zinc, the difference between safe and fatal copper concentrations is quite small (Brungs, 1969).

Iron is unlikely to be a problem in marine systems unless highly contaminated well water is piped directly into an occupied exhibit. Iron precipitates in alkaline seawater (Anon, 1972) and the iron hydroxide flocs are generally removed by mechani-cal filtration. At SeaWorld Ohio iron removal was accomplished using a water softener that improved iron-laden well water from 3.0-5.0 mg l^{-1} to $\leq$ 0.30 mg l^{-1}. This water was considered acceptable for makeup and was routinely used for water exchanges in freshwater systems. The tendency for iron to precipitate in seawater means that small amounts of exposed rebar should not be viewed as an important source of iron contamination.

Arsenic contamination is a potential concern when chronic leaks occur in roofs constructed from chemically treated lumber containing chromated copper arsenate (CCA). Ash from burnt CCA lumber is highly toxic: one tablespoon contains a lethal human dose. The construction of outdoor shade structures using CCA-treated wood is contraindicated unless these shelters are designed to minimize the introduction of condensate and rainwater into system water. Fly ash collected by electrostatic precipitators on coal-burning power plants contains high levels of arsenic. The no-effect level for fish exposure to disodium arsenate is <1.0 mg l^{-1} (Sorensen, 1991). Arsenic is known to replace phosphate in normal

metabolic reactions, interfering with respiration and other processes. Death usually occurs as a result of excess mucus production, especially on the gills, and subsequent suffocation (i.e., coagulation film anoxia).

Interactions between different metals are a concern, especially those involving copper, zinc, and perhaps nickel, all of which may be found at low levels in most marine aquarium systems. In laboratory studies using highly toxic concentrations of metals, a synergistic effect between zinc and copper has been reported (LaRoche, 1972). At the doses of metals likely to be encountered in aquariums, the effects of copper and zinc are probably additive, rather than synergistic (Lloyd, 1961). The presence of excess zinc in a quarantine system will therefore increase the toxicity of copper added as a therapeutic. For this reason, we recommend that metal levels in aquariums be checked before the initiation of any copper therapy. The removal of excess heavy metal ions prior to copper addition can be accomplished using activated carbon (Davis, pers. com.). It may be wise to discontinue the use of vitamins and trace element solutions containing heavy metals during such treatments.

Chlorine

Shocking system water with chlorine, to sterilize the water or reduce nitrogen levels (i.e., through breakpoint chlorination), should be avoided where possible. Incomplete neutralization of chlorinated water introduces toxic chlorine into elasmobranch systems. Less well known is the danger of using excessive amounts of sodium thiosulfate to neutralize chlorinated or chloramine-treated water. Reactions resulting in dangerously low pH and dissolved oxygen levels have been observed in systems where residual thiosulfate is present (Linn, pers. com.). Chen (1974) notes that slightly acidified dilute solutions of thiosulfates decompose to sulfite, free sulfur, and polythionate. Sulfur formation is signaled by the precipitation of a milky-white colloid.

If chlorinated or chloramine-treated city water is used for large water exchanges, it should be passed through a carbon filter prior to use. If chlorine alone is present, aeration can be employed for its removal (Wheaton, 1977). The judicious use of chlorinated tap water during routine pressure-washing of rockwork is usually not harmful. For example, two pressure washers (DSL 4200E, Clarke-Delco, USA) were routinely used to clean the Shark Experience at SeaWorld Ohio adding 3.6 m^3 (i.e., <0.25% exhibit volume) of chlorinated (i.e., without chloramines) domestic water to the exhibit. These cleaning sessions occurred on a weekly basis and lasted for two hours duration.

ORP (Oxidative Redox Potential)

As a chemical process, reduction-oxidation (redox) reactions are those in which electrons are transferred back and forth between chemical species. An oxidized species is one that donates an electron, a reduced species is one that accepts it. When a pathogen is oxidized a chemical component in its cell wall gives up an electron. When sufficient electrons have been lost, the cell's functions deteriorate or the cell wall disintegrates and eventually the organism is killed. Water that has been treated with an oxidant such as chlorine or ozone has a greater opportunity to allow these kinds of reactions to occur. ORP (oxidative redox potential) is a measure of the potential for oxidation-reduction reactions to take place and is measured in millivolts (mV).

Cohrs (2002) provides a good introduction and reference to ORP. Informally, ORP is used in the industry to describe the relative cleanliness of seawater. Raw or new artificial seawater has an ORP of ~250 mV. When aquatic animals and food are added to this water, ORP will drop further (i.e., the opportunity for redox reactions to occur will decrease). Therefore, pathogens and other unwanted chemical species are less likely to be oxidized. Conversely, when an oxidant such as ozone or chlorine is added to the water, ORP rises and the likelihood of pathogens and other unwanted chemical species being oxidized increases.

Different contaminants require different levels of oxidation for destruction. Many bacteria will be killed at an ORP as low as 400 mV, while other micro-organisms require an ORP as high as 800-900 mV and exposure for several minutes before they are destroyed. Unfortunately, an ORP of 800 mV is extremely unhealthy for the aquatic species on display. Even an ORP of 450 mV is harmful if exposure lasts for more than a few hours. Therefore, if oxidants (e.g., chlorine, ozone, etc.) are applied, they must be applied with a strategy to return oxidized water to the exhibit at a low ORP and toxic residual oxidants must be absent. The safe upper limit for ORP within an exhibit is generally regarded to be ~380 mV or less. On

the other hand, an ORP of ≤ 250 mV typically indicates an aquatic environment containing excess dissolved organics or other unwanted constituents.

LIFE SUPPORT SYSTEM COMPONENTS

Water supply

Water supplies for recirculating aquarium LSSs can be classified into three basic types: (1) flow-through systems; (2) closed systems; and (3) semi-closed (or semi-open) systems.

Flow-through systems

Flow-through systems receive a constant flow of new seawater that continually displaces water recirculating in the tank. The volume of influent replacement water (measured per day), compared to the total system volume, defines the percentage of flow-through. For example, 150 m^3 of new water added to a 1,000 m^3 exhibit each day is equivalent to a 15% flow-through or blow-down. These systems are typically outfitted with minimal water treatment, often limited to mechanical filtration, relying instead on the steady flow of replacement water to maintain water quality. Although, theoretically, the water source could be artificial, large elasmobranch systems usually draw new water from a natural source.

Closed systems

At the other end of the theoretical spectrum are closed systems, which by definition never undergo any water change or receive any replacement water. In reality, these systems do not exist. The replacement of losses resulting from backwash recovery, or evaporation, prevents any system from ever being truly closed.

Semi-closed systems

Semi-closed systems are the most common. Although these systems are not provided with a steady flow of replacement water, they do receive periodic water changes (natural or artificial) in batches. The volume and frequency of water changes vary greatly from system to system, and depends largely on system design and stocking levels. Aquariums with semi-closed systems designed to receive large contributions of natural seawater are sometimes supplied with under-engineered LSSs. These systems may prove to be inadequate during emergency periods when they must operate closed (LaBonne, pers. com.).

The maximum time a semi-closed system may be required to operate closed should be clearly quantified during LSS design. To achieve this goal, system design should be based on defined biological loads. Where natural seawater is used, the expected need for nitrification and gas balance should be weighed against historical data for quality of source water. If interruptions in source water are common, the exhibit may need to be designed as a fully functional closed system.

Mechanical filtration

If no provision for particulate removal is made, water clarity will be severely compromised, particularly for systems with long sight-lines.

Bubble-bead filters trap particulates but are designed for high-load systems (e.g., high density aquaculture) and are not as effective for particulate removal in low-load systems (i.e., a typical elasmobranch exhibit). Diatomaceous earth (DE) filters were extremely common in large systems built for marine mammals in the 1960's, but are infrequently used today because of operational difficulty (i.e., rapid soiling creating low flow conditions) and hazardous material concerns (i.e., from ultra-fine DE powder). Gravity sand filters have been widely used in Asian facilities. While effective at removal of particulates, gravity sand filters tend to be labor intensive, are prone to organic fouling, and require as much space as the exhibits they serve (Gendron, pers. com.).

Rapid sand filters are the most appropriate and most common form of mechanical filtration used for large elasmobranch systems. Rapid sand filters are designed to trap particulate material, improving water clarity, but are not capable of removing dissolved organic molecules. Foam fractionators will remove both dissolved organics and particulates. However, foam fractionators are not as efficient as rapid sand filters at removing all types and sizes of particulate contaminants. Thus, acting alone, foam fractionators may not produce sufficient clarity for large elasmobranch exhibits.

In general, a disadvantage of mechanical filters is that water is continually filtered through

contaminants trapped in the media (i.e., sand, diatomaceous earth, beads, etc.). Trapped accumulated particulates will release organic carbon back into solution and thus system water. Films of heterotrophic bacteria consume the hydrolyzed carbon, release carbon dioxide, and thereby lower system pH (Hovanec, 1995). Thus, although rapid sand filters are the most appropriate means for particulate removal for elasmobranch systems, some provision for facilitating gas exchange and removing dissolved organics must be considered.

There are two ways to alleviate the negative effects of trapped contaminants in mechanical filters. First, perform filter backwashes (or exchange media) on a timed interval rather than relying on the cue of pressure differentials. Employing this type of schedule will reduce the period of time that water is flowing through increasing amounts of trapped contaminants. Second, incorporate foam fractionators and/or biotowers (equipped to allow the mixing of ambient air with system water) into the LSS. If no provision is made for ambient air mixing or dissolved organics removal, the signs of accumulated CO_2 and dissolved organics will begin to occur—i.e., pH and ORP will have a tendency to drop. Adequate ventilation is an important component of any gas-balance device (Powell, pers. com.). These principles have been successfully applied at SeaWorld Ohio for the Shark Encounter exhibit, where each of the four sand filters (surface area 10.2 m^2) were operated on a staggered 140-hour backwash cycle. This schedule is a compromise between daily backwashing to remove organic solids, and a longer interval to optimize water clarity. Sand filtration for the exhibit has been used in conjunction with foam fractionators, resulting in acceptable water clarity, a stable pH of 8.0, and minimal nitrate (NO_3^-) accumulation. If no provision is made for the removal of dissolved organics and ambient air mixing, signs of both accumulated organo-carbon compounds and carbon dioxide will be evident (i.e., decreasing pH and ORP).

Highly loaded rapid sand filters (e.g., rapid sand filters for stingray pools where the public is encouraged to feed the animals) should be emptied and re-bedded periodically. Lightly loaded filters may not become fouled, but need to be re-bedded at some point in their operational life as media gradually wears away during backwashes (Linn, pers. com.).

Biological filtration (nitrifying filters)

Nitrifying bacteria form biological films on filter media and oxidize the accumulated toxic nitrogen compounds, ammonia (NH_3/NH_4^+) and nitrite (NO_2), into nitrate. This process is known as nitrification. Feeding rates (i.e., amount of food added per day) will determine initial ammonia production, and minimum biological media requirements can be calculated if ammonia conversion rates for specific media are known.

Hovanec (1995) contains a good review of the various types of biological filtration currently in use. Rapid sand filters provide nitrification in most traditional systems. Other LSSs may have under-gravel filtration, or dedicated wet-dry trickle biological filters or biotowers.

Reverse flow under-gravel filtration is practical if clean, filtered water can be delivered to the substrate bed. Many under-gravel filtration systems, constructed with sand beds poured directly over a sparger (i.e., a network of water distribution pipes), have become rapidly fouled because of inefficient and uneven water distribution and shifting substrate. When buried sparger systems are used, non-calcareous pea gravel should be employed to cover the pipe network before sand beds are poured. During the construction of their elasmobranch exhibit, SeaWorld Ohio installed a perforated FRP (fiber-reinforced polyester) plate above the LSS sparger before a uniform layer of substrate was poured. The sand bed within this dependable design did not require cleaning during its 11 years of operation.

Nitrification produces carbon dioxide (as does elasmobranch respiration) so any LSS must include provisions for gas exchange or system pH will be depressed. Biological filters are susceptible to fouling with organic matter, channeling (i.e., short-circuiting) through densely packed media, and oxygen depletion because of microbial respiration. The use of biotowers is a good way to mitigate these negative effects. Biotowers designed with specific hydraulic flow rates allow water to pass in thin films over media surfaces. At ambient pressures this process facilitates a highly efficient exchange of carbon dioxide and oxygen, returning dissolved gases to natural levels, and helps relieve the negative effects of biodegradation in sand filters. Where possible, biotowers are best placed after some type of mechanical filtration component (e.g., foam fractionators or rapid sand filters) to

minimize fouling. Biotowers are a critical addition to systems where sand filtration has been reduced to a side stream process as they provide both nitrification and gas exchange.

The effects of therapeutics on nitrification

Hawke (1991) reviews the effects of therapeutic agents on biological filtration systems. Although copper sulfate is rarely used in systems with elasmobranchs, it is worth noting that copper sulfate can affect LSSs in unpredictable ways. Copper treatment can provoke a spike in ammonia concentrations, even in systems that have been treated without incident in the past. One of the authors (Mohan) has observed this phenomenon in two systems at SeaWorld Ohio, and Roger Klocek (pers. com.) reported a similar incident at the John G. Shedd Aquarium, Chicago, USA in the 1980's.

Antibiotic bath therapy is rarely used for large elasmobranch systems or quarantine pools, but it may be practical for smaller systems. Gentamycin, nifurpirinol, sulfamerazine, tetracycline, and trimethoprim appear to have little or no effect on biological filtration (Hawke, 1991). However, other antibiotics (e.g., chloramphenicol and erythromycin) may seriously inhibit nitrification.

Praziquantel (Sigma, USA), an antihelminthic treatment, has recently been used to treat large systems at the Epcot Center's Living Seas Pavilion (Orlando, USA), SeaWorld Ohio, and Omaha's Henry Doorly Zoo (Nebraska, USA). Concentrations of ≤2 mg l^{-1} praziquantel were applied for more than one week and no adverse effects on biological filtration were observed.

Denitrification

Over time, nitrification leads to the accumulation of nitrates. Although the toxicity of nitrates is low, they should not be permitted to reach high concentrations in an elasmobranch system. The biological process of removing nitrates is called denitrification and relies on either heterotrophic or autotrophic bacterial populations.

Heterotrophic denitrification systems require a highly concentrated source of organic carbon as an energy supply for the anoxic, reducing bacteria. The concentration of organic carbon in elasmobranch LSSs is not adequate to support the heterotrophic bacteria required to reduce nitrate concentrations. An additional source of organo-carbon (e.g., methanol) must be injected prior to the denitrification reactors. Although heterotrophic systems are more efficient than autotrophic systems, they are inherently difficult to operate because of the heavy biomass generated. Biological films clog and foul pipes, valves, flow meters, and other instrumentation within the denitrification system. Operators must frequently dismantle the system in order to prevent bio-fouling which may result in plumbing obstructions and flooding. For these reasons, heterotrophic systems are labor-intensive. In addition, many state fire codes require special handling and safety procedures for the use of methanol. Aiken (1995a) reports on a successful heterotrophic denitrification system built at the National Aquarium in Baltimore.

Autotrophic denitrification systems typically use sulfur as an energy source for the autotrophic denitrifying bacteria (i.e., *Thiobacillus* spp.). Autotrophic systems are less efficient than heterotrophic systems, but the process is simpler, less expensive, and less labor-intensive. Nitrate reduction occurs while sulfur is oxidized to sulfate. The resulting sulfate does not seriously increase sulfate concentrations within the LSS, however the resulting acid production does reduce system alkalinity. Low pH and high DO levels decrease filter efficiency by promoting nitrite accumulation (Zhang and Lampe, 1999). Thus, buffering media such as oyster shells or limestone must be added to an autotrophic denitrification system in order to maintain pH. Buffering material can either be incorporated into the sulfur reactor or added as a second component following the reactor. Furumai et al. (1996) suggest maintaining a pH above 7.4 within the denitrification vessel. Zhang and Lampe's (1999) study suggests that optimal results can be obtained using sulfur:limestone ratios of between 1:1 to 3:1 and a hydraulic retention time (i.e., the time required for a volume of water, equivalent to the size of the reactor, to pass through the reactor) of 3-10 hours. Zhang and Lampe (1999) found that autotrophic denitrification will proceed in both aerobic and anaerobic conditions, although oxygen appeared inhibitory to the process.

The first sulfur-based denitrification system employed in a public aquarium was started in 1993 at the MAAO (Musée National des Arts d'Afrique et d'Oceanie) Aquarium in Paris, France. This system was installed on a 60.6 m^3 exhibit with a resultant reduction of nitrate from >300 mg l^{-1} to <10 mg l^{-1}. No negative

water quality sideeffects were observed (Hignette et al., 1997). Successful sulfur denitrification systems have been employed at the London Aquarium (UK), Tennessee Aquarium (Chattanooga, USA), SeaWorld Orlando, Discovery Cove (Orlando, USA), and the National Aquarium in Baltimore.

High biomass and/or organo-carbon concentrations typically characterize the effluent of denitrification systems. By passing denitrification effluent through foam fractionators the vast majority of organic loads will be captured and removed, preventing its release into system water. Existing foam fractionators may be employed for this purpose, or an additional foam fractionator dedicated to the denitrification plant can be used.

Algal turf scrubbers

Another means to remove nitrogen contaminants is the use of plants to naturally consume excess nutrients. Algal turf scrubbers rely on rapid growth of cultivated algae, the associated uptake of nutrients, and subsequent removal of the alga biomass to reduce nutrient levels. Reef HQ in Townsville, Australia uses both foam fractionation and frequent small water changes in conjunction with their algal turf scrubbers, and much of the harvested algae is disposed (Czaja, pers. com.). In its pure research form, this type of biological filter has been used without the export of harvested algae and protein (Adey and Loveland, 1991). As a result, experimental systems at the Smithsonian Institute (Washington, USA), the Pittsburgh Zoo (USA), and the St. Louis Zoo (USA) developed an increasingly yellow coloration over time. While algal turf scrubbers are effective at removing nutrients from system water, maintenance of the scrubbers is labor-intensive and large numbers of units would be required for a typical elasmobranch system.

Foam fractionation (protein skimming)

Foam fractionators (also known as protein skimmers) remove organic contaminants from solution while at the same time providing some disinfection via the removal of bacteria that are trapped in the skimmed foam matrix (Conway and Ross, 1980). Foam fractionators work by taking advantage of the natural attraction of surface active organic compounds to air bubbles. Air bubbles are injected into the bottom of a reactor and as dissolved organic compounds continue to collect on the surface of the bubbles, the bubbles become stickier, stronger, and coalesce into a wet foam. The foam matrix is thus formed by the adsorption of surfactants onto the surface of the bubbles and by micro-flocculation. Foam fractionators are typically equipped with a cone and chimney designed to allow rising foam to condense and exit the system as it overflows out of the stack. The addition of relatively small doses of ozone will enhance coagulation and increase the removal of dissolved organics and bacteria.

Foam fractionation provides significant benefits to water quality, including: increased pH and a reduced dependency on buffers, increased DO concentrations, increased redox potential, improved water clarity, reduced turnover rates, reduced dependency on sand filters, and reduced frequency of sand filter backwashes (Aiken, 2000).

It is important to maintain a high air:water ratio in order to obtain maximum performance from a foam fractionator. A 1:10 air:water ratio is considered ideal when bubble size is ≤0.5 mm (Rozenblum and LaBonne, 1995). Restricting airflow to the venturi through the use of a valve will decrease the volume of air entering the venturi and decrease bubble size. The result is an overall increase in the total surface area of bubbles within the foam fractionator and an increase in operational efficiency. Bubble size, contact time (i.e., bubble retention within the water column), ozone dosage rates, and foam removal are other factors that will affect the design, size, and efficiency of a foam fractionator (Rozenblum and LaBonne, 1995).

Foam production rates appear to depend on the relative concentration of organics and antifoaming agents. The foam fractionators at Discovery Cove vary in efficiency according to a diurnal cycle that may be related to the input of body oils and suntan products from human bathers. These products appear to collapse foam columns when the exhibit is in peak use. Maximum foam production is seen during non-public hours presumably after antifoaming agents have been broken down.

One disadvantage of foam fractionators is that they require relatively frequent adjustment to ensure proper operation, unlike pressurized components such as rapid sand filters that can be left to operate without adjustment. Optimum operation can be obtained by ensuring that water

flow to the unit varies as little a possible. Stabilizing water flow to a foam fractionator can be accomplished by providing dedicated pumps that draw water directly from the exhibit, supplying water from a header tank with fixed water levels, using modulated pumps linked to level sensors within the fractionator, etc.

Supersaturation may become a problem if bubbles are allowed to exit the foam fractionator and enter a pressurized process. LaBonne (pers. com.) suggests that situations where bubbles are accidentally re-circulated through a pump and venturi should be avoided. If a fractionator discharges directly into an exhibit, pressure differentials across the venturi of ≤103-117 kPa are recommended. Usually this means that the venturi intake pressure should be ≤138 kPa (LaBonne, pers. com.).

Flocculating agents and other additives

The use of the strong flocculent aluminum sulfate (alum) has generally been avoided in fish systems. However, injection of alum prior to a mechanical filter may make limited use of this material possible as the floc is captured and then removed by backwashing. Organic, chitin-based flocculants, such as Sea-Klear (Vanson HaloSource, USA), are safer alternatives to alum but may be somewhat less effective. Recently, a number of facilities have used lanthanum chloride (ZeroPhos™, Vanson HaloSource, USA) to reduce phosphate levels, providing some relief from excessive algal growth, especially in outdoor exhibits. Ferric chloride has been used successfully for large elasmobranch systems, injected in-line before rapid sand filtration (Smith, pers. com.).

Ozone

Tri-atomic oxygen, or ozone, is a strong oxidant and useful tool for the treatment of water in large elasmobranch systems. When used correctly ozone can contribute to superior water quality, when used incorrectly it can result in the mortality of valuable specimens.

Throughout this section, it is important to remember the dual role of ozone as both a flocculent and disinfectant. When the ozone:organics ratio is low (i.e., g l^{-1} ozone << g l^{-1} dissolved organics), ozone will act as a flocculent, destabilizing dissolved organic molecules in suspension and bringing about their coalescence. This process leads to the formation of a foamy mass at the water surface. As the ozone:organics ratio increases the flocculating properties of ozone are lost, giving way to direct oxidation and disinfection. There is a transition range where both effects can occur and thus, depending on the applied dose and organic load, ozone can function as a flocculent, a disinfectant, or both.

Micro-flocculation

When used in conjunction with a foam fractionator, flocculation removes unwanted dissolved organic molecules and other particles caught in the foam matrix. Removal of the foam matrix is important since dissolved organics will consume DO and depress pH. Thus, micro-flocculation results in higher ORP, higher pH, and clearer water.

It is important to tailor ozone dosing rates to achieve the intended function. Applying disinfection doses of ozone in foam fractionators will result in poor foam formation and possibly an unsafe concentration of ozone in the surrounding environment (e.g., the pump room). Micro-flocculation typically requires only 0.03-0.10 mg l^{-1} of ozone, although different systems may require slightly different ranges (Rozenblum, pers. com.).

When ozone dosing favors flocculation, foam will collect on the water surface inside ozone contact chambers, as well as the interior of foam fractionators. Unfortunately ozone contact chambers have no way to discharge the foam and early designs were outfitted (or retrofitted) with sprayers designed to liquefy the foam so that it would not accumulate. This process is not recommended since it effectively puts unwanted organic molecules back into solution.

Disinfection

While disinfection through the removal of microorganisms does occur in foam fractionators, not all pathogens, viruses, and bacteria are of suitable dimensions to be harvested in the foam matrix. More complete sterilization of water for large elasmobranch exhibits may be achieved through the use of high concentrations of ozone applied in ozone contact chambers. The disinfection capacity of ozone contact chambers is greater and more cost effective than sterilization with ultraviolet light (UV), especially for large elasmobranch systems (Aiken, 1995b).

Ozone contact chambers are designed specifically for water sterilization whereby system water is subjected to high doses of ozone and very high ORP (i.e., 800-900 mV) for several minutes. In this environment, a high kill ratio is achieved (i.e., >99%) as microorganisms are destroyed via cell lysis. This kind of disinfection is not possible in foam fractionators. Conversely, collection and removal of dissolved organic molecules through micro-flocculation are not effectively achieved in ozone contact chambers (Aiken, 2000).

Sterilizing concentrations of ozone typically start at ~1.0 mg l^{-1}, with an application range of 0.3-1.5 mg l^{-1} (Rozenblum and LaBonne, 2001). Contact chamber design has a significant impact on the dose required for sterilization. Disinfection should occur in sealed contact chambers preventing high concentrations of ozone from escaping into the surrounding environment. Ozone destruction units and proper ventilation are mandatory.

Microorganisms destroyed in ozone contact chambers are not removed from the LSS. The biomass remains in solution to be assimilated into biological films, consuming DO, and releasing carbon dioxide via degradation and microbial respiration. Incorporating foam fractionators into the LSS can mitigate these effects.

ORP monitoring and control

If ozone is used as a disinfectant in an ozone contact chamber (or for that matter as a micro-flocculent in a foam fractionator) it is recommended that ORP control be used. The higher dosages of ozone required for disinfection means that ORP readings can rapidly rise to dangerous levels. Poorly controlled ORP threatens fish health and can be responsible for elasmobranch mortality.

An informed understanding of the relationship between ORP, ozone, and residual oxidants is necessary for any curator, aquarist, or LSS operator who uses ozone on an elasmobranch system. When an operator is experiencing problems or is unfamiliar with an ozonation system, we recommended a cautious approach be applied until it is possible to operate the system correctly. Advice from experienced operators MUST be sought. Cohrs (2002) provides an excellent introduction and reference to ORP and how ozone affects ORP in aquarium exhibit waters.

Residual oxidants

Ozone acts as molecular O_3 at pH <7.0 and undergoes a decomposition reaction yielding hydroxyl radicals, the primary oxidants, at pH >9.0. Between a pH of 7.0 and 9.0, ozone exists in a transition range where both forms are present.

During seawater hyper-ozonation, and the associated elevation of ORP, a whole family of residual oxidants is produced. One of the better known residual oxidants is hyperbromous acid (HOBr), formed when bromide is oxidized by ozone. In natural seawater, there is a high concentration of bromide (~67.0 mg l^{-1}) available to be oxidized. The germicidal activity of HOBr is analogous to that of hyperchlorous acid (HOCl) and it actively participates in disinfection (Johnson, 2001). Hyperbromous acid is extremely toxic to fishes and under no circumstances should it be allowed to enter an exhibit with elasmobranchs.

There are two general approaches to protecting elasmobranch exhibits from overexposure to ozone residuals or high ORP:

1. Mix high ORP water (e.g., from an ozone contact chamber) with sufficient non-oxidized water to give a final combined ORP of ~350 mV. Usually a 10:90% or 20:80% ozonated:non-ozonated ratio is used. In this case, residual oxidants are consumed by organic molecules in the untreated portion of the water, once the waters are re-mixed.

2. Pass ozone contact chamber effluent water through an activated carbon filter. Activated carbon will consume residual ozone (or other oxidant) molecules left in solution.

Both strategies have been successfully used in public aquariums for many years. While the latter method is much safer, it occupies more floor space and implies greater capital and operational expense.

ORP does not directly measure the concentration of ozone-related oxidants and should never replace direct testing for potentially toxic residual oxidants (e.g., ozone, bromine, hyperbromous acid, etc.). A total DPD chlorine test (Hach Company, Loveland, Colorado, USA) provides a good qualitative measure of residual oxidants and a modified indigo method, as described by Chiou et al. (1995), is often used for quantitative ozone analyses.

Should residual oxidants enter an elasmobranch exhibit it is possible to neutralize them quickly

using sodium thiosulfate. Approximately one milligram of sodium thiosulfate is capable of eliminating a similar amount of ozone oxidants in seawater prepared with natural levels of bromine (Hemdal, 1992). Emergency additions of sodium thiosulfate should be preceded by preparations for rapid pH changes and followed by continuous pH monitoring.

Effluent treatment

Designers of elasmobranch exhibit LSSs should recognize that regulations governing the disposal of waste seawater vary widely. It is incumbent upon the designer to know the regulations governing their region and specify the LSS accordingly. In some cases, seawater disposal regulations can be extremely stringent. For example, SeaWorld Orlando is required to operate reverse osmosis plants to concentrate effluent from its closed systems and thus avoid the disposal of large volumes of wastewater from backwashes, etc.

Monitoring and record keeping

A thorough water monitoring protocol should be implemented and exhibit water should be rigorously screened for critical parameters (e.g., DO, temperature, ORP, residual oxidants, etc.) on a regular basis. The importance of comprehensive water monitoring, record keeping, and a LSS evaluation program cannot be overemphasized.

LIFE SUPPORT SYSTEM PHILOSOPHIES

A number of paradigms for the LSSs of large elasmobranch systems have been created over the years. Existing LSSs tend to fall into one of these basic models. The rapid sand + ozone model was used by most facilities constructed during the 1980's and early 1990's in the USA. In the past decade, however, alternative LSSs favoring foam fractionation, denitrification, and other technologies have been added to the mix.

Rapid sand filter + ozone contact chamber

Typically rapid sand + ozone systems were designed to recycle exhibit water every 60-90 minutes, a rule of thumb that has been in use for at least 30 years (Hagen, 1970) (Figure 6.1). A number of newer designs use faster turnover times. Ozone treatment of system water was accomplished in contact chambers. Early versions of these systems used air-stones as the ozone introduction device. Newer systems featured entrainment of smaller gas bubbles using venturis.

A portion of the rapid sand + ozone LSS must be devoted to the restoration of gas balance (LaBonne, pers. com.). This equilibration process includes off-gassing of carbon dioxide and nitrogen, and the restoration of oxygen levels lowered by animal and bacterial respiration. In some systems, degas towers are equipped with media to assist gas exchange and optimize gas balance (Johnson, pers. com.). Structurally these towers are akin to biotowers, without the biological function. Piedrahita and Grace (1991) describe a carbon dioxide removal system that consists of a packed column supplied with countercurrent aeration.

Although LSS design is moving away from systems dominated solely by rapid sand filtration, significant amounts of sand filtration may still be required for exhibits with long sight-lines, and exhibits where large quantities of particulates are produced such as ray feeding pools (Johnson, pers. com.).

While exhibits employing early versions of the rapid sand + ozone model tended to accumulate nitrogenous wastes, later versions avoided this problem through the addition of efficient foam fractionation and frequent backwashing (e.g., SeaWorld Ohio's shark exhibit's LSS incorporated foam fractionation on a 10% side-stream and backwashing on a 140 hour cycle).

Denitrification has been added, as a side-stream process, to a number of recently constructed rapid sand + ozone systems.

Foam fractionator-dominated

Some designers now favor LSS configurations where-by foam fractionators comprise ≥ 50% of the total filtration capacity of the system (LaBonne, pers. com.). These systems are frequently referred to as fractionator-dominated (Figure 6.2). Rapid sand filters are relegated to a side-stream loop and ozone additions, where needed, are minimal. LaBonne (pers. com.) notes that the need for denitrification systems is reduced because organics are removed before the nitrification process, leading to nitrate accumulation, has taken place. LaBonne (pers. com.) considers the use of foam fractionators, to

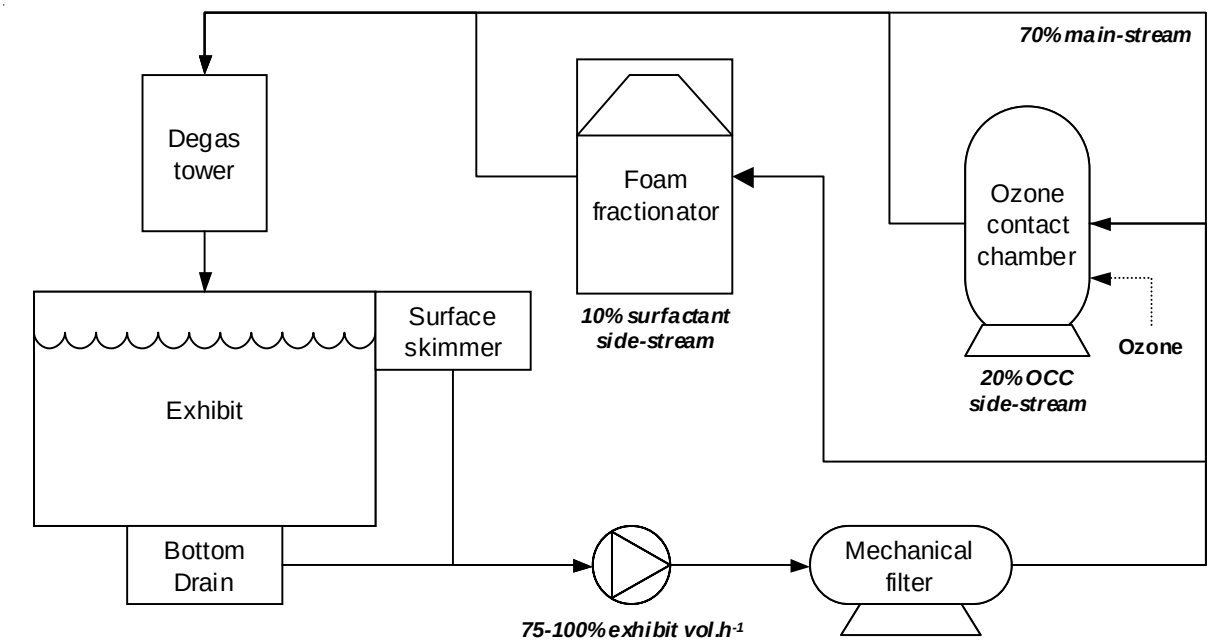


Figure 6.1. Basic process diagram of a rapid sand filter + ozone contact chamber LSS.

the exclusion of ozone contact chambers and traditional mechanical filtration systems, to be a design and operation goal for modern elasmobranch LSSs.

Some LSS designers apply foam fractionators before rapid sand filters. In such applications, the foam fractionator will act as a site for micro-flocculation, and will remove some of the flocs before they reach the rapid sand filters.

Parallel flow

The term “parallel flow” is traditionally used to describe any LSS where parallel treatment

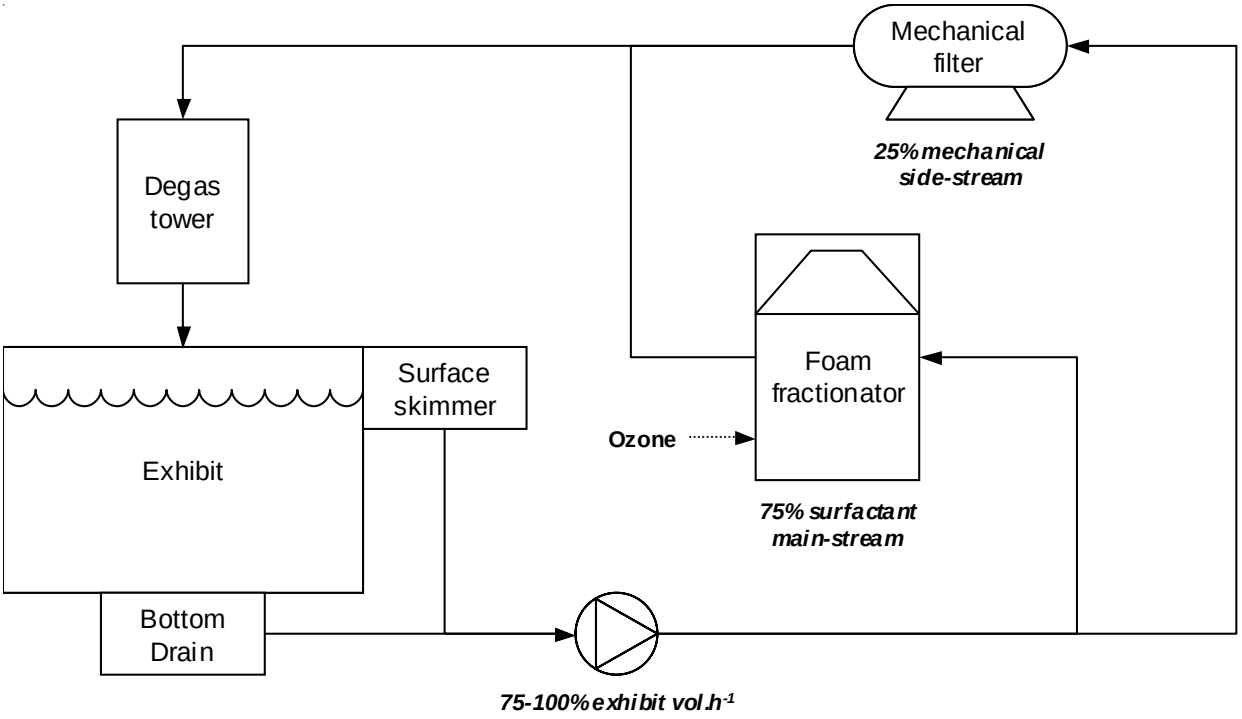


Figure 6.2. Basic process diagram of a fractionator-dominated LSS.

streams are employed. More recently the term has been used to denote a specific combination of separate rapid sand filter and foam fractionator streams (Johnson, pers. com.). This scheme uses less floor space and energy than the traditional rapid sand filter model. Parallel flow LSSs use particular water treatment processes where they are most needed: oils and surfactant-rich water from surface skimmers are directed to foam fractionators, while particulate-loaded bottom drain water is directed to rapid sand filters (Figure 6.3). Water flow is divided more or less equally between the two treatment streams and joins at a common degas chamber. Ozone can be added to a dedicated reaction chamber or foam fractionators as described above. A good example of this LSS philosophy may be found at Ripley's Aquarium of the Smokies, Gatlinburg, USA (Johnson, pers. com.).

Pre-ozonation

Pre-ozonation has been applied at Discovery Cove and is intended to address bacteriological issues having regulatory significance for the immersion of guests within exhibit water. The entire process stream receives ozone, injected into an open contactor via venturis, prior to particulate removal (Figure 6.4). Ozone acts as a micro-flocculent, improving mechanical filtration. An up-flow fluidized carbon bed is used to strip residual ozone and oxidants before water returns to the pool. A packed column degas chamber is employed to prevent supersaturation and to balance all dissolved gases (Johnson, pers. com.; Linn, pers. com.). It is believed that ozone reacting with carbon in the fluidized bed may be creating hydroxyl ions that enhance disinfection through a process known as advanced oxidation (Johnson, pers. com.).

ACKNOWLEDGEMENTS

We would like to express our special thanks to David LaBonne, Joel Johnson, John Linn, and Dave Powell for their input.

REFERENCES

Adey, W. H. and K. Loveland. 1991. Dynamic Aquaria - Building Living Ecosystems. Academic Press, San Diego, USA. 643 p.

Aiken, A. B. 1995a. Side-stream denitrification for large scale, high-nitrate aquarium exhibits. *In:* AZA Annual Conference Proceedings, September 15-19, Seattle, Washington, p. 10-

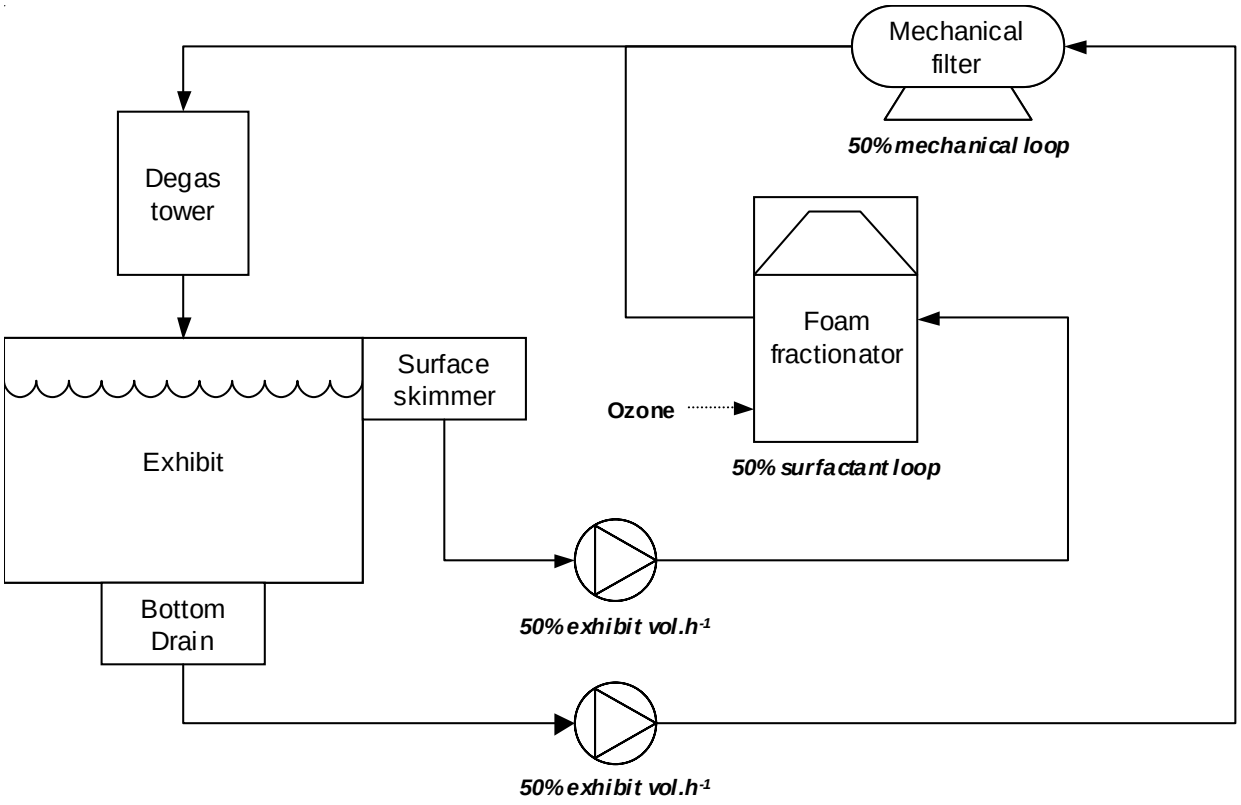


Figure 6.3. Basic process diagram of a parrallel flow LSS.

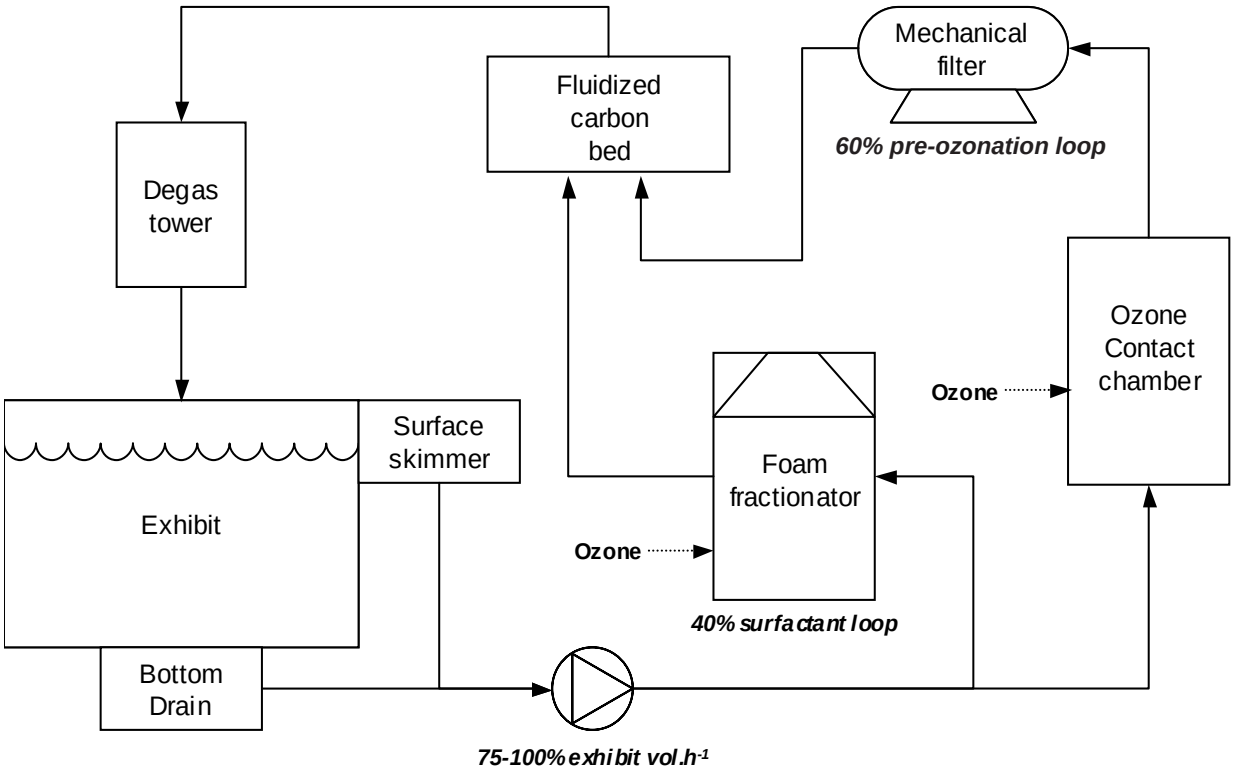


Figure 6.4. Basic process diagram of a pre-ozonation LSS.

16. American Zoo and Aquarium Association, Silver Spring, Maryland.

Aiken, A. B. 1995b. Use of ozone to improve water quality in aquatic exhibits. International Zoo Yearbook 34: 106-114.

Aiken, A. B. 2000. Application and benefits of retrofitting foam fractionators to existing life support systems. *In:* AZA Annual Conference Proceedings, September 24-28, Orlando, Florida, p. 237-241. American Zoo and Aquarium Association, Silver Spring, Maryland.

Anon. 1972. Water Quality Criteria. United States Government Printing Office, Washington, D. C. 593 p.

Atz, J. W. 1970. Water quality. *In:* Aquarium Design Criteria (Drum and Croaker Special Edition), p. 15-20. W. Hagen (ed.). National Fisheries Center and Aquarium, Washington D.C. (www6).

Bouck, G. R. 1980. Etiology of gas bubble disease. Transactions of the American Fisheries Society 109: 703-707.

Bouck, G. R. 1982. Gasometer: An inexpensive device for continuous monitoring of dissolved gases and supersaturation. Transactions of the American Fisheries Society 111: 505-516.

Brown, V. M., V. V. Mitrovic, and G. T. C. Stark. 1968. Effects of chronic exposure to zinc on toxicity of mixtures of detergent and zinc. Water Research 2(4): 225-263.

Brungs, W. A. 1969. Chronic toxicity of zinc to the fathead minnow, *Pimphales promelas* Rafinesque. Transactions of the American Fisheries Society 98(2): 272-279.

Chen, K. Y. 1974. Chemistry of sulfur species and their removal from water supplies. *In:* Chemistry of Water Supply, Treatment, and Distribution, p. 109-135. A. J. Rubin (ed.). Ann Arbor Science Publishers, Ann Arbor, Michigan.

Chiou, C-F., B. J. Mariñas, and J. Q. Adams. 1995. Modified indigo method for gaseous and aqueous ozone analyses, Ozone Science and Engineering 17: 329-344.

Cohrs, D. 2002. An introduction to ORP. Flow (Aquatic Animal Life Support Operators newsletter) 3(1): 8-12.

Colt, J. 1984. Computation of Dissolved Gas Concentrations in Water as Functions of Temperature, Salinity, and Pressure. Special Publication No. 14. American Fisheries Society, Bethesda, Maryland. 154 p.

Colt, J. 1986. Gas supersaturation - impact on the design and operation of aquatic systems. Aquacultural Engineering 5: 49-85.

Compagno, L. J. V. 1984. Sharks of the World, An annotated and Illustrated Catalogue of Shark Species Known to Date, Part 2 - Carcharhiniformes, FAO Species Catalogue, Volume 4, United Nations Development Program, Food and Aqriculture Organization of the United Nations, Rome, Italy. 655 p.

Conway, R. A. and Ross, R. D. 1980. Handbook of Industrial Waste Disposal. Van Nostrand Reinhold Company, New York, USA. 565 p.

Craig, H. and Weiss, R. F. 1971. Dissolved gas saturation anomalies and excess helium in the ocean. Earth and Planetary Science Letters 10: 289-296.

Crespo, S., Flos, R., Balasch, F., and Alonso, G. 1979. Zinc in the gills of the dogfish (*Scyliorhinus canicula* L.) related to experimental aquatic zinc pollution. Comparative Biochemistry and Physiology 63C: 261.

Furumai, H., H. Tagui, and K. Fujita. 1996. Effects of pH and alkalinity on sulfur-denitrification in a biological granular filter. Water Science and Technology 34(1-2): 355-362.

Grguric, G., J. A. Komas, and L. A. Gainor. 1999. Differences in major ions composition of artificial seawater from aquarium tanks at the New Jersey State Aquarium. Aquarium Sciences and Conservation 2: 145-159.

Hagen, W. 1970. Water Systems. *In:* Aquarium Design Criteria (Drum and Croaker Special Edition), p. 21-23. W. Hagen, (ed.). National Fisheries Center and Aquarium, Washington D.C. (www6).

Hawke, J. P. 1991. Potential effects of therapeutic agents on biological filtration in closed aquaculture systems. *In:* Proceedings of the Design of High-Density Recirculating

Aquaculture Systems Workshop, September 25-27, Baton Rouge, Louisiana, p. 56-60. Louisiana State University.
Hemdal, J. F. 1992. Reduction of ozone oxidants in synthetic seawater by use of sodium thiosulfate. Progressive Fish Culturist 54: 54-56.
Hignette, M. B. Lamont, M. Langouet, S. Leroy, and G. Martin. 1997. The removal of nitrates via biological sulfur based autotrophic denitrification in seawater. *In:* Proceedings of the European Union of Aquarium Curators Congress, Munich, Salzburg & Innsbruck, 1996, p. 7-13. P. Richard (ed.). Monaco l'Institut Océanographique: Memoires.
Hovanec, T. A. 1995. New trends in biological filtration. In: AZA Annual Conference Proceedings, September 15-19, Seattle, Washington, p. 217-223. American Zoo and Aquarium Association, Silver Spring, Maryland.
Hughes, J. S. 1968. Toxicity of some chemicals to striped bass (*Roccus saxitalis*). *In:* Proceedings of the 22nd Annual Conference of the Southeastern Association of Game and Fish Commissioners, 5 p. Louisiana Wildlife and Fisheries Commission, Baton Rouge.
Johnson, J. V. 2000. Application of ozone in marine parks and large aquariums. *In:* Proceedings of the 5th International Aquarium Congress), Bulletin de l'Institut Océanographique, Monaco. n° special 20 vol. 1, 430 p.
LaBonne, D. L. 2001. Aquarium life support system design entering the 21st century. Marine Technology Society Journal 35(1): 69-75.
LaRoche, G. 1972. Biological effects of short-term exposure to hazardous materials. *In:* Control of hazardous material spills. Proceedings of the 1972 National Conference on Control of Hazardous Material Spills, March 21-23, Houston, Texas, p. 199-206. Environmental Protection Agency, University of Houston.
Lloyd, R. 1961. The toxicity of mixtures of zinc and copper sulphates to rainbow trout (*Salmo gairdnerii* Richardson). Annals of Applied Biology 49: 535-538.
Merson, R. R. and H. L. Pratt, Jr. 2001. Distribution, movements and growth of young sandbar sharks, *Carcharhinus plumbeus*, in the nursery grounds of Delaware Bay. Environmental Biology of Fishes 61: 13-24.
Piedrahita, R. H. and G. R. Grace. 1991. Carbon dioxide removal for intensive aquaculture. *In:* Proceedings of the Design of High-Density Recirculating Aquaculture Systems Workshop, September, 25-27, Baton Rouge, Louisiana, p. 99-108. Louisiana State University.
Rasmussen, J. M., and M. M. Garner. 1999. Presumptive volatile organic compound intoxication of sharks and teleost fish in a newly constructed aquarium. *In:* AZA Annual Conference Proceedings, September 24-28, Minneapolis, Minnesota, p. 51-52. American Zoo and Aquarium Association, Silver Spring, Maryland.
Rozenblum, M. and D. L. LaBonne. 1995. Optimizing the use of air flotation in the design of aquarium life support systems. *In:* AZA Annual Conference Proceedings, September 15-19, Seattle, Washington, p. 446-454. American Zoo and Aquarium Association, Silver Spring, Maryland.
Rozenblum, M., and D. L. LaBonne. 2001. Bringing aquatic life indoors. Consulting-Specifying Engineer 9: 26-32.
Sorensen, E. M. B. 1991. Metal Poisoning in Fish. CRC Press, Boca Raton, Florida, USA. 374 p.
Spotte, S. 1992. Captive Seawater Fishes. John Wiley & Sons, New York, USA. 942 p.
Stonecypher, R. W., J. D. Fernandez, W. A. Hubert, and W. A. Gern. 1992. Increased copper concentration in water from chilling units with copper evaporator coils. The Progressive Fish Culturist 54: 61-63.
Wedemeyer, G. A., F. P. Meyer, and L. Smith. 1976. Diseases of Fishes. Book 5: Environmental Stress and Fish Diseases. S. F. Snieszko and H. R. Axelrod (eds.). T. F. H. Publications, Neptune City, NJ. 192 p.
Weitkamp, D. E. and M. Katz, 1980. A review of dissolved gas supersaturation literature. Transactions of the American Fisheries Society 109: 659-702.
Wheaton, F.W. 1977. Aquacultural Engineering, John Wiley & Sons, New York, USA. 708 p.
Zhang, T. C. and D. G. Lampe. 1999. Sulfur:limestone autotrophic denitrification processes for treatment of nitrate-contaminated water; batch experiments. Water Research 33(3): 599-608.
Zitko, V. and W. G. Carson. 1976. A mechanism of the effects of water hardness on the lethality of heavy metals to fish. Chemosphere 5: 299.

PERSONAL COMMUNICATIONS

Bailey, S. 2001. The New England Aquarium, Boston, MA 02110, USA.
Barber, T. 2000. Reef Ball Development Group, Woodstock, GA 30188, USA.
Billin, K. 2002. Landry's Downtown Aquarium, Houston, TX 77002, USA.
Choromanski, J. 1998. Ripley Aquariums, Inc., Orlando, FL 32819, USA.
Clarkson, P. 2002. Aquarium of the Pacific in Long Beach, Long Beach, CA 90802, USA.
Czaja, R. 2002. The Florida Aquarium, Tampa, FL 33602, USA.
Davis, R. 2002. SeaWorld Florida, Orlando, FL 32821, USA.
Gendron, S. 2001. Ocean Park, Aberdeen, Hong Kong, PRC.
Henningsen, A. 2001. National Aquarium in Baltimore, Baltimore, MD 21202, USA.
Jewell, J. 2001. Shark Reef at Mandalay Bay, Las Vegas, NV 89119, USA.
Johnson, J. 2001. Enartec, Inc., San Diego, CA 92111, USA.
Klocek, R. 1989. John G. Shedd Aquarium, Chicago, IL 60605, USA.
LaBonne, D. 2001. IDEA, Cambridge, MA 02138, USA.
Lerner, R. Colorado's Ocean Journey, Denver, CO 80211, USA,
Linn, J. Busch Entertainment Corporation, Clayton, MO 63105, USA.
Powell, D. 2001. Monterey Bay Aquarium (Retired), Monterey, CA 93940, USA.
Rozenblum, M. Syska Hennessy Group, Cambridge, MA 02142, USA.
Smith, M. F. L. 2001. IDEA, Cambridge, MA 02138, USA.
Thoney, D. 2002. Humboldt State University, California 95570, USA.
Violetta, G. 2001. SeaWorld Florida, Orlando, FL 32821, USA.
Zoller, C. 2002. The Rainforest and Aquarium at Moody Gardens, Galveston, TX 77554, USA.

INTERNET RESOURCES

www1 http://wlapwww.gov.bc.ca/wat/wq/BCguidelines/tgp

www2 http://www.fish-news.com/PH200.html

www3 http://www.epa.gov/cgi-bin/ecotox_quick_search

www4 http://www.toxnet.nlm.nih.gov

www5 http://www.reefball.com

www6 http://www.colszoo.org/internal/drumcroaker.htm

The Elasmobranch Husbandry Manual: Captive Care of Sharks, Rays and their Relatives, pages 89-103.
© 2004 Ohio Biological Survey

Chapter 7
Elasmobranch Capture Techniques and Equipment

ALLAN MARSHALL

Pittsburgh Zoo and PPG Aquarium
One Wild Place,
Pittsburgh, PA 15206, USA.
E-mail: allanm@zoo.pgh.pa.us

Abstract: Captive elasmobranchs are often collected using techniques modified from commercial fishing practices. However, elasmobranch species fill diverse niches within the marine environment and additional specialized fishing techniques have been developed. In general, there are four basic methods suitable for live capture: netting, trapping, hooking, and targeting. Hand-drawn nets are more forgiving than mechanically trawled nets, and when carefully deployed little physical injury will result. Small, baited traps are effective for catching sedentary elasmobranchs, but are not recommended for pelagic species. Larger, non-baited oceanic traps cause little or no injury to open water animals, as specimens are contained without restricting their swimming patterns. Angling with rod and reel allows an animal to be landed immediately, reducing capture-induced stress. Hooking with long-line can be effective if soak times are limited to less than two hours. Targeting may be employed if netting, trapping, and angling are not sufficiently discriminating to catch the desired elasmobranch. Targeting techniques include, among others, dip netting, SCUBA divers with catch bags, treble hooks cast over and snagging specific specimens, set hooking, and hooping.

Acquiring healthy elasmobranch specimens for aquariums and live research can be a labor-intensive operation. Contrary to popular belief, most elasmobranchs are extremely delicate and when removed from their natural habitat may suffer lethal physiological stress responses (Rasmussen and Rasmussen, 1967; Piiper and Baumgarten, 1969; Piiper et al., 1972; Mazeaud et al., 1977; Gruber, 1980; Holeton and Heisler, 1983; Wood et al., 1983; Cliff and Thurman, 1984; Meroz, 1990; Murru, 1990; Wood, 1991; Smith, 1992; Stevens, 1994). It is generally not practical to rely on by-catch specimens from commercial or recreational fisheries, as survival rates can be unacceptably low. Thus, it is usually necessary to conduct fishing operations using equipment and techniques specific to the species intended for capture. In some cases it may be possible to establish mutually beneficial relationships between aquariums and commercial fishing operations, taking advantage of both commercial fishing equipment and specialized animal handling techniques. In all cases, minimal handling and the minimization of injury to specimens will increase the chances of a successful acquisition.

PLANNING

Extensive planning is required to ensure the acquisition of appropriate animals for a given exhibit. Lists of potential species should take into consideration the size and physical limitations of an exhibit, the geographical area represented, species compatibility, species availability, and budgetary constraints (refer to Chapter 2 of this manual for more information about species selection). It is essential to understand the permitting processes as they pertain to the species selected (refer to chapter 3 of this manual for more information about permitting). Once a species list has been determined, further research into the requirements and habitat of each individual species should be undertaken. This research should yield information about possible commercial collectors, suitable fishing techniques, effective bait, specific handling and

equipment requirements, and appropriate fishing locations.

COMMERCIAL COLLECTORS

There are many professional commercial collectors of marine animals. Unfortunately, a few unscrupulous individuals mar the work of many excellent suppliers, so it is important to get references. The selected commercial collector must have the knowledge and skills to capture and, if required, transport elasmobranchs correctly. Where possible, their facilities should be inspected and the commercial collectors queried about their specific collection and transportation techniques. This information will provide a better understanding of their infrastructure and knowledge limitations. Having an experienced staff member accompany the commercial collector throughout the process will help ensure that best practices are used and provide reliable intelligence about how specimens were caught and subsequently treated (i.e., feeding techniques employed, medications given, etc.). It is imperative that the commercial collector has all the required permits prior to the commencement of fishing operations on behalf of the aquarium. All agreements should be in writing to avoid misunderstandings.

LOCATING SUITABLE SPECIMENS

Each elasmobranch species has specific habitat requirements. In addition, specific parameters (e.g., depth ranges, habitat preferences, and geographic locations) may vary seasonally (Gruber, 1980; Rupp, 1984; Burgess, 1985; Wisner, 1987; Boggs, 1992; Martin and Zorzi, 1993; Di Giacomo et al., 1994; Nakano et al, 1997; Fahy, pers. com.; Human, pers. com.). Understanding these parameters and how they relate to local conditions will increase the chances of successfully locating and capturing required specimens. For example, it is understood that sand tiger sharks (*Carcharias taurus*) undertake annual migrations within their home range and that these migrations usually correlate with seasonal changes in water temperature. The following migration routes are known for this species: Massachusetts to Northern Florida in the USA; Southern New South Wales to Southern Queensland in Australia; and Cape Town, South Africa to Southern Mozambique. Fishermen in the USA have noted that sand tigers will rarely take baited hooks at temperatures below 17°C (Barnhart, pers. com.). In Australia, the sand tiger shark prefers rocky reefs and in particular gutters or channels at depths of ~18 m.

COLLECTION TECHNIQUES

There are four distinct techniques used to capture live elasmobranchs: netting, trapping, hooking, and targeting. Table 7.1 summarizes the methods used to successfully collect different elasmobranch species for aquariums and live research. Quoted techniques are not a guarantee of survival. Capture technique, specimen size, handling time, transport regime, and water quality will all impact specimen survivability. Minimizing capture, handling, and transport times should be the ultimate goal of any expedition.

Netting

Nets can be used to capture a wide variety of elasmobranchs. When nets are carefully deployed, little physical injury results. Hand-drawn nets are more forgiving than mechanically operated and hauled nets, such as otter trawls. However, some sedentary species are hardy enough to survive trawled nets and these nets can therefore be an effective method for collecting resilient animals.

Seine netting

Seine nets are regularly used to collect many species of shallow water sharks and rays. A seine net consists of a length of mesh with sufficient dimensions to reach from the surface to the seabed. Lead weights are attached to the lower edge of the net, keeping it in contact with the bottom, while floats hold the upper edge of the net at the surface (Figure 7.1). If the water is clear and potential specimens can be seen, the net may be deployed, usually by a boat, in a circular pattern around the target animal(s). The net is laid in the boat in such a way as to let it progressively peel out, without tangling, as the boat moves forward. Once the animal is fully encircled, the net is slowly drawn in. The area in which the elasmobranch is swimming gradually decreases until it is fully confined and can be transferred to a transport container. In turbid water, the net can be deployed where target animals are suspected to be present and the net drawn in until capture has been verified. With a proficient knowledge of both target

Table 7.1. Elasmobranchs captured for aquariums and live research, showing successful techniques used. Many of the entries for otter trawl and gillnet are the result of by-catch from commercial fishing operations. All references are personal communications unless otherwise indicated by a date of publication.

Species name	Common name	Collection technique	Reference
Aetobatus narinari	Spotted eagle ray	Netting - cast net, otter trawl, seine	Marín-Osorno; Nemeth; Thomas
		Targeting - dip net, snagging, spearing	Christie; Henningsen; Long; Young
Aetomylaeus niehofii		Trapping - hadra	McEwan
Alopias vulpinus	Thintail thresher	Hooking - rod and reel	Thomas
Amblyraja radiata	Thorny skate	Netting - otter trawl	James; Kelleher
		Targeting - grabbing/hand net	Kelleher
Aptychotrema rostrata	Eastern shovelnose ray	Hooking - rod and reel	unpublished results
		Netting - otter trawl	
Asymbolus analis	Australian spotted catshark	Hooking - longline	Kinnunen
		Targeting - grabbing	Kinnunen
Bathyraja aleutica	Aleutian skate	Hooking - rod and reel	Thomas
		Netting - otter trawl	Thomas
Bathyraja interrupta	Sandpaper skate	Hooking - longline	Thomas
Brachaelurus waddi	Blind shark	Hooking - longline, rod and reel	Kinnunen
		Netting - gillnet	Kinnunen
		Targeting - grabbing	Kinnunen
		Trapping - baited trap	Kinnunen
Callorhinchus callorynchus	Cockfish	Netting - otter trawl	Di Giacomo et al., 1994
Carcharhinus acronotus	Blacknose shark	Hooking - longline, rod and reel	Christie; Henningsen; Marín-Osorno; Young
Carcharhinus altimus	Bignose shark	Hooking - longline, rod and reel	Powell; Powell, 2001
Carcharhinus amblyrhynchoides	Graceful shark	Hooking - longline, rod and reel	Stevens, et al., 2001
		Netting - gillnet	Stevens, et al., 2001
Carcharhinus amboinensis	Pigeye shark	Hooking - block line, longline, rod and reel	Ballard, 1989; Stevens, et al., 2001
		Netting - gillnet	Stevens, et al., 2001
Carcharhinus brachyurus	Copper shark	Hooking - longline, rod and reel	Kinnunen; Thomas
Carcharhinus brevipinna	Spinner shark	Hooking - longline, rod and reel	Henningsen; Kinnunen; Marín-Osorno
Carcharhinus dussumieri	Whitecheek shark	Hooking - rod and reel	McEwan
		Netting - otter trawl	McEwan
Carcharhinus falciformis	Silky shark	Hooking - longline, rod and reel	Christie; Marín-Osorno; Thomas; Young
Carcharhinus galapagensis	Galapagos shark	Hooking - rod and reel	Arai, 1997
Carcharhinus leucas	Bull shark	Hooking - block line, longline, rod and reel	Denton, et al., 1987; Ballard, 1989; Henningsen; Marín-Osorno; Thomas; Young
Carcharhinus limbatus	Blacktip shark	Hooking - block line, free float, longline, rod and reel	Christie; Henningsen; Mc Court; Marín-Osorno; Nemeth; Newman; Thomas; Young
		Netting - cast net, gillnet	Henningsen; Nemeth
Carcharhinus longimanus	Oceanic whitetip shark	Hooking - rod and reel	Powell
Carcharhinus macloti	Hardnose shark	Hooking - longline, rod and reel	Stevens, et al., 2001
		Netting - gillnet	Stevens, et al., 2001
Carcharhinus melanopterus	Blacktip reef shark	Hooking - rod and reel	unpublished results
		Netting - otter trawl	McEwan
		Targeting - chasing	Wisner, 1987
		Trapping - hadra	McEwan, 2000
Carcharhinus obscurus	Dusky shark	Hooking - block line, longline, rod and reel	Cliff and Thurman, 1984; Denton, et al., 1987; Ballard, 1989; Henningsen; Kinnunen; Steslow; Thomas

Table 7.1 (continued). Elasmobranchs captured for aquariums and live research, showing successful techniques used. Many of the entries for otter trawl and gillnet are the result of by-catch from commercial fishing operations. All references are personal communications unless otherwise indicated by a date of publication.

Species name	Common name	Collection technique	Reference
Carcharhinus perezi	Caribbean reef shark	Hooking - longline, rod and reel	Christie; Henningsen; Nemeth
		Netting - purse seine	Nemeth
Carcharhinus plumbeus	Sandbar shark	Hooking - block line, longline, rod and reel	Ballard, 1989; Andrews and Jones, 1990; Arai, 1997; Henningsen; Kelleher; Thomas; Young
		Netting - gillnet	Henningsen
Carcharhinus sorrah	Spottail shark	Hooking - longline, rod and reel	Stevens, et al., 2001
		Netting - gillnet	Stevens, et al., 2001; Henningsen
Carcharhinus tilstoni	Australian blacktip shark	Hooking - longline, rod and reel	Stevens, et al., 2001
		Netting - gillnet	Stevens, et al., 2001
Carcharias taurus	Sand tiger shark	Hooking - longline, rod and reel	Visser, 1996; Ellis; Henningsen; Kinnunen; Young
		Netting - gillnet	Kelleher
		Targeting - hooping, feeding hook	Smith, 1992; Menzies
		Trapping - pound net	Ellis
Carcharodon carcharias	Great white shark	Hooking - longline, rod and reel	Kinnunen; Powell; Thomas
		Netting - gillnet, trammel net	Powell; Thomas
Cephaloscyllium laticeps	Australian swellshark	Netting - otter trawl	Kinnunen
Cephaloscyllium ventriosum	Swellshark	Targeting - grabbing, hand collected eggs	Howard; Thomas
Chiloscyllium arabicum	Arabian carpetshark	Hooking - rod and reel	McEwan
		Netting - otter trawl	McEwan
		Trapping - hadra	McEwan, 2002
Chiloscyllium punctatum	Brownbanded bambooshark	Targeting - hand net	unpublished results
Chlamydoselachus anguineus	Frilled shark	Netting - gillnet, otter trawl	Shiobara, et al., 1997
Dasyatis americana	Southern stingray	Hooking - longline, rod and reel	Choe; Henningsen; Marín-Osorno; Nemeth; Thomas; Young
		Netting - cast net, seine	Choe; Christie
		Targeting - drop net, snagging	Christie; Nemeth; Young
Dasyatis brevicaudata	Short-tail stingray	Hooking - longline, rod and reel	Kinnunen
Dasyatis brevis	Whiptail stingray	Hooking - longline	Thomas
Dasyatis centroura	Roughtail stingray	Hooking - longline	Henningsen; Young
		Targeting - grabbing	Henningsen
		Trapping - pound net	Steslow
Dasyatis lata	Brown stingray	Hooking - rod and reel	Wisner
Dasyatis marmorata	Marbled stingray	Netting - seine	Sabalones
Dasyatis sabina	Atllantic stingray	Hooking - longline, rod and reel	Choe
		Netting - cast net	Choe
Dasyatis say	Bluntnose stingray	Hooking - longline, rod and reel	Choe; Henningsen
		Netting - cast net	Choe
Dasyatis violacea	Pelagic stingray	Hooking - rod and reel	Thomas
		Targeting - dip net	Thomas
		Trapping - piscina	unpublished results
Dipturus batis	Blue skate	Hooking - rod and reel	James
Dipturus laevis	Barndoor skate	Netting - otter trawl	Kelleher
Echinorhinus cookei	Prickly shark	Targeting - feeding hook	Powell
Galeocerdo cuvier	Tiger shark	Hooking - block line, longline, rod and reel	Denton, et al., 1987; Ballard, 1989; Christie; Henningsen; Kinnunen; Marín-Osorno; Thomas

Table 7.1 (continued). Elasmobranchs captured for aquariums and live research, showing successful techniques used. Many of the entries for otter trawl and gillnet are the result of by-catch from commercial fishing operations. All references are personal communications unless otherwise indicated by a date of publication.

Species name	Common name	Collection technique	Reference
Galeorhinus galeus	Tope shark	Hooking - longline, rod and reel	Howard; James; Thomas; Whitehead
		Netting - otter trawl	Janse
Galeus melastomus	Blackmouth catshark	Netting - otter trawl	Whitehead
Ginglymostoma cirratum	Nurse shark	Hooking - block line, longline, rod and reel	Carrier; Christie; Henningsen; Nemeth; Young
		Netting - cast net, seine	Carrier
		Targeting - feeding hook, grabbing, hand net	Carrier; Nemeth; Young
		Trapping - baited trap	Carrier; Christie; Nemeth
Gymnura altavela	Spiny butterfly ray	Hooking - longline	Henningsen
		Netting - seine	Henningsen
Gymnura marmorata	California butterfly ray	Hooking - longline	Thomas
Gymnura micrura	Smooth butterfly ray	Hooking - longline	Henningsen
		Targeting - hand net	Henningsen; Young
Haploblepharus edwardsii	Puffadder shyshark	Targeting - grabbing	Dainty; Human; Sabalones
Haploblepharus fuscus	Brown shyshark	Targeting - grabbing	Sabalones
Haploblepharus pictus	Dark shyshark	Targeting - grabbing	Dainty; Human; Sabalones
Hemiscyllium ocellatum	Epaulette shark	Targeting - hand net	Squire
Heterodontus francisci	Horn shark	Targeting - grabbing	Thomas
Heterodontus galeatus	Crested bullhead shark	Hooking - longline	Kinnunen
		Netting - gillnet	Kinnunen
		Targeting - grabbing	Kinnunen
Heterodontus portusjacksoni	Port Jackson shark	Hooking - longline	Kinnunen
		Netting - gillnet, otter trawl	Kinnunen
		Targeting - grabbing	Kinnunen
Hexanchus griseus	Bluntnose sixgill shark	Hooking - longline	Thomas
Himantura bleekeri	Bleeker's whipray	Netting - otter trawl	McEwan
		Trapping - hadra	McEwan
Himantura gerrardi	Sharpnose stingray	Netting - otter trawl	McEwan
		Trapping - hadra	McEwan, 2000
Himantura imbricata	Scaly whipray	Netting - seine, otter trawl	McEwan
		Trapping - hadra	McEwan
Himantura schmardae	Chupare stingray	Targeting - hoop net	Christie
Himantura uarnak	Honeycomb stingray	Netting - otter trawl	McEwan
		Trapping - hadra	McEwan, 2000
Hypnos monopterygium	Australian numbfish	Netting - otter trawl	unpublished results
Isurus oxyrinchus	Shortfin mako	Hooking - longline, rod and reel	Kinnunen; Marín-Osorno; Powell; Steslow; Thomas
		Trapping - piscina	unpublished results
Lamna nasus	Porbeagle	Hooking - rod and reel	James; Thomas
Leucoraja erinacea	Little skate	Netting - otter trawl	Kelleher
		Targeting - grabbing/hand net	Kelleher
Leucoraja naevus	Cuckoo ray	Hooking - rod and reel	Whitehead
		Netting - otter trawl	James
Leucoraja ocellata	Winter skate	Hooking - rod and reel	Whitehead
		Netting - otter trawl	Kelleher
		Targeting - grabbing	Kelleher

Table 7.1 (continued). Elasmobranchs captured for aquariums and live research, showing successful techniques used. Many of the entries for otter trawl and gillnet are the result of by-catch from commercial fishing operations. All references are personal communications unless otherwise indicated by a date of publication.

Species name	Common name	Collection technique	Reference
Manta birostris	Giant manta	Hooking - longline	Marín-Osorno
		Netting - seine, purse seine	Christie; Marín-Osorno
Mobula munkiana	Munk's devil ray	Netting - gillnet	Powell
Mustelus antarcticus	Gummy shark	Hooking - rod and reel	Kinnunen
Mustelus asterias	Starry smooth-hound	Hooking - rod and reel	Kelleher; Whitehead
		Netting - otter trawl	James; Janse
Mustelus californicus	Grey smooth-hound	Hooking - longline	Thomas
Mustelus canis	Dusky smooth-hound	Hooking - longline, rod and reel	Ellis; Henningsen
		Trapping - pound net	Ellis
Mustelus henlei	Brown smooth-hound	Hooking - longline, rod and reel	Howard; Thomas
		Netting - otter trawl	Howard
Mustelus mustelus	Smooth-hound	Hooking - rod and reel	Whitehead
		Netting - otter trawl	James; Janse
Myliobatis aquila	Common eagle ray	Netting - seine	unpublished results
Myliobatis australis	Australian bull ray	Hooking - longline, rod and reel	Kinnunen
Myliobatis californica	Bat eagle ray	Hooking - block line, longline, rod and reel	Howard; Powell; Thomas
		Netting - otter trawl	Howard
Myliobatis freminvillii	Bullnose eagle ray	Hooking - longline, rod and reel	Henningsen
Narcine brasiliensis	Brazilian electric ray	Targeting - hand net	Young
Nebrius ferrugineus	Tawny nurse shark	Targeting - hand net	unpublished results
Negaprion brevirostris	Lemon shark	Hooking - block line, longline, rod and reel	Henningsen; Nemeth; Thomas; Young
		Netting - cast net, dip net, gillnet	Gruber, 1980; Henningsen; Nemeth
Notorynchus cepedianus	Broadnose sevengill shark	Hooking - block line, longline, rod and reel	Rupp, 1984; Howard; Kinnunen; Powell; Thomas
		Netting - otter trawl	Howard; Kinnunen
Orectolobus maculatus	Spotted wobbegong	Hooking - longline, rod and reel	Kinnunen
		Netting - otter trawl	unpublished results
		Targeting - grabbing, hand net	Kinnunen
Orectolobus ornatus	Ornate wobbegong	Hooking - longline, rod and reel	Kinnunen
		Netting - otter trawl	unpublished results
		Targeting - grabbing, hand net	Kinnunen
Paragaleus randalli	Slender weasel shark	Netting - otter trawl	McEwan
Pastinachus sephen	Cowtail stingray	Netting - otter trawl	McEwan
		Trapping - hadra	McEwan
Platyrhinoidis triseriata	Thornback guitarfish	Hooking - longline	Thomas
Poroderma africanum	Striped catshark	Targeting - grabbing	Dainty; Human; Sabalones
Poroderma pantherinum	Leopard catshark	Targeting - grabbing	Dainty; Human; Sabalones
Potamotrygon spp.	Freshwater rays	Hooking - longline	Dowd
		Netting - cast net, gillnet, seine	Dowd
		Targeting - dip net, spearing	Dowd
Prionace glauca	Blue shark	Hooking - longline, rod and reel	James; Kinnunen; Powell; Steslow; Thomas
		Targeting - dip net	Howard; Powell
		Trapping - piscina	unpublished results
Pristis pectinata	Smalltooth sawfish	Netting - gillnet, otter trawl	Christie; Henningsen; Young
		Targeting - hand net, spearing	Christie; Young

Table 7.1 (continued). Elasmobranchs captured for aquariums and live research, showing successful techniques used. Many of the entries for otter trawl and gillnet are the result of by-catch from commercial fishing operations. All references are personal communications unless otherwise indicated by a date of publication.

Species name	Common name	Collection technique	Reference
Pristis spp.	Sawfishes	Netting - gillnet, otter trawl	Squire
Raja binoculata	Big skate	Hooking - longline, rod and reel	Howard; Thomas
		Netting - otter trawl	Howard
Raja brachyura	Blonde ray	Hooking - rod and reel	James; Whitehead
		Netting - otter trawl	James
Raja clavata	Thornback ray	Hooking - rod and reel	Whitehead
		Netting - otter trawl	James
Raja eglanteria	Clearnose skate	Hooking - longline, rod and reel	Henningsen; Young
Raja microocellata	Small-eyed ray	Netting - otter trawl	James
Raja montagui	Spotted ray	Netting - otter trawl	James
Raja rhina	Longnose skate	Hooking - longline	Thomas
Raja stellulata	Starry skate	Hooking - longline, rod and reel	Howard; Thomas
		Netting - otter trawl	Howard
Raja undulata	Undulate ray	Hooking - rod and reel	Whitehead
		Netting - otter trawl	James
Rhina ancylostoma	Bowmouth guitarfish	Netting - otter trawl	McEwan; Squire
		Trapping - hadra	McEwan, 2000
Rhincodon typus	Whale shark	Targeting - Restrained	Kinnunen
Rhinobatos annulatus	Lesser sandshark	Netting - seine	Sabalones
Rhinobatos granulatus	Sharpnose guitarfish	Netting - otter trawl	McEwan
		Trapping - hadra	McEwan, 2000
Rhinobatos lentiginosus	Atlantic guitarfish	Targeting - grabbing, hand net	Henningsen; Young
Rhinobatos productus	Shovelnose guitarfish	Hooking - longline	Thomas
Rhinobatos typus	Giant shovelnose ray	Hooking - rod and reel	unpublished results
		Netting - otter trawl	Kinnunen
		Netting - otter trawl	unpublished results
Rhinoptera bonasus	Cownose ray	Hooking - longline, rod and reel	Marín-Osorno; Thomas
		Netting - seine	Thomas; Young
		Trapping - pound net	Henningsen; Kelleher
Rhizoprionodon acutus	Milk shark	Hooking - longline, rod and reel	Stevens, et al., 2001
		Netting - gillnet	Stevens, et al., 2001
Rhizoprionodon longurio	Pacific sharpnose shark	Hooking - longline, rod and reel	Powell, 2001; Thomas
Rhizoprionodon porosus	Carribean sharpnose shark	Hooking - longline, rod and reel	Henningsen; Nemeth
Rhizoprionodon taylori	Australian sharpnose shark	Hooking - longline, rod and reel	Stevens, et al., 2001
		Netting - gillnet	Stevens, et al., 2001
Rhizoprionodon terraenovae	Atlantic sharpnose shark	Hooking - longline, rod and reel	Christie; Henningsen; Marín-Osorno; Steslow
Rhynchobatus djiddensis	Giant guitarfish	Netting - otter trawl, seine	Kinnunen; McEwan
		Trapping - hadra	McEwan, 2000
Scyliorhinus canicula	Smallspotted catshark	Hooking - rod and reel	Whitehead
		Netting - otter trawl	Janse
		Targeting - grabbing	Whitehead
Scyliorhinus retifer	Chain catshark	Hooking - rod and reel	Nelson
		Netting - otter trawl	Kelleher
		Trapping - baited trap	Ellis; Kelleher

Table 7.1 (continued). Elasmobranchs captured for aquariums and live research, showing successful techniques used. Many of the entries for otter trawl and gillnet are the result of by-catch from commercial fishing operations. All references are personal communications unless otherwise indicated by a date of publication.

Species name	Common name	Collection technique	Reference
Scyliorhinus stellaris	Nursehound	Hooking - rod and reel	Whitehead
		Netting - otter trawl	James
		Targeting - grabbing	Whitehead
Somniosus pacificus	Pacific sleeper shark	Hooking - longline	Thomas
Sphyrna lewini	Scalloped hammerhead	Hooking - longline, rod and reel	Arai, 1997; Henningsen; Newman; Thomas; Young.
Sphyrna mokarran	Great hammerhead	Hooking - longline, rod and reel	Henningsen; Marín-Osorno; Young
		Netting - gillnet	Henningsen
Sphyrna tiburo	Bonnethead	Hooking - longline, rod and reel	Henningsen; Newman; Thomas; Young
		Netting - cast net, gillnet, seine	Henningsen; Powell; Thomas
Sphyrna zygaena	Smooth hammerhead	Hooking - rod and reel	Henningsen; Kinnunen; Thomas
Squalus acanthias	Spiny dogfish	Hooking - longline, rod and reel	Ellis; Howard; Kelleher; Thomas; Whitehead
		Netting - otter trawl	James; Janse; Whitehead
		Trapping - pound net	Ellis
Squatina australis	Australian angelshark	Hooking - longline	Kinnunen
Squatina californica	Pacific angelshark	Targeting - plastic bag	Howard
Squatina dumeril	Sand devil	Netting - otter trawl	Kelleher; Marín-Osorno
Squatina squatina	Angelshark	Netting - otter trawl	James
Stegostoma fasciatum	Zebra shark	Hooking - longline	Kinnunen
		Netting - gillnet, otter trawl	Henningsen; Kinnunen
		Targeting - grabbing, hand net	McEwan
Taeniura lymma	Bluespotted ribbontail ray	Targeting - hand net	McEwan
Torpedo californica	Pacific electric ray	Targeting - hand net	Howard
Torpedo marmorata	Marbled electric ray	Netting - otter trawl	James
Torpedo nobiliana	Electric ray	Netting - otter trawl	James; Kelleher; Whitehead
Torpedo panthera	Panther electric ray	Netting - otter trawl	McEwan
Triaenodon obesus	Whitetip reef shark	Hooking - rod and reel	unpublished results
		Targeting - hand net	McEwan
Triakis megalopterus	Sharptooth houndshark	Hooking - rod and reel	Sabalones
Triakis semifasciata	Leopard shark	Hooking - rod and reel	Howard; Thomas
		Netting - barrier net, otter trawl	Howard; Thomas
Trygonorrhina fasciata	Southern fiddler	Hooking - longline, rod and reel	Kinnunen
		Netting - otter trawl	unpublished results
		Targeting - hand net	Kinnunen
Urolophus halleri	Haller's round ray	Targeting - hand net	Henningsen; Thomas; Young
Urolophus jamaicensis	Yellow stingray	Targeting - hand net	Christie; Fahy; Thomas; Young

Figure 7.1. Seine net used to surround, enclose, and capture potential elasmobranch specimens.

species and fishing areas, this method can be productive.

Multi-strand nylon is an appropriate material for nets, and woven, knotless nets are preferred as they are less likely to injure a struggling animal. Mesh size for seine nets is usually small (~2.5 cm stretched mesh), but can be larger depending on the size of the target species. Oversized mesh is not recommended as an animal can push its head through the net (in a similar fashion to a gill net), become entangled, and ultimately injured.

Purse seine nets are used in a similar fashion to seine nets, however the bottom of the net is drawn together once deployed, when used in deeper water, to prevent animals from escaping.

Gill netting

Gill nets have a larger mesh size than seine nets. Gill nets are secured in an area, where the presence of a target species has been established, and are left in position for a set period of time. As the name implies, specimens are caught by entangling their heads in close proximity to the gills. Injuries sustained from gill nets are frequently lethal to captured specimens. Many species of elasmobranchs will die if constrained for extended periods, as gas exchange and blood circulation are impaired (Denton et al., 1987; Meroz, 1990; Smith, 1992). Gill nets are therefore not recommended for the live capture of elasmobranchs (Murru, 1990; Stevens, 1994; Shiobara et al., 1997) unless they are constantly monitored (Meroz, 1990; Stevens et al., 2000). If another option for the capture of specimens is available, it should be considered before gill netting is used. In some specific cases, where other options are not available, it is possible to work closely with commercial gillnet fishermen and harvest live specimens recently caught in their nets (Shiobara et al., 1997; Kelleher, pers. com.; Powell, pers. com.).

Sawfishes (Family: Pristidae) are easily ensnared and their numbers have dropped markedly in areas where commercial fishermen use gill nets. If a gill net is constantly monitored, live sawfish specimens may be readily caught, removed, and suffer little injury, as they are usually entangled around their robust saw (Squire, pers. com.; Young, pers. com.).

Cast netting

Cast nets have been employed to capture Atlantic (*Dasyatis sabina*), southern (*Dasyatis americana*), and bluntnose (*Dasyatis say*) stingrays, and other shallow water species of elasmobranch (Gruber, 1980; Murru, 1990; Choe, pers. com.). Cast nets may be thrown from a boat or the shoreline and are most effective when used in water <1 m deep (Figure 7.2). When a cast net has settled over a buried ray, gentle prodding will encourage it to rise out of the sand and move into the net.

Trawling

Otter trawl nets are commonly employed by the commercial fishery to collect crustaceans. Elasmobranchs are a frequent by-catch of the fishery. A conical shaped net is dragged along the sea floor by a boat on the surface. Angled boards are attached to each side of the net, holding the

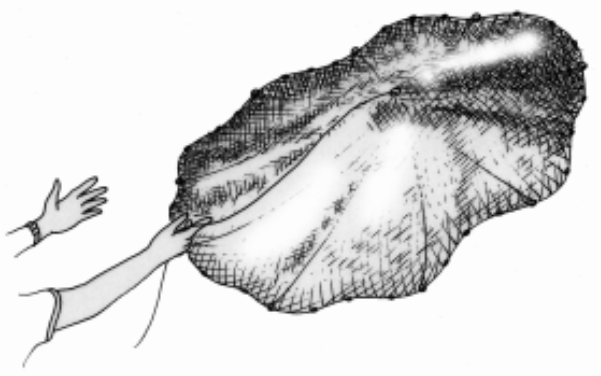


Figure 7.2. A cast net requires practice to be thrown correctly and is most effective in shallow water.

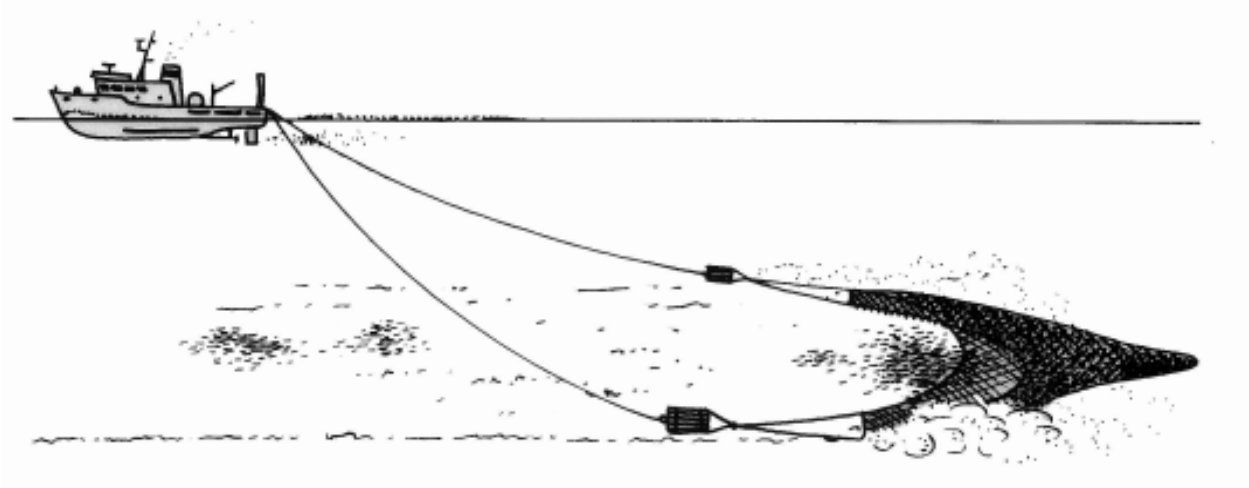


Figure 7.3. An otter trawl showing the otter boards on either side keeping the net open as it is towed.

net open during trawling (Figure 7.3). Animals displaced from the bottom are caught in the net and, following a set period of trawling time, brought to the surface. Although this method of fishing damages most animals, sedentary species of elasmobranchs (e.g., rays and guitarfishes) often fare well, providing an opportunity to acquire healthy specimens. Smaller versions of the otter trawl net have been used by aquariums to collect inshore coastal species for display (Howard, pers. com.). Di Giacomo et al. (1994) collected healthy specimens of the cock fish (*Callorhinchus callorynchus*) using otter trawl nets deployed from research fishing vessels in Patagonia. Towing time was limited to 30 minutes and net retrieval was deliberately slow, reducing possible damage to the animals as they were raised from a depth of 50-100 meters. Because of the risks to captured specimens, other capture techniques should be considered before trawling is employed.

Trapping

Trapping can be an effective technique to collect elasmobranchs, allowing extended fishing periods and minimizing stress on captured specimens. The basic premise of trapping is to confine animals in a small holding area where they can be collected at a later time. Some traps use bait, while others exploit the natural swimming behavior of target animals.

Baited trapping

Several designs of baited traps are used in the fishing industry to collect different species of animal. These traps usually consist of a box-like frame, over which is stretched a mesh of wire or netting, and one or two small, funnel-shaped openings. Animals can easily enter the mouth of the funnel from the exterior, but have difficulty exiting the narrow spout from the interior. Bait is usually placed inside the trap and it is then lowered to the sea floor for a predetermined period of time. Baited traps are effective for catching sedentary species of elasmobranch, but are not recommended for pelagic species.

Non-baited trapping

Larger traps capture animals by exploiting their natural behavior and herding them into a relatively small area. Native fishermen in Kuwait use a trap called a hadra (Figure 7.4) to collect a large variety of fishes (McEwan et al., 2000). The hadra has a design similar to pound nets used by professional fishermen on the East Coast of the USA (Murru, 1990). These traps consist of a long wall of partially submerged netting positioned perpendicular to the coastline. Fishes swimming parallel to the shore encounter the wall and instinctively turn toward deeper water to avoid the obstruction. When they reach the end of the wall, fishes are directed into a corral-shaped net where they continue to swim until removed by the fishermen. Hadras are positioned between the high and low tide marks of a gently sloping shoreline. As the tide recedes, fishes are left with no alternative but to enter a small section of the corral called the ser. In contrast, the corral of pound nets is positioned in deeper water, beyond the low tide mark, and have a floor of netting that can be raised by the fishermen to concentrate the

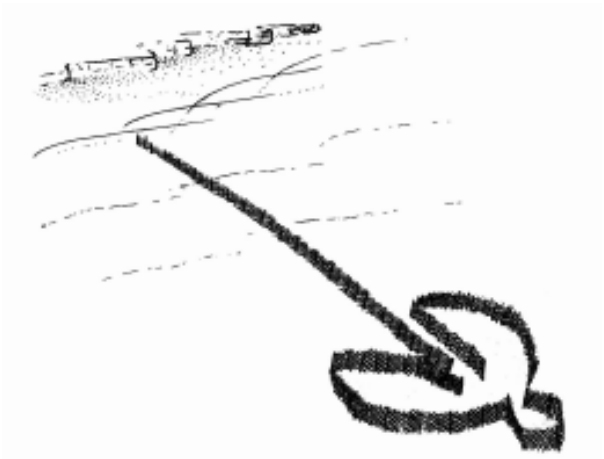


Figure 7.4. The "hadra" trap is positioned perpendicular to the shoreline. As fishes swim along the shoreline, they encounter the fence, head toward deeper water, and become trapped in the "ser".

animals in a smaller area. A larger version of these traps is used in the Mediterranean to capture tuna for the Japanese sushi market. These larger traps frequently collect free-swimming, demersal and pelagic sharks.

An advantage of these large traps is that they cause little or no injury to the animals. Specimens are contained without restricting their swimming patterns and stress is presumably limited to the time taken for handling and transport. Many elasmobranch species have successfully been collected using this technique. Most notable are the pelagic blue (*Prionace glauca*) and shortfin mako (*Isurus oxyrinchus*) sharks, notoriously difficult to capture alive using other methods.

Because of the complexity of these large traps and the labor required to keep them operational, it is usually not feasible for an aquarium to own and operate one. Rather, aquarium personnel should build a relationship with trap fishermen so that suitable animals may be acquired for display.

Hooking

The most common method for catching elasmobranchs is to fish them with a baited hook and line (Gruber, 1980; Cliff and Thurman, 1984; Rupp, 1984; Lawlor, 1985; Denton et al., 1987; Ballard, 1989; Jenkins, 1989; Andrews and Jones, 1990; Murru, 1990; Boggs, 1992; Stevens, 1994; Visser, 1996; Arai, 1997; Nakano et al., 1997; Stevens et al., 2000; Martin and Zorzi, 1993; Harrington, pers. com.; Wisner, pers. com.). Elasmobranchs are opportunistic feeders and will readily take bait when offered. Baited hooks may be monitored (e.g., angling) or set and left to fish remotely for a period of time (long-line fishing).

Angling

The advantage of angling with a rod and reel is that an animal can be landed as soon as it has been caught, reducing capture-induced stress (Murru, 1990; Stevens et al., 2000). The movements of live bait are an effective attractant to target elasmobranchs. The size and design of fishing tackle depends on the desired species and size ranges. A long wire trace is essential as the teeth and skin of most elasmobranchs can easily abrade and sever nylon fishing line, freeing the animal. Wire can, however, cause injury to the animal, so plastic-coated traces are recommended (Wisner, pers. com.).

Long-line fishing

Long-line fishing has been used for many years in the commercial fishing industry. Typical long-lines consist of a single line, up to several kilometers long, having hooks attached by short lines (gangions or snoods) at fixed distances along the line. During commercial fishing activities the line, having several thousand gangions, is left to soak for ~24 hours. Many marine animal collectors and aquarium staff have used modified long-line fishing techniques to capture elasmobranchs for display purposes (Rupp, 1984; Jenkins, 1989; Murru, 1990). Minimizing the time between hooking and landing an elasmobranch should be the top priority. This precaution reduces the chances of specimens succumbing to biochemical changes induced by extended capture stress. Thus, for the live capture of elasmobranchs, long-lines are typically reduced to a length of ~300-400 meters and ~50 hooks, and soak times restricted to less than two hours (Murru, 1990). Even fewer hooks can be used if the density of target species is known to be high. Capture stress can be further reduced by attaching the gangions to long leaders, allowing specimens to swim freely once hooked (Denton et al., 1987; Ballard, 1989; Murru, 1990; Boggs, 1992).

Gangions typically consist of a 2 m (x 4 mm diameter) length of nylon rope attached by a large swivel to an additional 1.5 m (x 3 mm diameter) length of heavy, plastic-coated wire. A hook is attached to the end of the wire trace. The heavy line and wire make it easier to haul in captured

specimens quickly. Gangions are usually attached to the long-line with a stainless steel clip. When a specimen is brought to the boat, it can be disconnected from the long-line and lifted into a transport container. The hook is then cut with a pair of bolt cutters, allowing it to be easily removed without further injury to the specimen. If the specimen is too large to be landed, the gangion may be detached from the long-line and the specimen guided to an area where it can be transferred to a transport container or a sea pen (Denton et al., 1987).

A variation of the long-line, whereby individual gangions are attached to individual floats, has been used successfully to collect blacktip sharks (*Carcharhinus limbatus*) in shallow waters (McCourt, pers. com.). This gear, referred to as a free float, is baited with live fish, set within a 300-meter radius of the fishing boat, and closely monitored. When a bait is taken the float is tracked until the boat can get close enough to snare it with a boat hook. The specimen is then landed and placed in a transport container. This technique has proven successful in shallow-water estuaries where it is often impractical to set long- lines. Capture success is high, when using this technique, as animals can run with the bait for a sufficient time to allow the hook to become properly set.

Block-line fishing

Denton et al. (1987) have captured elasmobranchs using a baited hook attached to a 50-meter rope and large, anchored buoy (Figure 7.5). Captured specimens were thus given sufficient freedom to maintain normal swimming patterns and this increased the time that viable specimens could remain on the line. This technique is called block-line fishing in the USA (Henningsen, pers. com.; Young, pers. com.) and drum-line fishing in Australia.

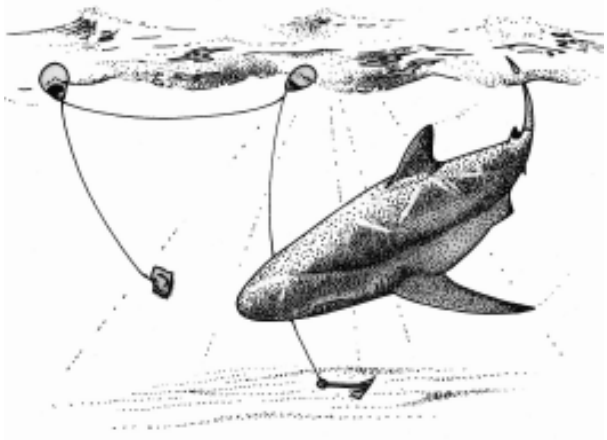


Figure 7.5. Block-line or drum-line fishing allows captured animals freedom to continue normal swimming patterns until retrieved.

Hooks

Any hooking technique has the risk of a specimen swallowing the hook and sustaining internal damage. Using a Mustad circle hook (O. Mustad & Son A. S., Gjøvik, Norway) (Figure 7.6) will reduce this risk as they embed primarily in the lower jaw or jaw hinge (Skomal, 2002; Harrington, pers. com.; Wisner, pers. com.). Wisner (pers. com.) suggests crimping down the barb of circle hooks to further reduce injuries incurred while angling.

Wisner (pers. com.) has used 85-113 gram lead sinkers, attached close to a hook, to prevent brown stingrays (*Dasyatis lata*) swallowing the hook when caught. The sinker is too large to fit into the mouth of the rays and therefore the hook does not get past the buccal cavity. Harrington (pers. com.) has adopted a similar technique, using a stainless steel rod (~30 cm long x 3-4 mm diameter) attached perpendicularly to the trace, to prevent hooks passing beyond the jaws of sand tiger sharks and sandbar sharks (*Carcharhinus plumbeus*) during long-line fishing operations.

Targeting

In some cases, the methods described above are not sufficiently discriminating to catch the size class or species of elasmobranch desired. In these cases, a more selective method of collection, e.g., targeting, may be required.

Blue sharks have been attracted to the side of a capture vessel using chum, dip-netted out of the water, and transferred directly into a transport tank on board (Howard, pers. com.).

Pacific angel sharks (*Squatina californica*) have been collected using large, perforated heavy-duty, plastic bags manipulated by a team of three or four SCUBA divers. Once a suitable specimen was located, two of the divers held the bag open near the shark and the other diver(s) maneuvered the shark into the bag (Howard, pers. com.).

Juvenile blacktip reef sharks (*Carcharhinus melanopterus*) have been chased through the reef-flat shallows of Christmas Island and successfully

Figure 7.6. Mustad circle hooks (left) of size 11/0 (top) and 12/0 (bottom), and Mustad number 9174 straight hooks (right) of size 6/0 (top) and 7/0 (bottom). A 1.0 cm scale is provided in the center of the figure. From Skomal et al. (2002).

caught with hoop nets (Wisner, 1987).

Striped catsharks (*Poroderma africanum*), leopard catsharks (*Poroderma pantherinum*), puffadder shysharks (*Haploblepharus edwardsii*), and dark shysharks (*Haploblepharus pictus*) are particularly easy to collect, as they are generally slow-moving and approachable. A mesh dive bag containing bait is tied to the holdfast of some nearby kelp. When the desired species comes in to feed they are simply caught by hand, just behind the head, and placed into a mesh dive bag. This method has been used to capture specimens ranging from 25-100 cm TL (Dainty, pers. com.; Human, pers. com.).

Sedentary tropical coral reef elasmobranchs are often selectively collected by SCUBA divers using hand nets. Once a specimen has been caught, mesh is twisted over the net entrance to prevent the animal from escaping (Squire, pers. com.; Young, pers. com.).

An older method of collecting large rays (e.g., spotted eagle rays, *Aetobatus narinari*) was to spear the selected specimen through the lateral margin of the pectoral fin. The spear was attached to the gun with a long cord, allowing the animal to be dragged to the boat and transferred to a transport container. Injuries to animals were considered relatively minor and wounds healed quickly. Prophylactic antibiotics were frequently given (Long, pers. com.).

A technique used to collect many species of ray is to locate the target specimen, cast a small barb-less treble hook over the animal, and snag the edge of the pectoral fin (Young, pers. com.). The animal is quickly brought to the boat with a rod and reel, as per normal angling. This technique has proven successful, causing minimal injury.

Sand tiger sharks swallow and store air in their stomach to aid buoyancy. If a sand tiger shark is brought to the surface from great depth, gastric emboli may result (Smith, 1992). It is therefore advantageous to burp the air from these animals prior to bringing them to the surface. Two capture methods adopted in Australia have been directed at addressing this issue: set hooking and hooping.

Set hooking uses a standard angling rig with a long steel trace (~3 m) and a toggle positioned close to the end of the line. A SCUBA diver takes the baited hook and feeds it to a selected shark. Once the shark has taken hold of the bait, the diver waits for a few moments before setting the hook by pulling on the toggle. The diver orients the hook so that it imbeds in the lower jaw, causing minimal injury to the specimen. It is important that the surface angler maintains tension on the line, as soon as the hook is set, to prevent the shark from dislodging or swallowing the hook (Menzies, pers. com.).

Hooping employs a rope noose suspended inside a large, rigid hoop. SCUBA divers swim above the shark and lasso the specimen as it swims through the hoop. When the shark is partially through the hoop, as far as the leading edge of its pectoral fins, the noose is pulled taut. The captured shark is thus restrained by the rope around the pectoral girdle. After a brief struggle, the animal is burped, brought to the surface, and transferred to a transport container (Smith, 1992).

CONCLUSIONS

Numerous techniques have been used to acquire live elasmobranchs for display or research purposes. In general, it is essential to understand

the habits and requirements of required species, and to select an appropriate capture method to ensure success. Although little has been published about elasmobranch capture techniques, much information is available from experienced collectors and aquarium personnel. Individuals intending to acquire elasmobranchs would do well to consult these people and heed their advice. Once caught, elasmobranchs need to be handled and transported with great care to avoid excess physical trauma and stress (please refer to Chapter 8 of this manual for more information about the handling and transport of elasmobranchs).

ACKNOWLEDGEMENTS

Aside from the people with whom I have had personal communication, thanks must go to all staff from the institutions cited below. Many of the techniques described in this chapter have arisen from the hard work and inspiration of these individuals.

REFERENCES

Andrews, J. C. and R. T. Jones. 1990. A method for the transport of sharks for captivity. Journal of Aquariculture and Aquatic Sciences 5: 70-72.

Arai, H. 1997. Collecting, transporting and rearing of the scalloped hammerhead. *In:* Proceedings of the Fourth International Aquarium Congress, June 23-27, 1996, Tokyo, p. 87-89. Tokyo, Japan by the Congress Central Office of IAC '96, Tokyo Sea Life Park. 402 pp.

Ballard, J. A. 1989. Some specimen collecting and transport methods. Bulletin de l'Institut Océanographique, Monaco, Nº spécial 5: 63-69.

Boggs, C. H. 1992. Depth, capture time, and hooked longevity of longline-caught pelagic fish: Timing bites of fish with chips. Fishery Bulletin 90(4): 642-658.

Burgess Jr., G. H. 1985. Biology of sharks as related to commercial shark fishing. *In:* Manual on Shark Fishing: a compilation of papers, p. 4-10. W. S. Otwell, (ed.). University of Florida, Sea Grant Extension Program, Gainesville.

Cliff, G. and G. D. Thurman. 1984. Pathological and physiological effects of stress during capture and transport in the juvenile dusky shark, *Carcharhinus obscurus*. Comparative Biochemistry and Physiology 78A: 167-173.

Denton, N. R., G. Cliff, A. P. Bowmaker, and G. D. Thurman, 1987. The capture, transport and maintenance in captivity of large sharks for experimental purposes in the field. Oceanographic Research Institute (Durban). Poster No. 2. Presented at the Sixth National Oceanographic Symposium, University of Stellenbosch, South Africa, July 6-10, 1987. FR 33(4).

Di Giacomo, E. E., A. M. Parma, and J. M. Orensanz. 1994. Food consumption by the cock fish, *Callorhinchus callorhynchus*, (Holocephali: Callorhynchidae), from Patagonia (Argentina). Environmental Biology of Fishes 40: 199-211.

Gruber, S. H. 1980. Keeping sharks in captivity. Journal of Aquariculture and Aquatic Sciences 1: 6-14.

Holeton, G. F. and N. Heisler. 1983. Contribution of net ion transfer mechanisms to acid-base regulation after exhausting activity in the larger spotted dogfish (*Scyliorhinus stellaris*). Journal of Experimental Biology 103: 31-46.

Jenkins, R. L. 1989. The captive biology of elasmobranchs at the National Aquarium in Baltimore. *In:* Annual Conference Proceedings, September 15-19, 1989, Pittsburgh, PA, p. 209-216. American Association of Zoological Parks and Aquariums (American Zoo and Aquarium Association, Silver Spring, Maryland).

Lawlor, F. J. 1985. Shark fishing methods and gear. *In*: Manual on shark fishing: a compilation of papers, p. 11-19. Otwell, W. S. (ed.). University of Florida, Sea Grant Extension Program, Gainesville.

Martin, L., and Zorzi, G. D. 1993. Status and review of the California skate fishery. *In*: Conservation Biology of Elasmobranchs, p. 39-52. S. Branstetter (ed.). US Department of Commerce, NOAA Technical Report, NMFS. Vol. 115.

Mazeaud, M. M., F. Mazeaud, and E. M. Donaldson. 1977. Primary and Secondary effects of stress in fish: Some new data, with a general view. Transactions of the American Fisheries Society 106(3): 201-212.

McEwan, T., S. A. F. Bell, and M. J. Dvornak. 2001. The "hadra" fish trap: a technique to acquire fishes for aquaria. *In:* Proceedings: Fifth International Aquarium Congress, November 20-25, 2000, Monaco. p. 205-208. Bulletin de l'Institute oceanographique, Monaco, No. spécial 20, part 1.

Meroz, A. 1990. Innovations in shark fishing. Journal of Aquariculture and Aquatic Sciences 5(4): 118. Abstract.

Murru, F. L. 1990. The care and maintenance of elasmobranchs in controlled environments. *In*: Elasmobranchs as Living Resources: Advances in Biology, Ecology, Systematics, and the Status of Fisheries, p. 203-209. H. R. Pratt, S. H. Gruber, and T. Taniuchi (eds.). US Department of Commerce, NOAA Technical Report. Vol. 90.

Nakano, H., M. Okazaki, and H. Okamoto. 1997. Analysis of catch depth by species for tuna longline fishery based on catch by branch lines. Bulletin - National Research Institute of Far Seas Fisheries (Japan) 34: 43-62.

Piiper, J., and D. Baumgarten. 1969. Blood lactate and acid-base balance in the elasmobranch *Scyliorhinus stellaris* after exhausting activity. Pubblicazioni della Stazione Zoologica 37: 84-94.

Piiper, J., M. Meyer, and F. Drees. 1972. Hydrogen ion balance in the elasmobranch *Scyliorhinus stellaris* after exhausting activity. Respiration Physiology 16: 290-303.

Powell, D. 2001. A Fascination for Fish. Adventures of an Underwater Pioneer. University of California Press/ Monterey Bay Aquarium Series in Marine Conservation, 3, Berkeley. 354 p.

Rasmussen, R. A. and L. E. Rasmussen. 1967. Some observations on the protein and enzyme levels and fractions in normal and stressed elasmobranchs. Transactions of the New York Academy of Science 29: 397-413.

Rupp, J. H. 1984. The Capture and Husbandry of Cowsharks, Family Hexanchidae. *In:* Annual Conference Proceedings, September 9-13, 1984, Miami, FL, p. 325-332. American Association of Zoological Parks and Aquariums (American Zoo and Aquarium Association, Silver Spring, Maryland).

Shiobara, Y., K. Suzuki, and S. Tanaka, 1997. Ecological notes and rearing on the frilled shark from Suruga Bay. *In:* Proceedings of the Fourth International Aquarium Congress, June 23-27, 1996, Tokyo. p. 75-78. Tokyo,

Japan by the Congress Central Office of IAC '96, Tokyo Sea Life Park. 402 pp.

Skomal, G. B., C. C. Bradford, and E. D. Prince. 2002. A comparison of circle hook and straight hook performance in recreational fisheries for juvenile Atlantic bluefin tuna. *In:* Catch and Release in Marine Recreational Fisheries, Symposium 30, p. 57-65. J. A. Lucy and A. L. Studholme (eds.). American Fisheries Society, Bethesda, Maryland.

Smith, M. F. L. 1992. Capture and transportation of elasmobranchs, with emphasis on the grey nurse shark (*Carcharias taurus*). Sharks: Biology and Fisheries 43(1): 325-343.

Stevens, J. E. 1994. The delicate constitution of sharks. Bioscience 44(10): 661-664.

Stevens, J. D., G. J. West, and K. J. McLoughlin. 2000. Movements, recapture patterns, and factors affecting the return rate of carcharhinid and other sharks tagged off northern Australia. Marine and Freshwater Research 51(2): 127-141.

Visser, J. 1996. The capture and transport of large sharks for Lisbon Zoo. International Zoo News 43(3): 147-151.

Wisner, M. 1987. Collecting and transporting blacktip reef sharks. Freshwater and Marine Aquarium 10: 16-17.

Wood, C. M., J. D. Turner, and M. S. Graham. 1983. Why do fish die after severe exercise? Journal of Experimental Biology 22: 189-201.

Wood, C. M. 1991. Acid-base and ion balance, metabolism, and their interactions, after exhaustive exercise in fish. Journal of Experimental Biology 160: 285-308.

PERSONAL COMMUNICATIONS

Barnhart, A. 2001. Dynasty Marine Associates, Inc., Marathon, Florida 33050, USA.

Carrier, J., 2002. Albion College, Albion, Michigan 49224, USA.

Christie, N. 2001. Atlantis Resort, Paradise Island, Bahamas.

Choe, K. 2001. University of Florida, Gainesville, Florida 32611, USA.

Dainty, A. 2001. University of Cape Town, Rondebosch, 7701, South Africa.

Dowd, S. A. 2001. New England Aquarium, Boston, Massachusetts 02110, USA.

Ellis, C. M. 2001. Mystic Aquarium, Mystic, Connecticut 06335, USA.

Fahy, D. P. 2001. Nova Southeastern University - Oceanographic Center, Dania Beach, Florida, 33004, USA.

Harrington, D. 2001. Professional Fisherman, Ocean City, Maryland 21842, USA.

Henningsen, A. 2001. National Aquarium in Baltimore, Baltimore, Maryland 21202, USA.

Howard, M. 2001. Aquarium of the Bay, San Francisco, California 94133, USA.

Human, B. 2001. University of Cape Town Medical School, Observatory, 7925, South Africa.

James, R. 2001. Sea Life Centre, Weymouth DT4 7SX, UK.

Janse, M. 2001. Burger's Zoo, 6816 SH Arnhem, The Netherlands.

Kelleher, M. 2001. New England Aquarium, Boston, Massachusetts 02110, USA.

Kinnunen, N. 2001. Sydney Aquarium, Sydney, New South Wales 2000, Australia.

Long, T. 2001. Sea World Australia, Surfers Paradise, Queensland 4218, Australia.

McCourt, S. 2001. Sea World Australia, Surfers Paradise, Queensland 4218, Australia.

McEwan, T. 2001. The Scientific Centre of Kuwait, Salmiya 22036, Kuwait.

Marín-Osorno, R. 2001. Acuario de Veracruz, Veracruz, 91700, Mexico.

Menzies, S. J. 2001. Shanghai Ocean Aquarium, Pudong New Area, 200120, Shanghai.

Nelson, B. D. 2001. New England Aquarium, Boston, Massachusetts 02110 USA.

Nemeth, D. 2001. Coral World, St. Thomas, 6450, Virgin Islands.

Newman, P. 2001. Sea World, San Antonio, Texas 78251, USA.

Powell, D. 2001. Monterey Bay Aquarium, Monterey, California 93940, USA.

Sabalones, J. 2001. Newport Aquarium, Newport, Kentucky 41071, USA.

Squire, L. 2001. Cairns Marine Aquarium Fish, Cairns, Queensland 4870, Australia.

Steslow, F. 2001 New Jersey State Aquarium, Camden, New Jersey 08103, USA.

Thomas, L. 2001. Squid Inc., Eden Prairie, Minnesota 55346, USA.

Whitehead, J. 2001. New England Aquarium, Boston, Massachusetts 02110, USA.

Wisner, M. 2001. Mauna Lani Bay Hotel, Hawaii 96743, USA.

Young, F. 2001. Dynasty Marine Associates, Inc., Marathon, Florida 33050, USA.

The Elasmobranch Husbandry Manual: Captive Care of Sharks, Rays and their Relatives, pages 105-131.
© 2004 Ohio Biological Survey

Chapter 8
Elasmobranch Transport Techniques and Equipment

MARK F. L. SMITH

Oceanário de Lisboa,
Esplanada D. Carlos I - Doca dos Olivais,
1990-005, Lisboa, Portugal.
E-mail: mark@cosestudi.com

ALLAN MARSHALL

Pittsburgh Zoo & PPG Aquarium,
One Wild Place,
Pittsburgh, PA 15206, USA.
E-mail: allanm@zoo.pgh.pa.us

JOÃO P. CORREIA

Oceanário de Lisboa,
Esplanada D. Carlos I - Doca dos Olivais,
1990-005, Lisboa, Portugal.
E-mail: jpcorreia@oceanario.pt

JOHN RUPP

Point Defiance Zoo & Aquarium,
5400 North Pearl St,
Tacoma, WA 98407, USA.
E-mail: jrupp@pdza.org

Abstract: Elasmobranchs are delicate animals and appropriate care should be observed during their transport or permanent damage and even death can result. Key considerations include risk of physical injury, elevated energy expenditure, impaired gas exchange, compromised systemic circulation, hypoglycemia, blood acidosis, hyperkalemia, accumulation of metabolic toxins, and declining water quality. Carefully planned logistics, appropriate staging facilities, minimal handling, suitable equipment, an appropriate transport regime, adequate oxygenation, comprehensive water treatment, and careful monitoring will all greatly increase the chances of a successful transport. In special cases the use of anesthesia and corrective therapy may be merited.

Despite common perception to the contrary, sharks and rays are delicate animals. This delicate nature is nowhere more evident than during the difficult process of capturing and transporting these animals from their natural habitat to a place of study or display. If sufficient care is not used, it is not uncommon for elasmobranchs to perish during transport or shortly thereafter (Essapian, 1962; Clark, 1963; Gohar and Mazhar, 1964; Gruber, 1980).

Any elasmobranch transport regime should take into account a number of considerations related to species biology and transport logistics. A list of

these considerations has been outlined in Table 8.1 and each will be covered in this chapter or elsewhere in this volume.

Table 8.1. Important issues to consider when formulating an elasmobranch transport.

1. Elasmobranch biology
2. Species selection (refer chapter 2)
3. Legislation and permitting (refer chapter 3)
4. Logistics and equipment preparation
5. Specimen acquisition (refer chapter 7)
6. Handling and physical activity
7. Staging
8. Oxygenation, ventilation and circulation
9. Transport regime
10. Water treatment
11. Anesthesia (see also chapter 21)
12. Corrective therapy
13. Monitoring
14. Acclimation and recovery
15. Quarantine (refer chapter 10)
16. Specimen introduction (refer chapter 11)

ELASMOBRANCH BIOLOGY

Before discussing elasmobranch transport techniques it is important to understand the basics of elasmobranch biology as they pertain to capture, restraint, and handling.

Cartilaginous skeleton

Unlike teleosts (i.e., bony fishes), sharks and rays have a skeleton made of cartilage and lack ribs. This characteristic means that the internal organs and musculature of elasmobranchs are poorly protected and susceptible to damage without horizontal support (Clark, 1963; Gruber and Keyes, 1981; Murru, 1990).

Integument

Like other fishes elasmobranch skin is delicate and susceptible to abrasions especially on the snout and distal section of the fins (Howe, 1988).

Lateral line

Elasmobranchs detect low-frequency vibrations and pressure changes using a sensory organ called the lateral line (Boord and Campbell, 1977).

Ampullae of Lorenzini

Elasmobranchs detect weak electromagnetic fields using a specialized sensory organ called the Ampullae of Lorenzini, allowing them to detect electrical signatures produced by potential prey. They are sensitive to electrochemical cells induced by metals immersed in seawater and intolerant of dissolved heavy metallic ions (especially copper) (Gruber, 1980).

Optimal swimming velocity

An elasmobranch has achieved optimal swimming velocity when its energy expenditure per unit distance traveled, or total cost of transport (TCT), is minimized (Parsons, 1990; Carlson et al., 1999). If the elasmobranch swims slower or faster than this speed it will consume more energy. Carlson et al. (1999) observed that the optimal swimming velocity for blacknose sharks (*Carcharhinus acronotus*) was at speeds of 36-39 cm s^{-1} where TCT was 0.9-1.0 cal g^{-1} km^{-1}. If the sharks slowed down to 25 cm s^{-1}, then energy expenditure increased to 1.7 cal g^{-1} km^{-1}.

Negative buoyancy

Elasmobranchs have no swim bladder and are negatively buoyant (Bigelow and Schroeder, 1948). Sharks maintain or increase their vertical position within the water column by using their caudal fin to provide thrust and their rigid pectoral fins and snout to generate lift.

One technique negatively buoyant fishes use to conserve energy is to powerlessly glide at an oblique angle, gradually increasing depth, then return to the original depth by actively swimming upward. An energy saving in excess of 50% has been calculated for animals that adopt this strategy referred to as the swim-glide hypothesis (Weihs, 1973; Klay, 1977). An uninterrupted horizontal distance is required for the completion of the glide phase of this swimming strategy. If this minimum horizontal distance is not available the fish will consume excess energy through the muscular contractions required to turn. In the case of extreme interruptions the fish may stall and consume valuable energy reserves as it attempts to regain its position within the water column and re-attain a near-optimal swimming velocity. If this situation persists, the animal can become exhausted and ultimately die (Klay, 1977; Gruber and Keyes, 1981; Stoskopf, 1993).

Anaerobic metabolism

Many sedentary elasmobranchs have a low aerobic capacity relying heavily on the anaerobic metabolism of white muscle to support activity (Bennett, 1978). This strategy provides a great opportunity for sudden bursts of activity but implies long periods of immobility during recovery. Conversely, pelagic elasmobranchs have an increased commitment to the aerobic red muscle more suitable for their constantly cruising lifestyle. If oxygen (O_2) demand increases to a point where an insufficient supply feeds the working tissue aerobic red muscle will also start to metabolize anaerobically.

The velocity beyond which a swimming elasmobranch starts to metabolize anaerobically is referred to as U_{crit} or the maximum aerobically sustainable swimming speed (Lowe, 1996). The U_{crit} for leopard sharks (*Triakis semifasciata*) has been measured at 1.6 L s^{-1} for 30-50 cm TL specimens (where L = a distance equivalent to one body length and TL = total length of the specimen). U_{crit} was found to vary inversely with body size; 120 cm TL sharks exhibit a U_{crit} of 0.6 L s^{-1} (Graham et al., 1990). It is believed that less-active sharks have a lower U_{crit} than species adapted to a cruising pelagic lifestyle (Lowe, 1996).

Once an elasmobranch starts to metabolize anaerobically, be it benthic or pelagic, it produces lactic acid as a byproduct (Bennett, 1978).

Ventilation

Elasmobranchs have a small gill surface and their blood has a low oxygen-carrying capacity (Gruber and Keyes, 1981). Most demersal and benthic species ventilate gas-exchange surfaces using movements of the mouth to actively pump water across their gills. Pelagic species often use ram ventilation (i.e., forward motion and induced head pressure to force water into their mouth and out across their gill surfaces) to improve their ability to extract oxygen from the water. Species that are obliged to swim forward for effective gas exchange are referred to as obligate ram ventilators (Hughs and Umezawa, 1968; Clark and Kabasawa, 1977; Gruber and Keyes, 1981; Hewitt, 1984; Graham et al., 1990; Lowe, 1996; Carlson et al., 1999). An increased dependence on ram ventilation by pelagic species is possibly related to their increased reliance on oxygenated red swimming muscle.

Muscular pumping and systemic circulation

Elasmobranchs have limited cardiac capacity, low blood pressure, and low vascular flow rates. They rely on vascular valves and muscular contractions to aid systemic circulation. Optimal oxygenation of musculature and removal of toxic metabolites therefore occurs while swimming rather than at rest (Gruber and Keyes, 1981; Murru, 1984; Baldwin and Wells, 1990; Lowe, 1996). Muscular-assisted circulation is particularly important for pelagic and some demersal elasmobranchs because their aerobic swimming muscle requires constant oxygenation (Hewitt, pers. com.; Powell, pers. com.).

Hypoglycemia

During hyperactivity blood-glucose concentrations tend to rise as liver glycogen stores are mobilized (Mazeaud et al., 1977; Cliff and Thurman, 1984; Jones and Andrews, 1990). Cliff and Thurman (1984) observed an increase in the blood-glucose concentration of dusky sharks (*Carcharhinus obscurus*) from 5 to 10 mmol l^{-1} following 70 minutes of hyperactivity. Blood-glucose concentrations remained at 10 mmol l^{-1} for three hours and then continued to rise to a level in excess of 15 mmol l^{-1} over the next 24 hours. Glucose enters muscle cells where it supplies energy and raw material to restore glycogen reserves. If hyperactivity is prolonged blood-glucose elevation can be followed by a decline as liver glycogen reserves become exhausted (Cliff and Thurman, 1984; Hewitt, 1984; Jones and Andrews, 1990; Wood, 1991; Wells et al., 1986). Cliff and Thurman (1984) observed a decrease in blood-glucose concentrations to 2.9 mmol l^{-1} and 5.7 mmol l^{-1} in two dusky sharks that had struggled to the point of death.

Acidosis

Blood acid becomes elevated (i.e., pH declines) during hyperactivity. Acidosis is the result of two processes: increased plasma carbon dioxide (CO_2) concentration, and anaerobic metabolism and lactic acid production (Piiper and Baumgarten, 1969; Holeton and Heisler, 1978; Cliff and Thurman, 1984).

When CO_2 production in the muscles exceeds excretion via the gills, blood pH declines (Murdaugh and Robin, 1967; Albers, 1970). This pH decline happens because CO_2 reacts with H_2O according to Equation 8.1. This process is fast, happening within minutes of hyperactivity (Piiper

and Baumgarten, 1969; Cliff and Thurman, 1984; Lai et al., 1990; Wood, 1991). Cliff and Thurman (1984) observed a sharp blood-pH decline in a dusky shark from 7.29 to 7.12 within 10 minutes of hyperactivity. This decline is equivalent to an effective 57% increase in hydrogen ions (H^+).

Lactic acid produced during anaerobic metabolism dissociates into lactate and H^+ further lowering blood pH (Piiper and Baumgarten, 1969; Albers, 1970; Piiper et al., 1972; Bennett, 1978; Martini, 1978; Holeton and Heisler, 1978; Wardle, 1981; Holeton and Heisler, 1983; Cliff and Thurman, 1984; Wood, 1991). Cliff and Thurman (1984) observed that blood pH continued to decline during hyperactivity in a dusky shark from 7.12 at 10 minutes to 6.96 by the 70th minute. This slow decline, following an initial CO_2-induced decline, was attributed to the formation of lactic acid, and thus, H^+.

It has been suggested that only 20% of H^+ produced during anaerobic metabolism is released from the cells. Therefore, extracellular acidosis may be indicative of a more profound intracellular acidosis (Wood et al., 1983).

Hyperkalemia

Hyperactivity and blood acidosis can result in elevated serum electrolytes—in particular potassium ion (K^+) concentration—through the effusion of cellular electrolytes into the blood serum (Wood et al., 1983; Cliff and Thurman, 1984; Wells et al., 1986; Jones and Andrews, 1990; Wood, 1991). Cliff and Thurman (1984) observed an increase in plasma K^+ concentration from 3.3 mmol l^{-1} to 5.3 mmol l^{-1} in a dusky shark during hyperactivity.

Increased plasma K^+ concentration can disrupt cardiac function and promote muscular tetanus, augmenting anaerobic metabolism and promoting acidosis (Cliff and Thurman, 1984; Wells et al., 1986).

Excretion of metabolites

Elasmobranchs excrete many biochemical metabolites into the surrounding environment via their gills. Amongst others CO_2, H^+, and ammonia ion (NH_4^+) are excreted during normal metabolism (Robin et al., 1965; Murdaugh and Robin, 1967; Albers, 1970). Unless they are diluted or removed environmental accumulation of these metabolites will become toxic. As elasmobranchs are hyperosmotic to their environment an elevated NH_4^+ concentration may induce or further promote acidosis (Murru, 1990).

Transport mortality

Although mortality during and after elasmobranch transport is not fully understood it appears to be due, at least in part, to: depletion of blood-glucose concentrations and starvation of muscle tissue; blood acidosis, circulatory disruption, and central nervous system damage; elevated plasma K^+ concentration and cardiac dysfunction; accumulation of toxic metabolites within the immediate environment; or a combination of all these factors (Mazeaud et al., 1977; Bennett, 1978; Gruber and Keyes, 1981; Wood et al., 1983; Cliff and Thurman, 1984; Hewitt, 1984; Murru, 1984; Wells et al., 1986; Dunn and Koester, 1990; Stoskopf, 1993). The effect of these processes may not always be immediately obvious and their contribution to post-transport mortality may be overlooked or underestimated (Gruber and Keyes, 1981).

In conclusion the following challenges need to be considered during the transport of elasmobranchs: (1) risk of injury to internal organs, delicate skin, and sensitive sensory organs; (2) increased turning frequency and elevated energy expenditure; (3) impaired ventilation and compromised systemic oxygenation; (4) decreased muscular pumping resulting in reduced vascular circulation, reduced muscular oxygenation, and reduced metabolite elimination; (5) hypoglycemia; (6) blood acidosis; (7) hyperkalemia; and (8) the excretion and environmental accumulation of CO_2 , H^+ and NH_4^+. An overview of these challenges and possible solutions has been outlined in Table 8.2.

LOGISTICS AND EQUIPMENT PREPARATION

A fundamental aspect of any elasmobranch transport, determining ultimate success or failure, is logistical planning and equipment preparation. Equipment malfunction, vehicular breakdowns,

Equation 8.1

$$CO_2 + H_2O \rightleftarrows H_2CO_3 \rightleftarrows HCO_3^- + H^+ \rightleftarrows CO_3^{2-} + 2H^+$$

Carbon dioxide	Water	Carbonic acid	Bicarbonate	Hydrogen ion	Carbonate	Hydrogen ion

Table 8.2. Biological characteristics of elasmobranchs and possible strategies to mitigate negative effects during transport.

Biological characteristics		Solutions
Cartilaginous skeleton	1.1	While handling, ensure horizontal support using water-filled plastic bags for small specimens or stretchers for large specimens.
	1.2	Always transport in a water-filled vessel.
Integument	2.1	Use plastic bags or soft knot-less nets when catching specimens, and flexible plastic stretchers when restraining them.
	2.2	Minimize handling and use sterilized plastic gloves if handling is absolutely necessary.
	2.3	Minimize obstructions to natural swimming patterns within transport tank.
	2.4	Construct transport tank from non-abrasive material.
	2.5	In extreme cases, consider the use of sedation to minimize handling time and reduce physical injury.
Lateral line	3.1	Minimize external physical impacts to the transport tank.
Ampullae of Lorenzini	4.1	Avoid using metallic objects, especially within transport tank.
	4.2	Avoid using copper as a chemico-therapeutic.
	4.3	Where possible minimize electric currents in and around the transport vessel.
Optimal swimming velocity and negative buoyancy	5.1	Generate gentle current in transport tank to facilitate hydrodynamic lift, maintain specimen buoyancy, and simulate swimming behavior.
	5.2	Minimize turning frequency by transporting small specimens and reducing obstructions within transport tank (e.g., LSS equipment, conspecifics, etc.).
Anaerobic metabolism	6.1	Minimize hyperactivity by catching and restraining specimens quickly and calmly.
	6.2	Minimize transport duration.
	6.3	In extreme cases, consider the use of anesthesia to slow metabolic rate and to reduce O_2 consumption and metabolic waste production.
	6.4	Maximize dissolved O_2 concentration (as per 12.2 and 12.3)
	6.5	Maximize ventilation and systemic circulation (as per 7 and 8)
Ventilation	7.1	Where possible, transport specimens in 'free-swimming' mode to allow natural ventilation.
	7.2	When transporting in 'restrained' mode simulate natural ventilation by directing a current of oxygenated water into the mouth of the specimen.
Muscular pumping and systemic circulation	8.1	Where possible, transport specimens in 'free-swimming' mode to allow natural muscular pumping.
	8.2	When transporting in 'restrained' mode periodically flex the trunk of the specimen to stimulate muscular pumping.
Hypoglycemia	9.1	Minimize hyperactivity (as per 6.1 - 6.3).
	9.2	Consider use of IV- or IP-administered glucose supplementation.
Acidosis	10.1	Minimize hyperactivity (as per 6.1 - 6.3).
	10.2	Maximize O_2 and CO_2 exchange by facilitating ventilation (as per 7.1 and 7.2), degassing transport water (as per 15.2), and oxygenating transport water (as per 12.2 and 12.3).
	10.3	Minimize blood $[CO_2]$ and $[H^+]$ increase by facilitating systemic circulation (as per 8.1 and 8.2).
	10.4	Consider use of IV- or IP-administered bicarbonate or acetate to buffer the blood.
Hyperkalemia	11.1	Minimize hyperactivity (as per 6.1 - 6.3).
	11.2	Minimize blood acidosis (as per 10.1 - 10.4).
Oxygen (O_2) depletion	12.1	Minimize hyperactivity (as per 6.1 - 6.3).
	12.2	Maximize dissolved oxygen concentration within transport vessel by supplementing with pure O_2 at ~120-200% saturation.
	12.3	Maximize dissolved oxygen concentration within transport vessel by degassing transport water and liberating excess CO_2.
Temperature fluctuation	13.1	Mitigate temperature fluctuations by insulating transport vessel.
	13.2	Ensure vessel is transported and staged in temperature-controlled environments.
	13.3	Modify temperature as required using bagged ice, immersion heaters, etc.
Excreted particulates and 'organics'	14.1	Minimize hyperactivity (as per 6.1 - 6.3).
	14.2	Minimize waste accumulation by applying water exchanges, mechanical filtration, adsorption or chemical filtration, and foam fractionation to transport water.
Carbon dioxide (CO_2) buildup and pH decline	15.1	Minimize hyperactivity (as per 6.1 - 6.3).
	15.2	Minimize pH decline by degassing transport water and liberating excess CO_2.
	15.3	Minimize pH decline by buffering transport water with bicarbonate, carbonate, or Tris-amino®.
Nutrient buildup	16.1	Minimize hyperactivity (as per 6.1 - 6.3).
	16.2	Minimize $[NH_3 / NH_4^+]$ accumulation by performing periodic water exchanges, using ammonia sponges (e.g., AmQuel), and applying adsorption or chemical filtration (as per 14.2).

Table 8.3. Basic logistics and equipment preparation required for a typical intercontinental elasmobranch transport. This basic model may be adapted and used as a checklist for any transport.

Research	1.1	Information is power! Research as much as possible about the chosen species - appropriateness for display, special requirements, reliable sources (e.g., collection sites / professional collectors / other aquaria), transportability, etc.
	1.2	Obtain references for professional collectors. Inspect collector's facilities. Finalize species list and fix agreement with professional collector (i.e., fees, dates, responsibilities, etc.). Ensure that both you and the collector have appropriate permits.
	1.3	Determine shipment routes, dates, and times. Obtain references for haulers, carriers, airports, etc. Ensure shipment route is as direct as possible, secure, and requires minimal cargo handling. Have a freight agent negotiate with carriers to determine the best routes, minimize costs, and handle paperwork. Strive for non-peak times and working days when customs / quarantine staff are readily available.
	1.4	Clarify available transport conditions: Is climate control available throughout? Can staff easily enter cargo areas? Can animals be accompanied and checked in-flight? What are the plane loading and unloading restrictions (e.g., door dimensions, lifting equipment, etc.) at both the origin and destination?
Plan	2.1	Think your way through the entire transport process considering all equipment, transport, and infrastructure requirements (e.g., forklifts, pallet jacks, power supplies, water exchanges, etc.). Ensure that transport tanks and ancillary equipment will fit on trucks, airplanes, forklifts, and through all doors and corridors. Consider urban restrictions between staging facility, airports, and destination aquarium. Verify your plan with an individual experienced in the type of transport you are undertaking. Formulate final plan and review with all parties.
	2.2	Make a manifest of all required equipment and transport tanks (refer Table 8.4). Ensure that all equipment conforms to regulation (e.g., HazMat of the International Civil Aviation Organization (ICAO) and the International Air Transport Association (IATA)) and understand any possible restrictions. Calculate required amount of consumables (e.g. oxygen, batteries, water conditioners, etc.).
	2.3	Make a comprehensive list of all required permits and other documentation for exportation and importation (e.g., conservation certification (federal, state, etc.), health certification, HazMat documentation, way-bills, customs declarations, insurance policies, passports, visas, etc.).
	2.4	Make a comprehensive list of all tasks with completion dates / times and individuals responsible. Establish teams of people for every task (e.g., catching specimens, handling specimens, transporting specimens, receiving and acclimatization, post-transport monitoring, etc.). Ensure that the plan is clearly understood by everyone. Build in critical 'mileposts' for 'go' / 'no-go' decisions (e.g., carrier confirmed landed = commence loading animals into transport tanks, etc.). Be reasonable with timing estimates for each step and build in extra time.
Contingency	3.1	Ensure suitable infrastructure is available at all points throughout the transport route (e.g., lifting equipment, power supplies, climate control, water for exchanges, security, police escorts, etc.). Identify areas of risk and ensure suitable contingencies are in place.
	3.2	Adapt! You have a clear plan but be prepared to change the plan if it is simply not workable. Ensure any changes to the plan are communicated to everyone.
Communicate	4.1	Establish clear channels of communication with all parties and make a comprehensive contact list. Circulate plan, equipment manifest, required documentation, task list, and contact sheet to appropriate parties. These will include, but may not be limited to: husbandry staff, local security, public relations staff, local constabulary, freight agent, hauler, hazmat specialist, airport cargo handlers, carrier administration, carrier cargo handlers, customs officials, US Fish and Wildlife Service (or equivalent), Department of Agriculture (or equivalent), Department of Transport (or equivalent) and Immigration. Appoint the freight agent, or other appropriate party, to be the primary liaison with transport personnel. Using one spokesperson will minimize omissions and conflicting information.
Educate	5.1	Be prepared to be a teacher! Educate all personnel along the route of the transport (as per list detailed in 4.1 above). Airport officials are always interested in a transport and will cooperate if they are correctly and politely apprised of the situation. Ensure that all are aware of the need for expeditious processing of any documents and loading of cargo, etc. Likewise, local constabulary, security guards, and company PR personnel will be more sympathetic if everything is explained clearly and politely.
Prepare	6.1	Acquire all equipment on manifest (as per 2.2 above) and ensure it functions correctly.
	6.2	Acquire required permits (as per 2.3 above). Carry originals and copies throughout transport.
	6.3	Collect specimens and allow to recuperate in staging facility.
	6.4	Fast specimens (48 - 72 hours) if appropriate and verify that they are healthy and ready for transport.
	6.5	Prepare equipment, double-checking the manifest (as per 2.2 above) to ensure you have everything - remember permits! Include backups. Include tools for every pump, filter, battery, oxygen bottle, bolt, nut, screw, fastener, etc. Wash filtration media and pack filters. Test all batteries and pumps. Check oxygen supplies. Ensure transport tanks are robust, leak-proof, and have no pipes protruding where they could be sheared off. Calculate required quantities of water conditioners (e.g., AmQuel®, etc.) and prepare in advance. Ensure all loose equipment is stored in robust waterproof boxes.
	6.6	Pack tanks and ancillary equipment on trucks so they form discrete self-contained units for ease of movement with forklifts, pallet jacks, etc. Orientate and securely strap down tanks to minimize surge and allow ease of access to all equipment.
	6.7	Allocate specimens to specific tanks and make a check-list for final loading.
	6.8	Final check! On the day before the transport ensure EVERYTHING is prepared! Ensure all contingencies are in place. Review tasks and timing with team members adjusting where necessary. Get some rest!

Table 8.3 (continued). Basic logistics and equipment preparation required for a typical intercontinental elasmobranch transport. This basic model may be adapted and used as a checklist for any transport.

Execute	7.1	Verify that all team members and infrastructures are ready.
	7.2	Start and verify that all LSSs (life support systems) are functioning (i.e., oxygen supplies, pumps, mechanical filtration, directed water currents, etc.).
	7.3	Capture and transfer specimens to transport tanks quickly and calmly. Have extra personnel available to lift and carry.
	7.4	When all specimens and equipment is loaded re-check LSSs. If all is OK transport specimens to airport for processing.
	7.5	At the airport ensure tanks are secure throughout processing and be prepared to perform water exchanges and equipment repairs before loading. Pack tanks in aircraft securely (as per 6.6). Pack tanks on the final pallet spaces so they are the first off the aircraft on arrival.
	7.6	Monitor specimens, LSSs, and water quality throughout transport and make appropriate adjustments (e.g., adjust oxygen regulators, add water conditioners, etc.).
	7.7	On landing re-check LSSs and water quality. Perform water exchanges if necessary. Expedite customs and airport processing.
	7.8	Load tanks on truck(s) (as per 6.6) and transfer to destination aquaria.
	7.9	On arrival off-load tanks and commence specimen acclimation. Apply prophylactic measures as appropriate. Transfer specimens to quarantine tanks.

belligerent officials, and replacement resources (e.g., water, filtration media, spares, etc.) should be taken into account and contingencies previewed (Young, pers. com.). Use a competent freight agent to handle negotiations, transport bureaucracy, hazardous material documentation, and logistics communication (McHugh, pers. com.). Logistical planning and equipment preparation for a typical elasmobranch transport can be divided into seven basic steps. These steps have been outlined in Table 8.3 and a corresponding equipment manifest detailed in Table 8.4.

HANDLING AND PHYSICAL ACTIVITY

When initially restraining a specimen it is often necessary to use a net made of soft knot-less nylon to prevent abrasions (Powell, pers. com.). Some sharks, in particular the sand tiger shark (*Carcharias taurus*), are prone to catching their teeth in nets. Care should be taken to minimize tooth entanglement as permanent damage to the upper jaw may result (Mohan, pers. com.).

Another consideration for the sand tiger shark is its unique ability to swallow air and store it in its stomach to assist buoyancy (Hussain, 1989). If a portion of the air is not removed before transport the sand tiger shark may float upside-down in the transport tank. It is preferable to induce the sand tiger to expel some of the air before transport commences.

At no stage should the body of an elasmobranch be allowed to hang loosely from the head or tail. Sharks and rays should always be maintained in a horizontal position (Clark, 1963; Gruber and Keyes, 1981; Andrews and Jones, 1990; Stoskopf, 1993). When lifting and moving large specimens a stretcher has been employed (Clark, 1963; Davies et al., 1963; Murru, 1984; Smith, 1992; Visser, 1996). A stretcher consists of a flexible reinforced vinyl or canvas sheet stretched between two parallel poles. The shark is lifted out of the water while lying in a horizontal position within the bag formed by the sheet (Figure 8.1).

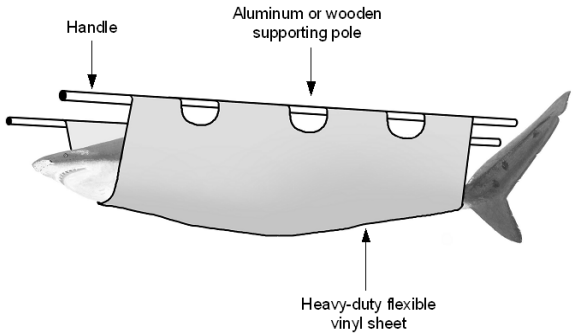


Figure 8.1. Flexible vinyl stretcher used to maintain sharks in a horizontal position while handling.

For small sharks horizontal support can be achieved by using a strong, rip-resistant, plastic bag. The water provides support while the flexible and smooth walls of the bag allow the specimen to move without damaging itself. The elasmobranch remains submerged and can continue to respire (Marshall, 1999; Ross and Ross, 1999). Double bagging provides security against breakage. Rays require support for their pectoral fins. Support can be achieved by using a vinyl or canvas sheet stretched within a rigid, circular, exterior hoop (Figure 8.2).

When handling elasmobranchs the use of sterile plastic gloves is recommended (Gruber, 1980;

Table 8.4. Comprehensive list of equipment considered necessary for the preparation and execution of a large-scale, long-duration elasmobranch transport. Short-term transports may be less demanding of resources and only require some of the equipment listed.

Transportation equipment	Tool kit	Medical equipment + Water analyses
Air pumps (battery operated)	Battery charger	Acidosis therapy (e.g. Bicarbonate)
Bags (plastic)	Block and tackle	Ammonia sponge (e.g., Amquel)
Bottles (plastic)	Cable ties	Anesthetic - IM (e.g., Ketamine, Xylazine, Yohimbine)
Buckets (plastic)	Double adapters / Power packs	Anesthetic - Immersion (e.g. MS-222)
Diving equipment (mask, snorkel, fins, etc.)	Drill - bits	Anesthetic - Local (e.g. Lignocaine)
Dry boxes	Drill - holesaw fittings	Anti-bleed therapy - IM (Vitamin K)
Electrical - batteries (12V) (sealed)	Drill - screwdriver fittings	Antiseptic - Topical (e.g., Betadine, Mercurochrome)
Electrical - cables + connectors + fusible links	Drill (electrical and battery operated)	Anti-shock therapy (e.g., Dexamethasone)
Electrical - inverter (DC 12V to AC?)	Electrical extension flex	Cardiac stimulant - (e.g., Adrenaline)
Electrical - transformer (AC? To DC 12V)	Fasteners, nails, screws & glues (assorted)	Catheters (14, 16, 18, 21G)
Filter (cartridge) - mechanical / chemical	Flashlights (+ batteries)	Dissection kit
Filter (cartridge) - media - activated carbon	Gloves	Hypoglycemia therapy (e.g., Glucose IV infusion)
Filter (cartridge) - media - pleated paper	Hammer	IV administration lines
First aid kit	Jigsaw (electrical)	Magnifying glass
Foam rubber	Knives	Measuring vials
Hose (flexible w/ reinforcing to avoid kinks)	Marker pens (permanent)	Needles (14, 18, 21, 22 and 24G)
Hose clamps	Multi-meter	Respiratory stimulant (e.g., Doxapram)
Ice box + ice packs	Pliers	Sample jars
Manifest	PVC pipes (+ fittings, glue and rags)	Scalpel blades
Money + credit cards	Ropes (assorted)	Scalpel handles
Net (cargo)	Saw (hack) (+ blades)	Sedative - IM (e.g. Valium)
Net (hand)	Saw (wood)	Stainless clamps (e.g., hemostat)
Net (shark restraining)	Screwdrivers	Staple gun + staples
Oxygen - airline + assorted connectors / manifolds	Silicone (+ gun)	Sterile swabs
Oxygen - cylinder + key + regulator + manifolds	Sponges	Sutures (nylon, stainless)
Oxygen - diffusers + weight	Stainless nuts + bolts + washers	Suturing needles
Oxygen - spare cylinder(s)	Tape (electrical, thread, duct)	Syringes (1, 2, 5, 10, 50 ml)
Pallet jack	Tie down straps	Tweezers
Pallets (allow access by forklift / pallet jack)	Tie Wire	Water pH buffer (e.g., Tris-Amino, Bicarbonate)
Slings (lifting)	Tool box	Lab wash bottles (for anesthetic administration)
Stretchers (shark + ray)	Towels	
Submersible pump (12V) - circulation	Vice grips	Ammonia test kit
Submersible pump (12V) - water exchanges	Wire cutters	Oxygen meter
Transport tanks(s) + life support system	Wrench (adjustable)	pH test kit
	Wrenches (assorted)	Refractometer
		Thermometer (+ spares)

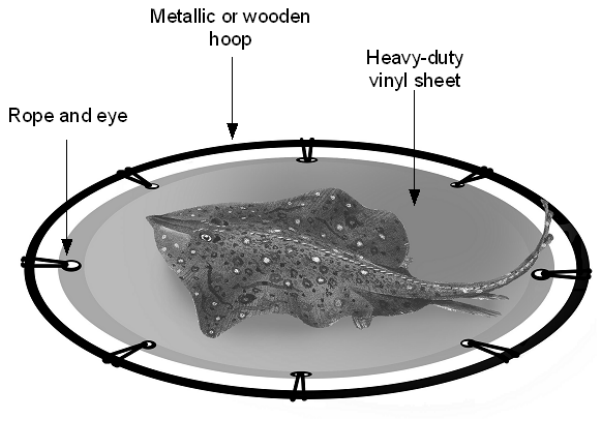


Figure 8.2. Rigid vinyl stretcher used to maintain rays in a horizontal position while handling.

Young et al., 2002). Sterile gloves will help prevent the development of post-handling infections of the skin. To minimize struggling during restraint it is possible to induce a trance-like state in many shark species by holding them in an upside-down position for a few minutes. This phenomenon is referred to as tonic immobility (Watsky and Gruber, 1990; Henningsen, 1994).

Throughout specimen restraint and transport physical activity must be minimized to reduce adverse biochemical reactions and the risk of specimens abrading their delicate snout and fin tips (Murru, 1990; Smith, 1992). A transport tank with smooth walls and a dark interior will reduce physical injury. Physical injury will be further reduced by the minimization of external stimuli and a reduction of surge by thoughtful design and positioning of the transport tank (i.e., placement of a rectangular tank transversely across the transport vehicle) (Smith, 1992; Arai, 1997).

STAGING

Staging refers to the process of temporarily maintaining a specimen in a large secure recovery enclosure close to the collection site. This process is important, as the biochemical changes induced during elasmobranch capture are often profound. Staging a specimen for at least 24 hours before transport commences will dramatically increase the chances of success (Cliff and Thurman, 1984; Murru, 1984; Wisner, 1987; Andrews and Jones, 1990; Arai, 1997; Young et al., 2002). It is critical that the staging facility has sufficient dimensions and water parameters to adequately maintain the specimen(s). If not, you will simply compound the already damaging biochemical changes.

A typical temporary staging facility comprises a plastic-lined pool supplied with raw seawater via an offshore intake and pumping system. A secure fence, for security from onlookers, and roofing to cut down radiant energy is beneficial (Visser, 1996). Water parameters, especially oxygen, temperature, pH, and nitrogenous wastes become a critical issue if water is not being constantly replenished. A re-circulating water treatment system capable of maintaining optimal water parameters is then required.

OXYGENATION, VENTILATION AND CIRCULATION

Oxygenation

The importance of oxygenation cannot be over-emphasized. Fishes consume up to three times their normal oxygen requirement under transport conditions. Exhausted fishes may consume as much as 5-10 times their normal requirement (Wardle, 1981; Froese, 1988). The greater a commitment to aerobic respiration, the more important an unimpeded oxygen supply (Wardle, 1981). This increasing demand for oxygen is illustrated by adjusted oxygen consumption rates for increasingly pelagic elasmobranchs (i.e., 296, 395 and 849 mg of O_2 kg^{-1} h^{-1} for *Sphyrna tiburo*, *Carcharhinus acronotus*, and *Isurus oxyrinchus*, respectively) (Parsons, 1990; Graham et al., 1990; Carlson et al., 1999).

The ability of elasmobranch blood to carry oxygen to the tissues, as demonstrated in the white shark (*Carcharodon carcharias*), is determined by: (1) the amount of oxygen transferred across the gills; (2) the oxygen-carrying capacity of the blood; and (3) the ability of the circulatory system to deliver oxygen to the tissues (Emery, 1985). These restrictions emphasize the importance of a steady supply of dissolved oxygen, adequate gill ventilation, and unimpaired systemic circulation to achieve normal metabolic respiration (Butler and Taylor, 1975; Wardle, 1981; Cliff and Thurman 1984; Lai et al., 1990; Smith, 1992).

Dissolved oxygen concentrations of 120-160% saturation have been reported as appropriate for the transport of elasmobranchs (Thoney, pers. com.). Other workers recommend concentrations as high as 200% saturation (i.e., 15 mg l^{-1} at 20°C) (Stoskopf, 1993). It has been suggested that higher concentrations may cause neurological damage or may be responsible for burning or

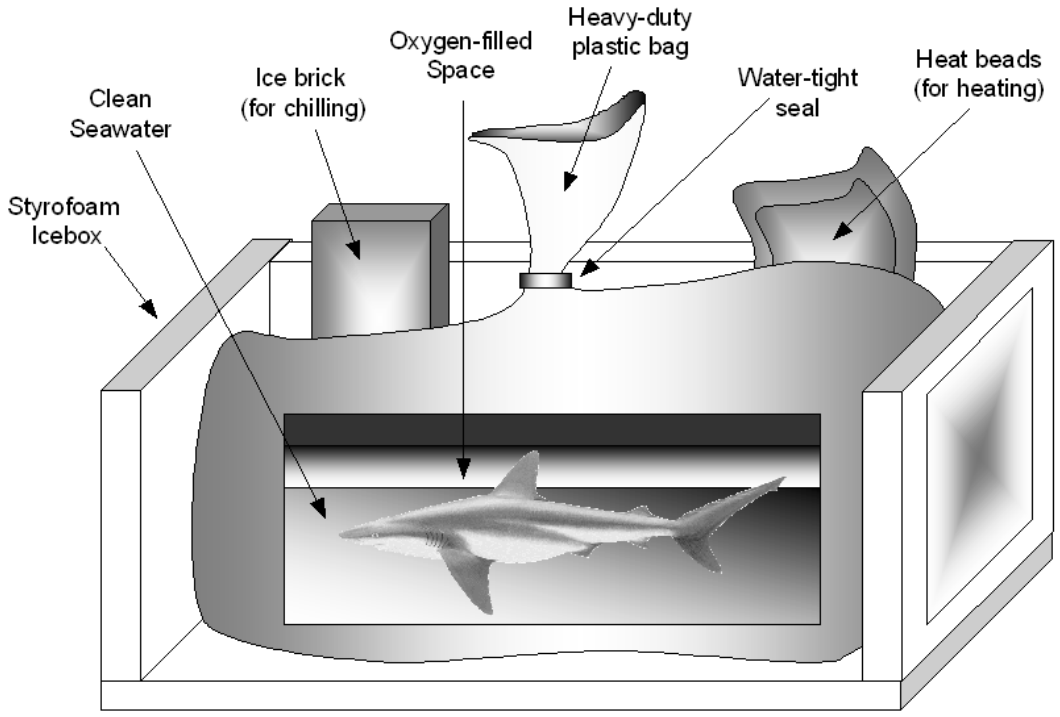


Figure 8.3. Sealed bag and insulated box transport regime.

oxidizing the gills (Stoskopf, 1993). However other workers have supersaturated transport water to 30 mg l^{-1} and observed no apparent adverse reactions (Hewitt, pers. com.; Powell, pers. com.). Regardless, caution should be exercised as hyperoxygenation may depress respiration and reduce offloading of CO_2 at the gill surface. This process will induce acidosis, one of the biochemical changes that oxygenation is intended to reduce.

Ventilation

Water flow across the gills is normally quite high in the shark. In the dogfish (*Squalus acanthias*) water flow across the gills can equal half the body weight of the animal every minute (Murdaugh and Robin, 1967). Water movement within the transport tank enhances ventilation and helps reduce anaerobic respiration by allowing the specimen to take advantage of the oxygen-rich environment. By directing currents so they flush across the gills of a stationary shark, it is possible to approximate ram ventilation (Ballard, 1989; Smith, 1992).

Circulation

When an elasmobranch is prevented from normal locomotion, muscular pumping of blood and lymphatic vessels can be impaired. If restraint is prolonged, lack of systemic circulation may result in both anaerobic metabolism and a buildup of toxic metabolites. Increased concentrations of metabolites can be associated with an increased rigidity progressing forward from the caudal region along the length of an animal. Another sign of this condition may be a reddening of the skin on the ventral surface (Powell, pers. com.). In extreme cases the animal can become completely immobile and die (Gruber and Keyes, 1981; Cliff and Thurman, 1984; Stoskopf, 1993).

When transporting non-sedentary elasmobranchs, in a restrained manner, consideration should be given to gently massaging the musculature by flexing the caudal peduncle and stroking the dorsal surface (Gruber and Keyes, 1981; Dunn and Koester, 1990; Powell, pers. com.; Young, pers. com.). Care should be exercised during this operation to repeat the process regularly. Delays may allow the accumulation of toxic metabolites that could be inadvertently introduced into systemic circulation in a single bolus dose (Smith, 1992; Stoskopf, 1993).

TRANSPORT REGIME

Transport regimes may be grouped into three basic types: (1) sealed bag and insulated box; (2) free-swimming; and (3) restrained.

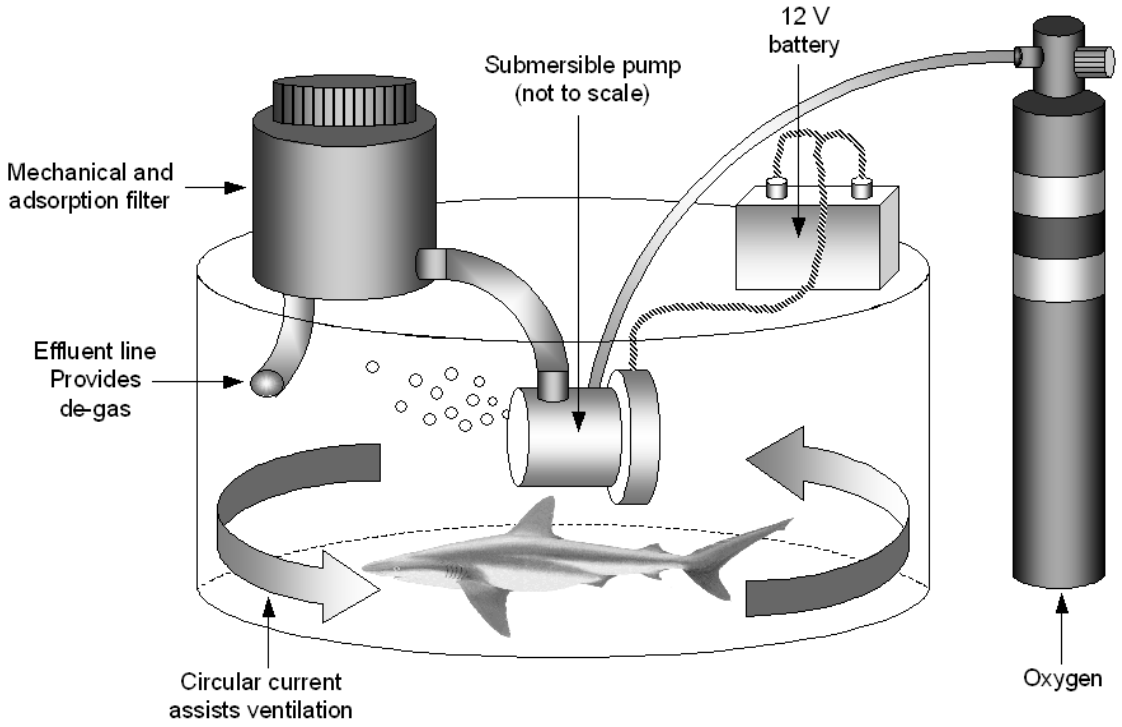


Figure 8.4. Free-swimming transport regime.

Sealed bag and insulated box

One of the simplest means to transport elasmobranchs is using the sealed bag and insulated box technique (Figure 8.3) (Gruber and Keyes, 1981; Wisner, 1987; Froese, 1988; Stoskopf, 1993; Ross and Ross, 1999). This option is appropriate for small benthic and some small demersal species (i.e., < 1 m TL).

The specimen is placed in a large, tough, plastic bag (e.g., 0.25 mm polyethylene) half filled with seawater. The dimensions of the bag should prevent the specimen from turning and getting wedged in an awkward position, or alternatively, be sufficiently large that it may swim gently (Gruber and Keyes, 1981). Double or triple bagging is preferred as it will help prevent leaks due to tearing. The upper half of the bag is filled with pure oxygen and sealed. The specimen remains in the bottom-half of the bag, covered with water, while the void above is filled with oxygen. Oxygen slowly diffuses into the water throughout the transport. The bagged shark or ray is placed into an insulated box (e.g., Styrofoam icebox) providing thermal isolation and protection against physical and visual stimuli. It is possible to include a basic water treatment system in the form of an ammonia scrubber (e.g., Poly-Filter®, Poly-Bio-Marine®, USA) and microwave heat beads or ice packs to maintain an appropriate temperature (Wisner, 1987).

Prepared in this manner a small elasmobranch can travel for 24-48 hours. An advantage of this technique is that specimens can be moved unattended, allowing a greater range of transport options and reduced expense.

Free-swimming

The second transport option, used principally for smaller pelagic elasmobranchs, is the free-swimming technique (Figure 8.4) (Hiruda et al., 1997; Marshall, 1999; Young et al., 2002). This technique consists of a circular, smooth-walled, plastic or fiber-reinforced polyester tank (e.g., 2.5 m diameter x 1 m deep) within which the elasmobranchs are free to move. The animals are often encouraged to swim against a gentle current generated by a 12 Volt submersible pump. In general, the pump should be capable of moving water equivalent to the volume of the tank every hour. In the example detailed above this requirement would necessitate a pump capable of moving 5 m^3 $hour^{-1}$ (e.g., Model 02, Rule ITT Industries, USA). Pumped water is sent to a water treatment system and returned to the transport tank via a directed line. The transport vehicle may supply energy for the pump, however, it is wise to have an independent and portable supply of energy (e.g., 12 Volt deep-cycle battery, Model 800-S, Optima, USA). This alternative supply

provides an energy source during transfers between vehicles and can be used as a backup in emergencies.

The submersible pump is usually suspended from the underside of the transport tank lid, keeping it off the floor where it could disrupt the swimming pattern of the specimens. It is possible to have a pump mounted externally, with the intake line draining from the side of the tank. If such is the case, care must be exercised to ensure the intake line is flush with the interior wall of the tank and covered with a large, smooth, protective mesh manifold. This manifold prevents animals from becoming trapped by the suction of the intake line. Care must be taken to ensure that external pipes and fittings cannot be sheared off in transit.

The water treatment system typically consists of a canister filter filled with mechanical and adsorption media (see below). Once the water is filtered, it is returned above the surface to facilitate gas exchange (i.e., O_2 dissolution and CO_2 liberation) and drive the gentle current. During long transports it may be necessary to use additional diffusers (i.e., air-stones) to eliminate accumulated CO_2. Monitoring pH will give an indication of changes in dissolved CO_2 concentration (Thoney, pers. com.). Dissolved oxygen is increased using bottled O_2, a reliable regulator, and a diffuser situated in the center of the tank (Smith, 1992). Oxygen may be introduced directly into the suction line of the submersible pump for better dissolution (Powell, pers. com.).

The transport tank should be leak-proof but allow ventilation for the elimination of excess CO_2. Ventilation can be achieved with both breather holes and a centrally located access hatch. The hatch may be opened periodically to flush out CO_2 and to manipulate equipment or animals as required. A robust side-mounted inspection window is useful. The entire top of the tank should be strong enough to withstand the weight of a person and provide a good seal against leaks. The top of the tank should be easy to remove for effective handling of specimens on arrival at the final destination.

The free-swimming technique is preferred for those species that are reliant on aerobic respiration, ram ventilation, and muscular-assisted vascular return. When formulating a free-swimming transport regime it is important to consider: (1) swimming behavior; (2) specimen size; (3) specimen number; (4) tank shape; (5) tank size; and (6) the number of obstructions within the tank (Young et al., 2002). These factors will determine the number of interactions with obstructions or conspecifics, turning frequency, and therefore energy expenditure. The less encumbered an environment, the lower the consumption of vital energy reserves, the lower the production of toxins, and the lower the risk of metabolic shock. An example of a transport regime with a good safety margin would be three 0.5 m TL scalloped hammerheads (*Sphyrna lewini*), transported for 48 hours, in a tank of dimensions 2.5 m diameter x 0.65 m deep (Young et al., 2002).

Some workers have found that circular tanks work well for short-duration, free-swimming transports where specimens potentially impacting the walls is of primary concern (Wisner, 1987; Marshall, 1999). However for long-term transports, where biochemical changes become increasingly important, the continuous turning required to negotiate a circular tank may be too energetically challenging (Powell, pers. com.). It has been equated to the energetic inefficiency of a constantly turning aircraft (Klay, 1977; Gruber and Keyes, 1981). It has been suggested that a larger rectangular tank, allowing animals to swim normally for short distances and then opt for discrete turns, will be less energetically challenging during long-term transports.

Restrained

Some elasmobranchs are simply too large to transport free-swimming. In such cases it may be possible to transport them in a restrained manner (Gruber, 1980; Gruber and Keyes, 1981; Hewitt, 1984; Ballard, 1989; Andrews and Jones, 1990; Murru, 1990; Smith, 1992). This technique consists of a smooth-walled rectangular plastic or fiber-reinforced polyester transport tank, only slightly larger than the specimen to be transported (Figure 8.5). A typical tank size would be 2.5 m x 0.5 m x 0.5 m. The dimensions should be such that the animal cannot turn easily and lies in the same position throughout the transport. The interior, especially the wall in front of the specimen's snout, may be padded with a soft flexible material (e.g., neoprene). Degassing and water treatment are performed in a similar manner to that described for the free-swimming technique. A pump is often employed to drive oxygenated water from the rear to the front of the tank, where it is gently jetted into the mouth of the specimen. This water circulation system is referred to as a raceway and is designed to approximate ram ventilation. In some cases a custom-designed harness has been employed to maintain the position of the shark relative to both the tank and

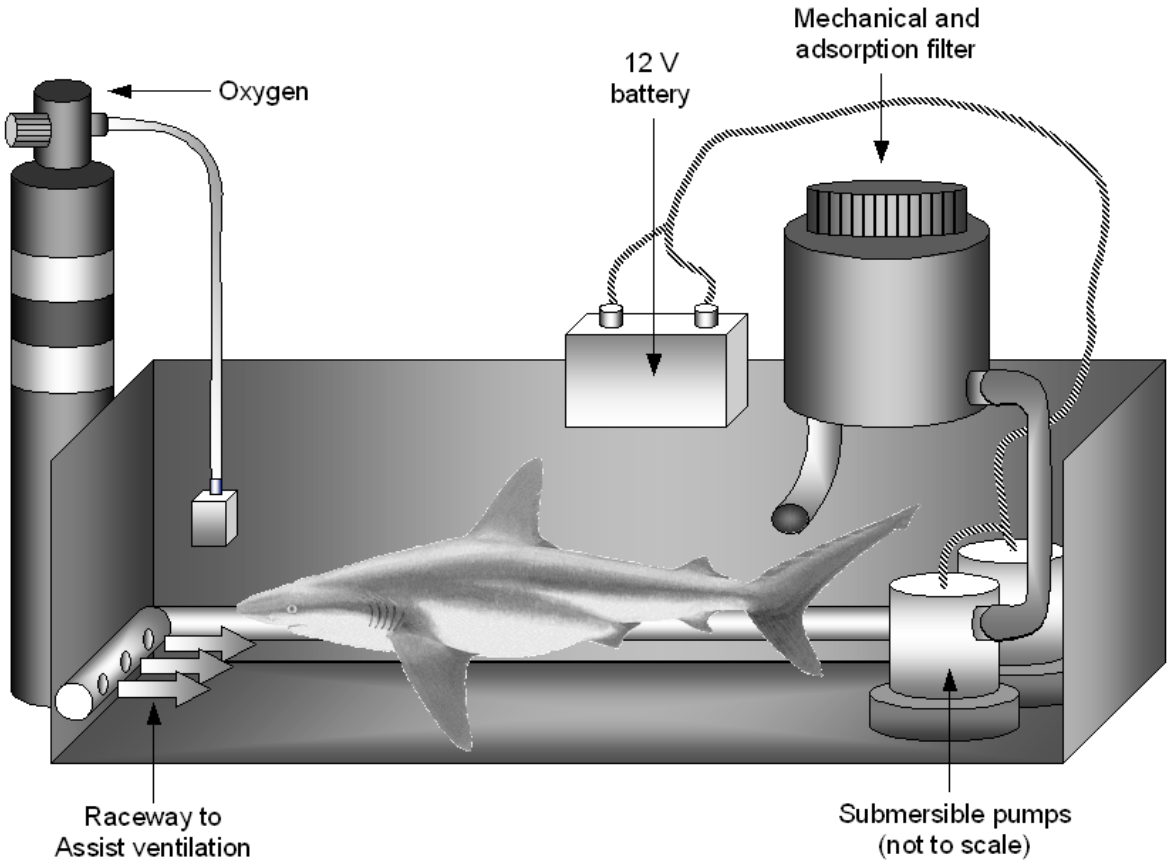


Figure 8.5. Restrained transport regime.

water flow (Hewitt, 1984; Andrews and Jones, 1990; Jones and Andrews, 1990; Murru, 1990).

Table 8.5 details a list of elasmobranch species that have been transported successfully using the three techniques described. Quoted durations are not a guarantee of survival. Capture technique, specimen size, handling technique, and water quality will all impact the success of the transport. Minimizing transport times should be the ultimate goal of any transport regime.

It is important to note that many workers reported limited success transporting the following species: thresher sharks (*Alopias spp.*), white sharks, mako sharks, porbeagle sharks (*Lamna nasus*), and blue sharks. Transport of these species should only be attempted by very experienced personnel.

WATER TREATMENT

Elasmobranchs continually excrete waste products that contaminate water in a transport tank. Decreasing water quality may be responsible for more losses during transport than any other factor (Murru, 1990). For a successful transport, water quality must be monitored closely and adjusted where necessary.

Before starting, transport tanks should be scrubbed thoroughly with seawater. Ensure that all traces of possible contaminants are removed. Transport water should be clean and preferably from the same source as the specimens to be transported. When transporting animals from a staging facility it is preferable to fast them for 48-72 hours beforehand. Referred to as conditioning, this process reduces water contamination via regurgitation and defecation in transit (Stoskopf, 1993; Sabalones, 1995; Ross and Ross, 1999). Once specimens are loaded and settled into the transport tank, a comprehensive water exchange (i.e., >75%) is recommended before transport commences. The water exchange will dilute stress-related metabolites and other contaminants, and greatly extend the period of time before water quality starts to decline (James, pers. com.). If transporting by boat there may be access to a continuous supply of fresh seawater; precluding the need to treat the water further. Land and air transports, on the other hand, may require a water

Table 8.5. Successfully transported elasmobranchs, showing technique and duration of transport; (j) refers to juvenile and (t) refers to a towed sea-cage. If more than one reference is available, durations are given as a range showing minimums and maximums. All references were personal communications unless otherwise indicated by a date of publication.

Species name	Common name	Technique	Duration (h)	Reference
Aetobatus narinari	Spotted eagle ray	Sealed bag and box	12 - 30 (j)	Thomas; Violetta; Young
		Free-swimming	21 - 56	Henningsen; Thomas; Violetta; Young; Marshall
		Restrained	7	Henningsen
Aetomylaeus niehofii	Banded eagle ray	Free-swimming	24 - 48	McEwan
Aptychotrema bougainvillii	Short-snouted shovelnose ray	Restrained	26	Hiruda et al., 1997
Aptychotrema rostrata	Eastern shovelnose ray	Sealed bag and box	2	Kinnunen
Asymbolus analis	Australian spotted catshark	Sealed bag and box	6	Kinnunen
Bathyraja aleutica	Aleutian skate	Free-swimming	3	Thomas
Bathyraja interrupta	Sandpaper skate	Free-swimming	3	Thomas
Brachaelurus waddi	Blind shark	Sealed bag and box	1	Kinnunen
		Free-swimming	1	Kinnunen
Carcharhinus acronotus	Blacknose shark	Sealed bag and box	24 (j)	Young
		Free-swimming	10 - 54	Henningsen; Violetta; Young; Young et al., 2001
		Restrained	1	Christie
Carcharhinus altimus	Bignose shark	Restrained	12	Powell
Carcharhinus brachyurus	Copper shark	Free-swimming	2.5	Kinnunen
Carcharhinus brevipinna	Spinner shark	Free-swimming	1	Kinnunen
Carcharhinus dussumieri	Whitecheek shark	Free-swimming	24 - 48	McEwan
Carcharhinus falciformis	Silky shark	Free-swimming	26 - 30	Young; Young et al., 2001
		Restrained	2	Christie
Carcharhinus leucas	Bull shark	Restrained	4 - 36	Gruber and Keyes, 1981; Ballard, 1989; Smith, 1992; Thomas; Violetta
Carcharhinus limbatus	Blacktip shark	Free-swimming	8 - 56	Thomas; Young et al., 2001; Christie; Violetta
		Restrained	1	Christie
Carcharhinus longimanus	Oceanic whitetip shark	Free-swimming	64	Ezcurra
Carcharhinus melanopterus	Blacktip reef shark	Sealed bag and box	14 - 35 (j)	Wisner, 1987; James; McEwan; Romero; Violetta
		Free-swimming	5 - 56	Barthelemy; Henningsen; Janse; Marshall
Carcharhinus obscurus	Dusky shark	Free-swimming	2	Kinnunen
		Restrained	6 - 8	Cliff and Thurman, 1984; Ballard, 1990; Steslow
Carcharhinus perezi	Caribbean reef shark	Restrained	1 - 2	Sabalones, 1995; Christie
Carcharhinus plumbeus	Sandbar shark	Sealed bag and box	16 - 30 (j)	Henningsen; James; Young
		Free-swimming	3 - 47	Barthelemy; Choromanski; Henningsen; James; Thomas; Violetta; Young
		Restrained	4 - 40	Gruber and Keyes, 1981; Andrews and Jones, 1990; Choromanski; Thomas; Violetta;
Carcharias taurus	Sand tiger shark	Sealed bag and box	5 - 15	Martel Bourbon
		Free-swimming	3 - 48	Hiruda et al., 1997; Choromanski; Farquar; Henningsen; Kinnunen; Thomas; Violetta
		Restrained	2 - 84	Smith, 1992; Choromanski; Henningsen; Romero; Thomas; Violetta; Marshall
Carcharodon carcharias	Great white shark	Free-swimming	2	Kinnunen
		Restrained	16 - 24	Gruber and Keyes, 1981; Hewitt, 1985
Cephaloscyllium laticeps	Australian swellshark	Free-swimming	7	Kinnunen
Cephaloscyllium ventriosum	Swellshark	Sealed bag and box	12 - 36	Howard; Thomas; Marshall
		Free-swimming	40	Thomas
Chiloscyllium plagiosum	Whitespotted bambooshark	Sealed bag and box	20 - 24	Christie; Violetta
Chiloscyllium punctatum	Brownbanded bambooshark	Sealed bag and box	24	Christie
Dasyatis americana	Southern stingray	Sealed bag and box	12 - 36	Henningsen; Thomas; Violetta; Young
		Free-swimming	10 - 70	Henningsen; Thomas; Violetta; Young
		Restrained	54	Marshall
Dasyatis brevicaudata	Short-tail stingray	Free-swimming	2 (t)	Kinnunen
		Restrained	26	Hiruda et al., 1997

Table 8.5 (continued). Successfully transported elasmobranchs, showing technique and duration of transport; (j) refers to juvenile and (t) refers to a towed sea-cage. If more than one reference is available, durations are given as a range showing minimums and maximums. All references were personal communications unless otherwise indicated by a date of publication.

Species name	Common name	Technique	Duration (h)	Reference
Dasyatis brevis	Whiptail stingray	Free-swimming	3	Thomas
Dasyatis centroura	Roughtail stingray	Sealed bag and box	30	Young
		Restrained	28	Steslow
Dasyatis chrysonota		Restrained	84	Unpub. Results
Dasyatis marmorata	Marbled stingray	Sealed bag and box	20 - 30	Farquar; Sabalones
		Free-swimming	84	Marshall
Dasyatis pastinaca	Common stingray	Free-swimming	5 - 21	James; Janse
Dasyatis violacea	Pelagic stingray	Free-swimming	9	Thomas
(= *Pteroplatytrygon*)		Restrained	8	Marshall
Dipturus batis	Skate	Free-swimming	14	James
Echinorhinus cookei	Prickly shark	Restrained	2	O'Sullivan
Galeocerdo cuvier	Tiger shark	Free-swimming	1 - 3	Kinnunen; Marín-Osorno
		Restrained	2 - 24	Gruber and Keyes, 1981; Ballard, 1989; Christie; Marín-Osorno; Thomas
Galeorhinus galeus	Tope shark	Free-swimming	6 - 26	James; Thomas
		Restrained	2 - 6	Engelbrecht; Howard; Thomas
Ginglymostoma cirratum	Nurse shark	Sealed bag and box	24 - 48 (j)	Carrier; Violetta; Young
		Free-swimming	12 - 50	James; Thomas; Violetta; Young
		Restrained	1 - 36	Clark, 1963; Christie; Marín-Osorno; Thomas; Violetta
Gymnura altavela	Spiny butterfly ray	Free-swimming	3	Henningsen
		Restrained	5	Marshall
Gymnura micrura	Smooth butterfly ray	Sealed bag and box	24	Young
		Free-swimming	7 - 9	Henningsen
Haploblepharus edwardsii	Puffadder shyshark	Sealed bag and box	20 - 30	Farquar; Sabalones
		Free-swimming	42	Marshall
Haploblepharus fuscus	Brown shyshark	Sealed bag and box	30	Sabalones
		Free-swimming	42	Marshall
Haploblepharus pictus	Dark shyshark	Sealed bag and box	20 - 30	Farquar; Sabalones
		Free-swimming	42	Marshall
Hemiscyllium ocellatum	Epaulette shark	Sealed bag and box	14 - 20	McEwan; Violetta
Heterodontus francisci	Horn shark	Sealed bag and box	14 - 36	James; Thomas; Violetta; Marshall
		Free-swimming	48	Thomas
Heterodontus galeatus	Crested bullhead shark	Restrained	2 - 26	Hiruda et al., 1997; Kinnunen
Heterodontus japonicus	Japanese bullhead shark	Free-swimming	56	Marshall
Heterodontus portusjacksoni	Port Jackson shark	Sealed bag and box	14 - 38	James; McEwan; Romero
		Free-swimming	10	Marshall
		Restrained	26	Hiruda et al., 1997
Hexanchus griseus	Bluntnose sixgill shark	Free-swimming	3	Thomas
		Restrained	3	Engelbrecht; Thomas
Himantura bleekeri	Bleeker's whipray	Free-swimming	24 - 48	McEwan
Himantura fai	Pink whipray	Restrained	56	Marshall
Himantura gerrardi	Sharpnose stingray	Free-swimming	24 - 48	McEwan
Himantura imbricata	Scaly whipray	Free-swimming	24 - 48	McEwan
Himantura schmardae	Chupare stingray	Free-swimming	2	Christie
Himantura uarnak	Honeycomb stingray	Free-swimming	24 - 48	McEwan
		Restrained	10 - 56	Marshall
Himantura undulata	Leopard whipray	Restrained	56	Marshall
Hydrolagus colliei	Spotted ratfish	Sealed bag and box	12	Marshall
		Free-swimming	44	Correia, J. 2001

Table 8.5 (continued). Successfully transported elasmobranchs, showing technique and duration of transport; (j) refers to juvenile and (t) refers to a towed sea-cage. If more than one reference is available, durations are given as a range showing minimums and maximums. All references were personal communications unless otherwise indicated by a date of publication.

Species name	Common name	Technique	Duration (h)	Reference
Isurus oxyrinchus	Shortfin mako	Free-swimming	2 - 7 (j)	Kinnunen; Steslow; Thomas
		Restrained	1.5	Powell
Manta birostris	Giant manta	Free-swimming	1 - 3	Christie; Marín-Osorno
Mobula munkiana	Munk's devil ray	Free-swimming	12	O'Sullivan
Mustelus antarcticus	Gummy shark	Free-swimming	8	Kinnunen
Mustelus asterias	Starry smooth-hound	Free-swimming	10	Janse
Mustelus californicus	Grey smooth-hound	Sealed bag and box	36	Thomas
		Free-swimming	36	Thomas
		Restrained	6	Engelbrecht
Mustelus henlei	Brown smooth-hound	Sealed bag and box	36	Thomas
		Free-swimming	1 - 36	Howard; Thomas
Mustelus mustelus	Smooth-hound	Sealed bag and box	12	James
		Free-swimming	10 - 50	Janse; Romero
Myliobatis aquila	Common eagle ray	Free-swimming	20	Farquar
		Restrained	84	Marshall
Myliobatis australis	Australian bull ray	Free-swimming	8	Kinnunen
Myliobatis californica	Bat eagle ray	Sealed bag and box	30 (j)	Thomas
		Free-swimming	1 - 36	Howard; Thomas
		Restrained	3	Engelbrecht
Myliobatis freminvillii	Bullnose eagle ray	Free-swimming	3	Henningsen
		Restrained	84	Marshall
Narcine brasiliensis	Brazilian electric ray	Sealed bag and box	24	Young
Nebrius ferrugineus	Tawny nurse shark	Sealed bag and box	14	McEwan
		Restrained	56	Marshall
Negaprion acutidens	Sicklefin lemon shark	Restrained	32	Engelbrecht
Negaprion brevirostris	Lemon shark	Sealed bag and box	14 - 48	Henningsen; Young
		Free-swimming	30 - 36	Thomas; Violetta
		Restrained	23 - 36	Gruber and Keyes, 1981; Henningsen; Thomas; Young
Notorynchus cepedianus	Broadnose sevengill shark	Free-swimming	3 - 5	Kinnunen; Thomas
		Restrained	1 - 6	Engelbrecht; Howard
Orectolobus maculatus	Spotted wobbegong	Sealed bag and box	24 - 30	Christie; Romero
		Free-swimming	8 - 10	Kinnunen; Marshall
		Restrained	4 - 26	Hiruda et al., 1997; Marshall
Orectolobus ornatus	Ornate wobbegong	Sealed bag and box	18 - 24	Christie; Violetta
		Restrained	3 - 26	Hiruda et al., 1997; Kinnunen
Paragaleus randalli	Slender weasel shark	Free-swimming	24 - 48	McEwan
Pastinachus sephen	Cowtail stingray	Free-swimming	24 - 56	McEwan; Marshall
Platyrhinoidis triseriata	Thornback guitarfish	Free-swimming	4	Thomas
Poroderma africanum	Striped catshark	Sealed bag and box	20 - 30	Farquar; Sabalones
		Free-swimming	56	Marshall
Poroderma pantherinum	Leopard catshark	Sealed bag and box	20 - 30	Farquar; Sabalones
		Free-swimming	56	Marshall
Potamotrygon motoro	Ocellate river stingray	Free-swimming	4	Janse
Prionace glauca	Blue shark	Free-swimming	1.5 - 4 (j, t)	Kinnunen; Thomas
		Restrained	3 - 8	Howard; Powell; Steslow; Thomas
Pristis pectinata	Smalltooth sawfish	Sealed bag and box	12	Christie; Henningsen
		Restrained	12 - 24	Christie; Engelbrecht; Henningsen; Violetta

Table 8.5 (continued). Successfully transported elasmobranchs, showing technique and duration of transport; (j) refers to juvenile and (t) refers to a towed sea-cage. If more than one reference is available, durations are given as a range showing minimums and maximums. All references were personal communications unless otherwise indicated by a date of publication.

Species name	Common name	Technique	Duration (h)	Reference
Pristis pristis	Common sawfish	Restrained	32	Romero
Raja binoculata	Big skate	Sealed bag and box	12 (j)	Howard
		Free-swimming	1 - 5	Howard; Thomas
		Restrained	6	Engelbrecht
Raja clavata	Thornback ray	Sealed bag and box	24	Marshall
		Free-swimming	10	Janse
Raja eglanteria	Clearnose skate	Sealed bag and box	30	Young
Raja rhina	Longnose skate	Free-swimming	3 - 16	Howard; Thomas
Raja stellulata	Starry skate	Free-swimming	3 - 15	Howard; Thomas
Raja undulata	Undulate ray	Free-swimming	8	Marshall
Rhina ancylostoma	Bowmouth guitarfish	Restrained	5	Smith, 1992
Rhincodon typus	Whale shark	Restrained	2	Kinnunen
Rhinobatos annulatus	Lesser sandshark	Sealed bag and box	20 - 30	Farquar; Sabalones
		Free-swimming	42	Marshall
Rhinobatos granulatus	Sharpnose guitarfish	Free-swimming	24 - 48	McEwan
Rhinobatos lentiginosus	Atlantic guitarfish	Sealed bag and box	6 - 30	Christie; Young
Rhinobatos productus	Shovelnose guitarfish	Free-swimming	36	Thomas
Rhinobatos typus	Giant shovelnose ray	Restrained	56	Marshall
Rhinoptera bonasus	Cownose ray	Sealed bag and box	6 - 60	Christie; Violetta; Young
		Free-swimming	12 - 76	Henningsen; Young
Rhizoprionodon terraenovae	Atlantic sharpnose shark	Sealed bag and box	10	Henningsen
		Free-swimming	3 - 30 (j)	Christie; Henningsen; Violetta
		Restrained	6 - 8	Steslow
Rhynchobatus djiddensis	Giant guitarfish	Restrained	2 - 42	Kinnunen; Unpub. Results
Scyliorhinus canicula	Smallspotted catshark	Sealed bag and box	3 - 25 (j)	James; Janse; Marshall
		Free-swimming	26	James
Scyliorhinus retifer	Chain catshark	Sealed bag and box	18	Marshall
Scyliorhinus stellaris	Nursehound	Sealed bag and box	10 - 24	James; Marshall
		Free-swimming	3 - 26	James; Janse
Somniosus pacificus	Pacific sleeper shark	Free-swimming	5	Thomas
Sphyrna lewini	Scalloped hammerhead	Free-swimming	6 - 60 (j)	Arai, 1997; Young, 2002; Thomas; Violetta
Sphyrna mokarran	Great hammerhead	Free-swimming	12 - 21	Christie; Young
Sphyrna tiburo	Bonnethead	Sealed bag and box	8 - 48 (j)	Christie; James; Thomas; Violetta; Young
		Free-swimming	8 - 76	Christie; James; Henningsen; Thomas; Violetta; Young
Sphyrna zygaena	Smooth hammerhead	Free-swimming	8 (t)	Kinnunen
Squalus acanthias	Spiny dogfish	Free-swimming	1 - 36	Howard; James; Thomas
		Restrained	1 - 6	Engelbrecht; Howard
Squatina australis	Australian angelshark	Restrained	3.5	Kinnunen
Squatina californica	Pacific angelshark	Free-swimming	4	Howard
		Restrained	6	Engelbrecht
Squatina dumeril	Sand devil	Restrained	0.5 - 1	Marín-Osorno
Squatina squatina	Angelshark	Sealed bag and box	17	Romero
Stegostoma fasciatum	Zebra shark	Sealed bag and box	14 - 24	Christie; McEwan; Violetta
		Free-swimming	8 - 20	Kinnunen; Romero; Violetta
		Restrained	6 - 56	Smith, 1992; Hiruda et al., 1997; Marshall
Taeniura lymma	Bluespotted ribbontail ray	Sealed bag and box	14 - 24	McEwan; Marshall
		Restrained	56	Marshall

Table 8.5 (continued). Successfully transported elasmobranchs, showing technique and duration of transport; (j) refers to juvenile and (t) refers to a towed sea-cage. If more than one reference is available, durations are given as a range showing minimums and maximums. All references were personal communications unless otherwise indicated by a date of publication.

Species name	Common name	Technique	Duration (h)	Reference
Taeniura meyeni	Blotched fantail ray	Restrained	56	Marshall
Torpedo californica	Pacific electric ray	Free-swimming	2 - 6	Howard
Torpedo marmorata	Marbled electric ray	Free-swimming	8	Marshall
Torpedo nobiliana	Electric ray	Free-swimming	18	Janse
Torpedo panthera	Panther electric ray	Free-swimming	24 - 48	McEwan
Triaenodon obesus	Whitetip reef shark	Sealed bag and box	10 - 18 (j)	Henningsen; McEwan; Violetta
		Free-swimming	7 - 34	Barthelemy; Marshall
		Restrained	26 - 56	Hiruda et al., 1997; Marshall
Triakis megalopterus	Sharptooth houndshark	Free-swimming	20 - 30	Farquar; Sabalones
		Restrained	84	Marshall
Triakis semifasciata	Leopard shark	Sealed bag and box	24 - 36 (j)	Carrier; Thomas; Marshall
		Free-swimming	1 - 48	Howard; James; Thomas
		Restrained	6	Engelbrecht
Trygonorrhina fasciata	Southern fiddler	Sealed bag and box	3 - 10	Kinnunen; Marshall
		Free-swimming	10	Marshall
		Restrained	26	Hiruda et al., 1997
Urolophus halleri	Haller's round ray	Sealed bag and box	24 - 36	Thomas; Young
		Free-swimming	36	Thomas
Urobatis jamaicensis	Yellow stingray	Sealed bag and box	24 - 48	Thomas; Violetta; Young; Marshall
		Free-swimming	1 - 36	Christie; Thomas
Urolophus sufflavus	Yellowback stingaree	Restrained	26	Hiruda et al., 1997

treatment system. Throughout any transport critical water parameters to monitor and control include: (1) oxygen (described above); (2) temperature; (3) particulates and organics; (4) pH; and (5) nitrogenous wastes.

Temperature

When elasmobranchs go from a warmer to cooler environment they suffer a short-term thermal shock that can result in respiratory depression. Conversely, an increased temperature can promote and exacerbate hyperactivity (Stoskopf, 1993, Ross and Ross, 1999). Reducing temperature differentials at the source, in transit, and at the final destination, will increase the chances of a successful transport (Andrews and Jones, 1990; Stoskopf, 1993). Transport tanks should be well-insulated, and as much as possible, temperature-controlled environments should be used (e.g., air conditioned vehicles, covered airport hangars, etc.). In extreme cases water exchanges, bagged ice, bagged hot water, and heat beads may be used to minimize temperature changes depending on prevailing trends.

Particulates and organics

Particulate and dissolved organo-carbon compounds, or organics, are excreted by elasmobranchs during transport. In particular skates and rays produce copious amounts of a proteinaceous slimes when subjected to stress. Particulates, or suspended solids, will irritate the gills, reduce water clarity, and cause distress to specimens being transported (Ross and Ross, 1999). Dissolved organics will tend to reduce pH, increase ammonia (NH_3) concentration, and consume O_2. The concentration of both particulates and organics should therefore be minimized during transport. Dilution of particulates and organics can be achieved by water exchanges, mechanical filtration, adsorption or chemical filtration, and foam fractionation (Gruber and Keyes, 1981; Stoskopf, 1993; Dehart, pers. com.).

Mechanical filtration usually takes the form of a canister filter containing appropriate media (e.g., pleated paper, filter wool, etc.). Filtration may be enhanced by the addition of an adsorption or chemical filtration medium such as activated carbon (e.g., Professional Grade Activated Carbon, Aquarium Pharmaceuticals Inc, USA) or other chemical filter (e.g., Eco-lyte™, Mesco Aquatic Products, USA) (Marshall, 1999). Eco-lyte™ is particularly effective (i.e., ~100 times as effective as activated carbon) at removing dissolved organics (Gruber and Keyes, 1981). As Eco-lyte™ is an adsorption medium it will be more effective if preceded by a mechanical filter. When using Eco-lyte™ it should be borne in mind that it will remove medications from the water (e.g., anti-stress agents, anesthetics, etc.). Always pre-wash a medium before packing a filter. For long transports it is beneficial to completely replace the medium, in transit, once it has become heavily contaminated.

pH

Continued excretion of dissolved CO_2 and H^+ will drive pH in a transport tank down (i.e., the water will become more acidic). Efforts should be made to resist this trend and pH should be maintained at a level of 7.8-8.2 (Murru, 1990). One important way to counter pH decline is the continuous removal of CO_2 from transport water by de-gassing. Degassing is achieved by spraying re-circulated water into the tank and agitating the surface, or alternatively, bubbling the water surface with a diffuser. Air ventilation is critical during this process as it will carry away liberated CO_2 gas (Young et al., 2002).

Both sodium bicarbonate ($NaHCO_3$) and sodium carbonate (Na_2CO_3) have been added to transport water to successfully resist decreasing pH (Cliff and Thurman, 1984; Murru, 1990; Smith, 1992). A more efficient buffer is tris-hydroxymethyl aminomethane (e.g., Tris-amino®, Angus Chemical, USA). This compound is more effective within the expected pH range (i.e. 7.5-8.5) and is able to increase the acid-absorbing capacity of seawater by up to 50 times (McFarlane and Norris, 1958; Murru, 1990). It is important to remember that increased pH results in an increased proportion of the toxic form of ammonia according to the reaction given in Equation 8.2. Any corrective therapy applied to the pH of the water must therefore be coupled with the removal of excess ammonia (Ross and Ross, 1999).

Nitrogenous wastes (NH_3 / NH_4^+)

Ammonia constitutes approximately 70% of the nitrogenous wastes excreted by aquatic organisms (Ross and Ross, 1999). Some delicate

Equation 8.2

$$\underset{\text{Ammonium ion}}{NH_4^+} + \underset{\text{Hydroxide ion}}{OH^-} \leftrightarrows \underset{\text{Ammonia}}{NH_3} + \underset{\text{Water}}{H_20}$$

elasmobranchs are particularly nutrient-sensitive and ammonia concentrations should never be allowed to exceed 1.0 mg l^{-1}. The removal of ammonia from a transport tank should therefore be one of the principal objectives of any water treatment system. Ammonia can be removed successfully using periodic 50% water exchanges and the application of adsorption media (see above). Pre-matured biological filters may be employed if ammonia production levels during transport can be calculated and simulated beforehand (e.g., with ammonium chloride) (Dehart, pers. com.). Another option is to use an ammonia sponge such as sodium hydroxymethanesulfonate (e.g., AmQuel®, Novalek Inc., USA) (Visser, 1996; Young et al., 2002). AmQuel® inactivates ammonia according to the reaction given in Equation 8.3. The substance formed is stable and non-toxic, and will not release ammonia back into the water. It should be noted that this reaction will lower pH so the addition of AmQuel® should be accompanied by the careful application of a buffer as discussed above. Following the application of AmQuel®, only salicylate-based ammonia tests will yield accurate results.

Zeolite (e.g., Ammo-Rocks®, Aquarium Pharmaceuticals Ltd., USA), an ion-exchange resin used for the removal of nitrogenous wastes in freshwater systems, does not work well in seawater because the ammonia molecule is similar in size to the sodium ion. At a salinity of 36 ppt there is a 95% reduction in zeolite's ability to remove ammonia from the water—although its ability to remove organic dyes remains unchanged (Noga, 1996).

ANESTHESIA

The mechanics of anesthesia, appropriate sedatives for elasmobranchs, and corresponding dosage rates will be covered in Chapter 21 of this manual. We will therefore focus only on specific examples as they relate to elasmobranch transport.

Anesthesia may be valuable during specific transports as it can minimize handling times, reduce physical injury, slow metabolic rate and O_2 consumption, and reduce the production of metabolic wastes (Ross and Ross, 1999). If anesthesia is used, respiratory and cardiovascular depression becomes a risk and must be avoided (Tyler and Hawkins 1981; Dunn and Koester, 1990; Smith, 1992). Reduction of a specimen's metabolism by chemical immobilization will constitute a poor trade-off if circulation becomes so weakened that O_2 uptake and metabolite effusion at the gill surface are impaired (Smith, 1992). Additionally, the wide inter-specific diversity of elasmobranchs can make it difficult to predict dosage rates and possible sensitivity reactions to immobilizing agents (Vogelnest et al., 1994). Consequently anesthesia may be warranted in specific cases but is not advocated for general use during elasmobranch transports.

If anesthesia is used it will be more valuable if transport does not begin until the drug has taken its full effect and specimen stability has been assured (Smith, 1992). If defense reactions are not fully moderated the animal could respond to external stimuli and physically injure itself or personnel. Aggressive emergence reactions should be avoided for the same reasons so visual, auditory, and pressure stimuli should be minimized during recovery (Smith, 1992). Some means to adequately monitor depth of anesthesia must be employed throughout the transport so that corrective measures can be undertaken should system deterioration be observed (Dunn and Koester, 1990).

Inhalation anesthesia

A popular method of inducing anesthesia in small sharks is by immersing them in water containing an anesthetic agent. The thin gill membranes act as the site of adsorption and the anesthetic passes directly into arterial blood (Oswald, 1977; Stoskopf, 1986). It is possible to control the depth of anesthesia by adjusting the concentration of drug in the water. Immersion, or inhalation, anesthesia is often impractical for large sharks so a modification of the technique, irrigation anesthesia, may be used as an alternative. Irrigation anesthesia is achieved by spraying a concentrated solution of the anesthetic over the gills using a plastic laboratory wash bottle or pressurized mister. This system yields a rapid

Equation 8.3

$$NH_3 + HOCH_2SO_3^- \leftrightarrows H_2NCH_2SO_3^- + H_20$$

Ammonia	AmQuel	Aminomethanesulfonate	Water

induction but there is a risk of overdose and it can result in delayed recovery (Tyler and Hawkins, 1981).

Some filtration media (e.g., ion-exchange resins and activated carbon) may remove drugs used for inhalation anesthesia. Inhalation anesthesia cannot be employed in aquariums where contamination of the water is a consideration (Stoskopf, 1986).

A popular inhalation anesthetic for elasmobranchs is tricaine methanesulfonate or MS-222 (Finquel®, Argent Laboratories, USA) (Gilbert and Wood, 1957; Clark, 1963; Gilbert and Douglas, 1963; Gruber, 1980; Gruber and Keyes, 1981; Tyler and Hawkins, 1981; Stoskopf, 1986; Dunn and Koester, 1990). Unfortunately dosage rates for transport purposes are not well documented. Stoskopf (1993) has suggested an immersion induction dose of 100 mg l^{-1} MS-222 for the long-term anesthesia of small sharks; with an expected induction time of 15 minutes. For the transport of fishes in general, Ross and Ross (1999) advocate a lower immersion induction dose of 50 mg l^{-1} followed by a maintenance dosage of 10 mg l^{-1}. Brittsan (pers. com.) successfully restrained and transported two blacktip reef sharks (*Carcharhinus melanopterus*) for 24 hours using an induction dose of 48 mg l^{-1} MS-222. Initial induction time was approximately two minutes and both animals were transferred to the transport tanks within eight minutes. A maintenance dose was considered unnecessary and was not applied.

Injection anesthesia

Injection anesthesia may be administered intravenously (IV), intraperitonealy (IP), and intramuscularly (IM). IV injections result in a quick onset of anesthesia but can only be administered to restrained animals, allowing accurate location of appropriate blood vessels. IP administered sedatives must pass through the intestinal wall or associated membranes so induction time is often delayed. IM injections can be administered to slow-swimming sharks without previous restraint and induction time is usually quite fast. It is possible to administer IM injections to active elasmobranchs with pole syringes or similar remote-injection devices. A convenient site for IM injection is an area of musculature surrounding the first dorsal fin, referred to as the dorsal saddle (Stoskopf, 1993).

The tough nature of shark skin and denticles requires the use of a heavy-gauge needle to penetrate through to a blood vessel, body cavity or musculature (Stoskopf, 1986). The use of heavy-gauge needles may present problems as shark skin is not very elastic and drugs may leak from the injection site. If possible, gently massaging the injection site can reduce leakage. When using any form of injection anesthetic it may be difficult to control depth of anesthesia because once the drug has been introduced into systemic circulation it is not easily reversed. In addition, recovery from a heavy dose of an injected anesthetic may be prolonged if the drug is slowly released from non-nervous tissue.

Sedation is defined as a preliminary level of anesthesia where response to stimulation is reduced and some analgesia is evident (Ross and Ross, 1999). Sedation may be useful if a specimen is likely to struggle excessively during the initial stages of capture and preparation for transport. The sooner a specimen becomes quiescent the better the chances of its long-term survival (Dunn and Koester, 1990). One of the authors (Smith) has used Diazepam (Valium®, F. Hoffmann-La Roche Ltd, Switzerland) successfully at 0.1 mg kg^{-1} IM to mitigate hyperactivity in sand tiger sharks for short periods. Visser (1996) applied 5.0 mg of Diazepam to a 1.8 m sand tiger shark prior to transferring the animal from the site of capture to a staging facility. Within minutes of application the shark was sedated and could be handled safely for a period of approximately one hour.

A combination of ketamine hydrochloride (Ketalar®, Parke-Davis, USA) and xylazine hydrochloride (Rompun®, Bayer AG, Germany) has been used successfully to deeply anesthetize large elasmobranchs, for long periods, when administered IM (Oswald, 1977; Stoskopf, 1986; Andrews and Jones, 1990; Jones and Andrews, 1990; Smith, 1992). Stoskopf (1993) suggests the use of 12.0 mg kg^{-1} ketamine hydrochloride and 6.0 mg kg^{-1} xylazine hydrochloride for anesthetizing large sharks. Stoskopf (1986) further recommends using higher doses for more active sharks, such as the sandbar shark (*Carcharhinus plumbeus*) (i.e., 16.5 mg kg^{-1} and 7.5 mg kg^{-1}, respectively), and lower dosage rates for less active sharks like the sand tiger (i.e., 8.25 mg kg^{-1} and 4.1 mg kg^{-1}, respectively). The expected induction time at these dosage rates is ~8-10 minutes. Andrews and Jones (1990) successfully used 16.5 mg kg^{-1} ketamine hydrochloride and 7.5 mg kg^{-1} xylazine hydrochloride IM to anesthetize sandbar sharks for transport purposes. Similarly the sand tiger shark, bull shark, zebra shark (*Stegostoma*

fasciatum), and bowmouth guitarfish (*Rhina ancylostoma*) have been anesthetized and transported using a combination of 15.0 mg kg^{-1} ketamine hydrochloride and 6.0 mg kg^{-1} xylazine hydrochloride IM, in conjunction with 0.125 mg kg^{-1} IM of the antagonist yohimbine hydrochloride (Antagonil®, Wildlife Pharmaceuticals Inc, USA) at the conclusion of the operation (Smith, 1992). Visser (1996) has anesthetized two 1.8 m sand tiger sharks, for transport purposes, using 900 mg ketamine hydrochloride and 360 mg xylazine hydrochloride IM.

CORRECTIVE THERAPY

If a transport is extensive in duration, or preceded by specimen hyperactivity, an elasmobranch will consume a lot of its stored energy reserves. By administering glucose directly into the bloodstream it is possible to compensate for a drop in blood-glucose concentrations and decrease the need to mobilize valuable glycogen stores from the liver. Reduced mobilization of glycogen is particularly important if hyperactivity has proceeded to such an extent that glucocorticoids are depleted or blood-glucose reserves are nearing exhaustion (Smith, 1992).

Although elasmobranchs have a limited ability to buffer their blood, it has been observed that they are able to absorb bicarbonate (HCO_3^-) directly from the surrounding environment to help counteract acidosis (Murdaugh and Robin, 1967; Holeton and Heisler, 1978). This ability suggests an avenue for therapy directed at alleviating acid-base disruption in an acidotic elasmobranch: specifically, direct administration of HCO_3^- into the bloodstream (Cliff and Thurman, 1984). Acetate has been suggested as an effective alternative to bicarbonate (Hewitt, 1984), although there is some concern that acetate degradation may be more noxious than bicarbonate dissociation (Young, pers. com.). Nevertheless, an acetate-bicarbonate combination has been used successfully to revive a prostrated blacktip reef shark by injecting the mix directly into the bloodstream (Hewitt, pers. com.).

In practice, a corrective therapy for hypoglycemia and acidosis can be prepared by adding HCO_3^- or CO_3^{2-} (carbonate) to an IV drip-bag of glucose or dextrose (e.g., 100 ml of 8.4% $NaHCO_3$ mixed in a 1.0 l IV drip-bag of 5% glucose in saline). The resulting mixture is introduced into the specimen via an IV drip line and heavy-gauge catheter. The preferred site for therapy administration is the large dorsal blood sinus just under the skin and posterior to the first dorsal fin. This site allows the position of the catheter to be monitored closely (Murru 1990). If this blood vessel resists penetration, it is possible to use a caudal blood vessel just posterior to the anal fin or even to introduce the catheter IP. IV will be more effective than IP in the case of bradycardia (cardiac depression). Approximately 500 ml of the corrective therapy should be administered to a 100 kg shark every hour (i.e., 5 ml kg^{-1} h^{-1}). This dosage may be increased if the decline in blood pH is known to be profound (Smith, 1992).

Many workers have produced variations on this therapeutic recipe to make it less physiologically challenging to target elasmobranchs (Table 8.6). In some cases, urea has been added to equilibrate the osmotic pressure of the mixture with that of shark plasma (Murru, 1990; Andrews and Jones, 1990). This area of corrective therapy would benefit greatly from some structured research.

MONITORING

When transporting delicate species, or using an elaborate transport regime, it is important to frequently check specimen and equipment status (Cliff and Thurman, 1984; Smith, 1992; Ross and Ross, 1999). Many important factors should be verified and have been summarized in Table 8.7. If a problem is observed, corrective measures should be undertaken immediately. Tanks should be packed so that windows, access hatches, and critical equipment (e.g., valves, gauges, tools, etc.) are all easily accessible. Testing equipment to measure critical water parameters should be carried and used.

It is important to monitor ventilation rate. Ventilation and cardiac rates are functionally linked in many fishes and may be neurologically synchronized (Ross and Ross, 1999). Gilbert and Wood (1957) observed that heartbeat was synchronous with ventilation rate in anesthetized lemon sharks. Skin color should be monitored as it may be used to loosely assess biochemical impact on a specimen; increasing loss of skin color equating to an increased biochemical change (Cliff and Thurman, 1984; Smith, 1992). Stoskopf (1993) observed that hypo-oxygenation could cause sharks to turn blotchy and increase their respiration rate, while hyper-oxygenation caused them to become pale and occasionally cease ventilation altogether (Stoskopf, 1993).

Table 8.6. Corrective therapies applied to hypoglycemic and acidotic elasmobranchs during transportation showing formulations, dosage rates for adult specimens, mode of administration, and species treated.

Hewitt, 1984		**Ballard, 1989**		**Murru, 1990**		**Andrews and Jones, 1990**		**Smith, 1992**		**Visser, 1996**	
Dextrose	25.0 g l^{-1} (2.5%)	Dextrose	? [a]	Dextrose	20.0 g l^{-1} (2.0%)	Glucose	1.00 g l^{-1}	Glucose	50.0 g l^{-1} (5.0%)	Glucose	50.0 g l^{-1} (5.0%)
NaHCO3	? [a]	NaHCO3	0.42 g l^{-1}	NaCO3	? [b]	$NaHCO^3$	0.35 g l^{-1}	NaHCO3	0.84 g l^{-1} [d]	NaHCO3	0.84 - 3.36 g l^{-1} [e]
Amino acids	? [a]			Urea	300 mEq l^{-1}	Urea [c]	21.02 g l^{-1}				
Electrolytes	? [a]			Na^+	280 mEq l^{-1}	NaCl	16.00 g l^{-1}				
B-vitamins	? [a]			Cl^-	230 mEq l^{-1}	KCl	0.40 g l^{-1}				
				K^+	4.4 mEq l^{-1}	$CaCl_2$	0.14 g l^{-1}				
						$MgCl_2$	0.10 g l^{-1}				
						KH_2PO_4	0.06 g l^{-1}				
						$MgSO_4$	0.10 g l^{-1}				
						$NaHPO_4$	0.90 g l^{-1}				
? [a]		250 - 500 ml h^{-1}		40 - 100 ml h^{-1}		120 ml h^{-1}		500 ml h^{-1}		600 - 700 ml h^{-1}	
IV		IP		IP (or IV)		IP		IP (or IV)		IP	
Carcharodon carcharias		*Carcharhinus leucas*		*Carcharhinus leucas*		*Carcharhinus plumbeus*		*Carcharhinus leucas*		*Carcharias taurus*	
		Carcharhinus obscurus		*Carcharhinus obscurus*				*Carcharias taurus*			
				Carcharhinus plumbeus							

a: value unknown.
b: value unknown; sodium carbonate added to formulation until pH value of 8.4 attained.
c: urea added to formulation until osmotic pressure equilibrated with shark plasma.
d: quoted as 8.4 g $100ml^{-1}$ HCO_3^- added to 900 ml of 5% glucose in saline.
e: quoted as 8.4 g $100ml^{-1}$ HCO_3^- added to 900 ml of 5% glucose in saline and 16.8 g $200ml^{-1}$ HCO_3^- added to 800 ml of 5% glucose in saline.

Table 8.7. Important factors to monitor throughout an elasmobranch transport. Should any of these factors represent a progressive problem corrective measures should be undertaken immediately.

Specimens	1.1	Ataxia (uncoordinated movements) or disorientation.
	1.2	Partial or total loss of equilibrium.
	1.3	Tachy-ventilation or brady-ventilation (i.e. increased or decreased gill ventilation rates).
	1.4	Changes in muscle tone.
	1.5	Changes in shade and homogeneity of skin color.
	1.6	Possible physical injury.
Equipment	2.1	Bubbles emerging from oxygen diffuser.
	2.2	Uninterrupted power supply and power supply not overheating.
	2.3	Pump operating correctly and not overheating.
	2.4	Water flow constant and correctly orientated.
	2.5	Water level stable with no appreciable leakages.
	2.6	Water clear and uncontaminated.
	2.7	Water quality parameters within acceptable limits.

ACCLIMATIZATION AND RECOVERY

At the termination of a transport specimens should be acclimatized to local water parameters by slowly replacing the water in the transport tank with water from the quarantine facility (refer Chapter 11 for more information about specimen acclimatization). If the elasmobranch appears to be healthy, prophylactic treatments (e.g., anti-helminthic baths, antibiotic injections, etc.) may be applied (Mohan, pers. com.). Serious abrasions, punctures, or lacerations should be evaluated and may require the application of an antibacterial agent or possibly sutures (Murru, 1990). Handling should be kept to a minimum and excess external stimuli avoided.

Reversal of immobilizing drugs should be coincident with specimen release. Once a specimen starts to swim normally, muscle tissue will be flushed with fresh, oxygenated blood. This process will cause metabolic by-products sequestered in the tissues and extra-cellular spaces to move into circulation. High concentrations of toxins may enter delicate organs and possibly compromise recovery (Cliff and Thurman, 1984). In addition, immobilizing drugs may be flushed into the bloodstream and renew their paralyzing effects. During this period the animal may be disorientated and exhibit defense responses to external stimuli (Gruber and Keyes 1981; Smith, 1992).

When released, some pelagic and demersal elasmobranchs will lie on the bottom of the aquarium. Walking while holding the shark in the water column, flexing its caudal peduncle, and stroking its dorsal surface have all been recommended as techniques to increase ventilation, assist venous return, and facilitate recovery (Clark, 1963; Gruber and Keyes, 1981). These techniques require excess handling and do not simulate normal swimming behavior. In addition, these techniques may actually compound the effects of hyperactivity and prematurely flush systemic circulation with high concentrations of toxic metabolites. As long as an elasmobranch is ventilating voluntarily, allowing it to lie in a current of oxygen-rich seawater avoids these complications (Hewitt, 1984; Smith, 1992; Stoskopf, 1993).

It is preferable to allow specimens to recover in an isolated and unobstructed tank. Once a recovering elasmobranch is swimming freely it is important that a program of post-transport observation be implemented. Feeding the animal within 24 hours of transport is not recommended (Smith, 1992). If a suitable isolation facility is available, a comprehensive quarantine regime should be seriously considered before the specimen is introduced into the destination exhibit (Andrews, pers. com.).

ACKNOWLEDGMENTS

The following individuals made valuable suggestions to improve the contents of this chapter: Jackson Andrews, Geremy Cliff, Heidi Dewar, John Hewitt, Frank McHugh, Pete Mohan, Dave Powell, Juan Sabalones, Dennis Thoney, Marty Wisner, and Forrest Young. I would like to thank the staff of the Oceanário de Lisboa, and International Design for

the Environment and Associates, all of whom allowed me valuable time to work on the elasmobranch husbandry manual project.

REFERENCES

Albers, C. 1970. Acid-base balance. *In:* Fish Physiology, Vol. IV, p. 173-208. W. S. Hoar and D. J. Randall (eds). Academic Press, New York, USA.

Andrews, J. C. and R. T. Jones. 1990. A method for the transport of sharks for captivity. Journal of Aquariculture and Aquatic Sciences 5: 70-72.

Arai, H. 1997. Collecting, transporting and rearing of the scalloped hammerhead. *In:* Proceedings of the Fourth International Aquarium Congress, June 23-27, 1996, Tokyo, Japan, p. 87-89. Tokyo, Japan by the Congress Central Office of IAC '96, Tokyo Sea Life Park. 402 pp.

Baldwin, J. and R. M. G. Wells. 1990. Oxygen transport potential in tropical elasmobranchs from the Great Barrier Reef: relationship between hematology and blood viscosity. Journal for Experimental Marine Biology and Ecology 144: 145-155.

Ballard, J. A. 1989. Some specimen collecting and transport methods. Bulletin de l'Institut Océanographique, Monaco 5: 63-69.

Bennett, A. F. 1978. Activity metabolism of the lower vertebrates. Annual Review of Physiology 408: 447-469.

Bigelow, H. B., and W. C. Schroeder. 1948. Fishes of the Western North Atlantic. Volume 1. Memoir, Sears's Foundation for Marine Research, Yale University, New Haven, Connecticut, USA. 576 p.

Boord, R. L. and C. B. G. Campbell. 1977. Structural and functional organization of the lateral line system in sharks. American Zoologist 17: 431-441.

Butler, P. J. and E. W. Taylor. 1975. The effect of progressive hypoxia on respiration in the dogfish (*Scyliorhinus canicula*) at different seasonal temperatures. Journal of Experimental Biology 63: 117-130.

Carlson, J. K., C. L. Palmer, and G. R. Parsons. 1999. Oxygen consumption rate and swimming efficiency of the blacknose shark. *Carcharhinus acronotus*. Copeia 1999: 34-39.

Clark, E. 1963. The maintenance of sharks in captivity, with a report on their instrumental conditioning. *In:* Sharks and Survival, p. 115-149. P. W. Gilbert (ed.). D. C. Heath and Company, Boston, Massachusetts, USA.

Clark, E. and H. Kabasawa. 1977. Factors affecting the respiration rates of two Japanese sharks, *Triakis scyllium* and *Heterodontus japonicus*. O. N. R. Tokyo Scientific Bulletin 1: 1-11.

Cliff, G. and G. D. Thurman. 1984. Pathological and physiological effects of stress during capture and transport in the juvenile dusky shark, *Carcharhinus obscurus*. Comparative Biochemistry and Physiology 78: 167-173.

Correia, J. P. 2001. Long-term transportation of ratfish, *Hydrolagus colliei*, and tiger rockfish, *Sebastes nigrocinctus*. Zoo Biology 20: 435-441.

Davies, D. H., E. Clark, A. L. Tester, and P. W. Gilbert. 1963. Facilities for the experimental investigation of sharks. *In:* Sharks and Survival, p. 151-163. P. W. Gilbert (ed.). D. C. Heath and Company, Boston, Massachusetts, USA.

Dunn, R. F. and D. M. Koester. 1990. Anesthetics in elasmobranchs: A review with emphasis on halothane-oxygen-nitrous oxide. Journal of Aquariculture and Aquatic Sciences 5: 44-52.

Emery, S. H. 1985. Hematology and cardiac morphology in the great white shark, *Carcharodon carcharias*. Memoirs of the Southern California Academy of Sciences 9: 73-80.

Essapian, F. S. 1962. Notes on the behavior of sharks in captivity. Copeia 1962: 457-459.

Froese, R. 1988. Relationship between body weight and loading densities in fish transport using the plastic bag method. Aquaculture and Fisheries Management 19: 275-281.

Gilbert, P. W. and S. D. Douglas. 1963. Electrocardiographic studies of free-swimming sharks. Science 140: 1396.

Gilbert, P. W. and F. G. Wood. 1957. Method of anesthetizing large sharks and rays safely and rapidly. Science 126: 212 - 213.

Gohar, H. A. F. and F. M. Mazhar. 1964. Keeping elasmobranchs in vivaria. Publications of the Marine Biological Station Al-Ghardaqa (Red Sea) 13: 241-250.

Graham, J. B., H. Dewar, N. C. Lai, W. R. Lowell, and S. M. Arce. 1990. Aspects of shark swimming performance determined using a large water tunnel. Journal of Experimental Biology 151: 175-192.

Gruber, S. H. 1980. Keeping sharks in captivity. Journal of Aquariculture and Aquatic Sciences 1: 6-14.

Gruber, S. H. and R. A. Keyes. 1981. Keeping sharks for research. *In:* Aquarium Systems, p. 373-402. A. D. Hawkins (ed.). Academic Press, New York, USA.

Henningsen, A. D. 1994. Tonic immobility in 12 elasmobranchs: Use as an aid in captive husbandry. Zoo Biology 13: 325-332.

Hewitt, J. C. 1984. The great white shark in captivity: A history and prognosis. *In:* AAZPA Annual Conference Proceedings, September 9-13, 1984, Miami, FL, p. 317-323. American Association of Zoological Parks and Aquariums (American Zoo and Aquarium Association), Silver Spring, Maryland, USA.

Hiruda, H., I. Gordon, T. White, and M. Miyake. 1997. Long distance air transportation of large species of sharks. *In:* Proceedings of the Fourth International Aquarium Congress, June 23-27, 1996, Tokyo, p. 91-95. Tokyo, Japan by the Congress Central Office of IAC '96, Tokyo Sea Life Park. 402 pp.

Holeton, G. F. and N. Heisler. 1978. Acid-base regulation by bicarbonate exchange in the gills after exhausting activity in the larger spotted dogfish *Scyliorhinus stellaris*. Physiologist 21: 56.

Holeton, G. F. and N. Heisler. 1983. Contribution of net ion transfer mechanisms to acid-base regulation after exhausting activity in the larger spotted dogfish (*Scyliorhinus stellaris*). Journal of Experimental Biology 103: 31-46.

Howe, J. C. 1988. Oxygen consumption rate in juvenile scalloped hammerhead sharks *Sphyrna lewini* Griffith and Smith: a preliminary study. Journal of Aquariculture and Aquatic Sciences 5: 28-33.

Hughs, G. M., and S. Umezawa. 1968. Oxygen consumption and gill water flow in the dogfish, *Scyliorhinus canicula* L. Journal of Experimental Biology 49: 559-564.

Hussain, S. M. 1989. Buoyancy mechanism, and density of sand tiger shark *Eugomphodus taurus*. Indian Journal of Fisheries 36: 266-268.

Jones, R. T. and J. C Andrews. 1990. Hematologic and serum chemical effects of simulated transport on sandbar sharks, *Carcharhinus plumbeus* (Nardo). Journal of Aquariculture and Aquatic Sciences 5: 95-100.

Klay, G. 1977. Shark dynamics and aquarium design. Drum and Croaker 17: 29-32.

Lai, N. C., J. B. Graham, and L. Burnett. 1990. Blood respiratory properties and the effect of swimming on blood gas transport in the leopard shark, *Triakis semifasciata*. Journal of Experimental Biology 151: 161-173.

Lowe, C. G. 1996. Kinematics and critical swimming speed of juvenile scalloped hammerhead sharks. The Journal of Experimental Biology 199: 2605-2610.

Marshall, A. 1999. Transportation of pelagic fishes. Memoires de l'Institut Océanographique Paul Ricard, 1999: 115-121.

Martini, F. H. 1978. The effects of fasting confinement on *Squalus acanthias*. *In:* Sensory Biology of Sharks, Skates and Rays, p. 609-646. E. S. Hodgson and R. F. Mathewson (eds.). Office of Naval Research, U. S. Government Printing Office, Washington, D. C., USA.

Mazeaud, M. M., F. Mazeaud, and E. M. Donaldson. 1977. Primary and secondary effects of stress in fish: Some new data, with a general view. Transactions of the American Fisheries Society 106: 201-212.

McFarlane, W. N. and K. S. Norris, 1958. The control of pH by buffers in fish transport. California Fish and Game 44: 291-301.

Murdaugh, V. H. and E. D. Robin. 1967. Acid-base metabolism in the dogfish shark. *In:* Sharks, Skates and Rays, p. 249-64. P. W. Gilbert, R. F. Mathewson and D. P. Rall (eds.). Johns Hopkins Press, Baltimore, Maryland, USA.

Murru, F. L. 1984. Preliminary baseline parameters for several sharks. AAZPA Annual Conference Proceedings, September 9-13, Miami, FL, p. 334-337. American Association of Zoological Parks and Aquariums (American Zoo and Aquarium Association), Silver Spring, Maryland, USA.

Murru, F. L. 1990. The care and maintenance of elasmobranchs in controlled environments. *In:* Elasmobranchs as Living Resources: Advances in Biology, Ecology, Systematics, and the Status of Fisheries, p. 203-209. H. R. Pratt, S. H. Gruber, and T. Taniuchi (eds.). U.S. Department of Commerce, NOAA Technical Report 90.

Noga, E. J. 1996. Fish disease: Diagnosis and treatment. Mosby Year Book Inc., St. Louis, Missouri, USA. 367 p.

Oswald, R. L. 1977. Injection anesthesia for experimental fish studies. Comparative Biochemistry and Physiology 60C: 19-26.

Parsons, G. R. 1990. Metabolism and swimming efficiency of the bonnethead shark, *Sphyrna tiburo*. Marine Biology 104: 363-367.

Piiper, J. and D. Baumgarten. 1969. Blood lactate and acid-base balance in the elasmobranch *Scyliorhinus stellaris* after exhausting activity. Pubblicazioni della Stazione Zoologica, Napoli 37: 84-94.

Piiper, J., M. Meyer, and F. Drees. 1972. Hydrogen ion balance in the elasmobranch *Scyliorhinus stellaris* after exhausting activity. Respiration Physiology 16: 290-303.

Robin, E. D., H. V. Murdaugh, and J. E. Millen. 1965. Acid-base, fluid and electrolyte metabolism in the elasmobranch: III-oxygen, CO_2, bicarbonate and lactate exchange across the gill. Journal of Cellular Biology 67: 93-100.

Ross, L. G., and B. Ross. 1999. Anaesthetic & Sedative Techniques for Aquatic Animals. 2nd Edition. Blackwell Science Ltd, Oxford, UK. 176 p.

Sabalones, J. 1995. Considerations on the husbandry of sharks for display purposes. International Zoo Yearbook 34: 77-87.

Smith, M. F. L. 1992. Capture and transportation of elasmobranchs, with emphasis on the grey nurse shark (*Carcharias taurus*). Australian Journal of Marine and Freshwater Research (Sharks: Biology and Fisheries) 43: 325-343.

Stoskopf, M. K. 1986. Preliminary notes on the immobilization and anesthesia of captive sharks. Erkrankungen Zootiere 28: 145-151.

Stoskopf, M. K. 1993. Environmental requirements and diseases of sharks. *In:* Fish Medicine, p. 758-763. M. K. Stoskopf (ed.). W. B. Saunders Company, Harcourt Brace Jovanovich, Inc., Philadelphia, Pennsylvania, USA.

Tyler, P. and A. D. Hawkins. 1981. Vivisection, anesthetics and minor surgery. *In:* Aquarium Systems, p. 247-278. A.D. Hawkins (ed.). Academic Press, Harcourt Brace Jovanovich, London, England.

Visser, J. 1996. The capture and transport of large sharks for Lisbon Zoo. International Zoo News 43: 147-151.

Vogelnest, L., D. S. Spielman, and H. K. Ralph. 1994. The immobilization of spotted seven-gill sharks (*Notorynchus cepedianus*) to facilitate transport. Drum and Croaker 25: 5-6.

Wardle, C. S. 1981. Physiological stress in captive fish. *In:* Aquarium Systems, p. 403-414. A. D. Hawkins (ed.). Academic Press, Harcourt Brace Jovanovich, London, England.

Watsky, M. A., and S. H. Gruber. 1990. Induction and duration of tonic immobility in the lemon shark, *Negaprion brevirostris*, Fish Physiology and Biochemistry 8: 207-210.

Weihs, D. 1973. Mechanically efficient swimming techniques for fish with negative buoyancy. Journal of Marine Research 31: 194-209.

Wells, R. M. G., R. H. McIntyre, A. K. Morgan, and P. S. Davie. 1986. Physiological stress responses in big game fish after capture: Observations on plasma chemistry and blood factors. Comparative Biochemistry and Physiology 84: 565-571.

Wisner M. 1987. Collecting and transporting blacktip reef sharks. Freshwater and Marine Aquarium 10: 16-17.

Wood, C. M. 1991. Acid-base and ion balance, metabolism, and their interactions, after exhaustive exercise in fish. Journal of Experimental Biology 160: 285-308.

Wood, C. M., J. D. Turner, and M. S. Graham. 1983. Why do Fish die after Severe Exercise? Journal of Fish Biology 22: 189-201.

Young, F. A., S. M. Kajiura, G. J. Visser, J. P. S. Correia, and M. F. L. Smith. 2002. Notes on the long-term transportation of the scalloped hammerhead shark, *Sphyrna lewini*. Zoo Biology 21(3): 242-251.

Young, F. A., D. C. Powell, and R. Lerner. 2001. The transportation of live silky sharks (*Carcharhinus falciformis*) on standard palletized aircraft cargo positions for long distance and time period transports. Drum and Croaker 32: 5-7.

PERSONAL COMMUNICATIONS

Andrews, J. 2002. Tennessee Aquarium, Chattanooga, TN 37402, USA.

Barthelemy, D. 2001. Océanopolis, Brest, F-29275, France.

Brittsan, M. 2002. Columbus Zoo and Aquarium, Powell, OH 43065, USA.

Carrier, J. 2001. Albion College, Albion, MI 49224 -5011, USA.

Choromanski, J. 2001. Ripley Aquariums, Inc., Orlando, FL 32819, USA.

Christie, N. 2001. Atlantis Resort, Ft. Lauderdale, FL 33304, USA.

Dehart, A. 2001. National Aquarium in Baltimore, Baltimore, MD 21202, USA.

Ezcurra, M. 2001. Monterey Bay Aquarium, Monterey, CA 93940, USA.

Farquar, M. 2001. Two Oceans Aquarium, Waterfront, Cape Town, 8002, South Africa.

Henningsen, A. 2001. National Aquarium in Baltimore, Baltimore, MD 21202, USA.

Hewitt, J. 2000. Aquarium of the Americas, New Orleans, LA 70130, USA.

Howard, M. 2001. Aquarium of the Bay, San Francisco, CA 94133, USA.

James, R. 2001. Sea Life Centres, Dorset, DT4 7SX, UK.

Janse, M. 2001. Burger's Ocean, Arnhem, 6816 SH, Netherlands.

Kinnunen, N. 2001. Sydney Aquarium, Darling Harbor, Sydney, 2001, Australia.
Marín-Osorno, R. 2001. Acuario de Veracruz, Veracruz, CP 91170, Mexico.
Martel Bourbon, H. 2001. New England Aquarium, Boston, MA 02110, USA.
McEwan, T. 2001. The Scientific Centre, Salmiya, 22036, Kuwait.
McHugh, F. 2001. The Tower Group, Boston, MA 02150, USA.
Mohan, P. 2001. Six Flags Worlds of Adventure, Aurora, OH 44202, USA.
O'Sullivan, J. 2001. Monterey Bay Aquarium, Monterey, CA 93940, USA.
Powell, D. 2001. Monterey Bay Aquarium, Monterey, CA 93940, USA.
Romero, J. 2001. National Marine Aquarium, Plymouth, PL4 0LF, UK.
Sabalones, J. 2001. Newport Aquarium, Newport, KY 41071, USA.
Steslow, F. 2001. New Jersey State Aquarium, Camden, NJ 08103, USA.
Thomas, L. 2001. Oregon Coast Aquarium, Newport, OR 97365, USA.
Thoney, D. 2003. Humboldt State University, Trinidad, CA 95570, USA.
Young, F. 2001. Dynasty Marine Associates Inc., Marathon, FL 33050, USA.

The Elasmobranch Husbandry Manual: Captive Care of Sharks, Rays and their Relatives, pages 133-141.
© 2004 Ohio Biological Survey

Chapter 9
Identification of Individual Elasmobranchs

ALLAN MARSHALL

Pittsburgh Zoo and PPG Aquarium,
One Wild Place,
Pittsburgh, PA 15206, USA.
E-mail: allanm@zoo.pgh.pa.us

Abstract: The ability to identify individual elasmobranchs within a collection is an important aspect of successful husbandry. Most aquariums prefer to have an identification system that is subtle and not readily observed by the general public, the aim being to provide as natural a display as possible. Research scientists prefer a more obvious method of individual identification to reduce the possibility of mistaken identity. Many methods have been used successfully to identify individual elasmobranchs, including natural markings, fin clipping, branding, implants, and tags. Using natural markings to identify individuals is the least intrusive technique. Chemical branding has provided a reliable identification technique, is relatively easy to accomplish, but is not permanent and requires periodic re-application. PIT tags are reliable and accurate, with no chance of misidentification, and are usually relied on for accurate record keeping.

The ability to identify individual elasmobranchs within a collection is fundamental to implementing a successful husbandry protocol. Elasmobranchs notoriously display little or no sign of health problems until the ailment is at an advanced stage. Monitoring food intake, growth, behavior, health, and the medication of individual animals provides valuable information that will aid their ongoing care. A change in behavior or food intake, for example, can be an early sign of a health problem. The ability to closely monitor an individual and administer treatment, if necessary, can be the key to averting potential tragedy.

In some regions, the permit to collect wild elasmobranchs may be issued with the stipulation that each animal is identified using a unique tag, usually supplied by the government, verifying that the animals were captured legally.

Most public aquariums prefer to have an identification system that is subtle and not readily observed by the general public, providing as natural a display as possible. Research scientists prefer obvious and unambiguous identification techniques, reducing the risks of mistaken identity and bias in experimental results.

A variety of identification techniques have been used in the past, each having specific advantages and disadvantages. No single method is appropriate for all circumstances, therefore a technique should be chosen that is most suitable for the intended purpose. Nielsen (1992) identifies seven basic techniques for identifying fishes: external tags, external marks, internal tags, natural marks, biotelemetric tags, genetic identifiers, and chemical marking. Of these techniques, only a few are considered suitable for public aquariums.

Rounsefell and Kask (1945), Kelly (1967), Stott (1971), and Everhart and Youngs (1981) have listed characteristics and criteria of the ideal mark for identifying fishes. For the purposes of identifying individual fishes in a public aquarium, the following list of criteria should be considered ideal and will help with the selection of an appropriate technique: (1) uniquely identifies each individual fish; (2) remains unaltered on an individual throughout its lifetime; (3) has no effect on growth, behavior, mortality, or vulnerability to predators; (4) is nontoxic and nonirritating; (5) is easy and fast to apply, without anesthetic, and with minimal stress to the fish; (6) is not obvious to the public, while still unmistakable to

curatorial staff; and (7) is inexpensive, easily obtained, and requires little or no specialized equipment.

This chapter will be concerned, primarily, with identification methods suitable for public aquarium animals. Techniques used for research purposes are well described in the scientific literature, including several synopses (Nielsen, 1992; Rounsefell and Kask, 1945; Kelly, 1967; Stott, 1971; Everhart and Youngs, 1981; Basavaraju et al., 1998; Kohler and Turner, 2001), and shall be referred to only briefly. By contrast, there is a paucity of literature available for methods to identify aquarium animals; therefore much of the information herein has been collated from direct communications with experienced aquarium personnel. It is advisable to consult with as many people as possible when considering options for identifying elasmobranchs within a collection.

Natural differences

The most common and straightforward method of identifying individual elasmobranchs is to take note of natural differences in coloration, markings, size, and/or sex (Ellis, pers. com.; Lewand, pers. com.; Smith, pers. com.; Violetta, pers. com.). This technique is particularly effective with species of a mottled, spotted, or otherwise non-uniform coloration. For example, sand tiger (*Carcharias taurus*), broadnose sevengill (*Notorynchus cepedianus*), and whitetip reef (*Triaenodon obesus*) sharks can often be distinguished by the distribution of darker spots on their bodies. The shape of dorsal fins, and notches or scars thereon, have been used to identify individual white sharks (*Carcharodon carcharias*) in the wild (Klimley and Ainley, 1998).

Relative size differences between individuals may become less obvious as animals grow. However, it is unusual for an individual within a collection to completely change its size ranking relative to other members of the group. Of course a medical condition that affects appetite or food assimilation may change this equation.

Behavioral differences may be used as a natural identification technique. Janse (pers. com.) has noted a clear and reliable difference in the feeding behavior of two individual blacktip sharks (*Carcharhinus limbatus*). Individual animals may consistently choose a specific area of an exhibit to swim and/or rest, or have distinctly different behavior toward the presence of divers.

The use of natural differences to distinguish individual elasmobranchs is only feasible with a small group of animals. As it is a comparative method, one needs to observe all or most of the animals within a group to distinguish an individual. Many natural identifying features, such as injuries, scarring, and distinctive behavior, may change or disappear over time (Lewand, pers. com.; Violetta, pers. com.). By combining two or more identification criteria, it is usually possible to monitor and identify several individuals for extended periods. With experience and time, an aquarist can become familiar with individual animals using more subtle differences such as slight variations in body shape, fin shape, etc. (Cushing, pers. com.). These differences may not be easily discernable and may be of limited use (i.e., only to those people who have continual contact with the elasmobranchs).

The skin pigmentation of some elasmobranch species is patterned, variations of which are characteristic to individuals. The arrangement of white spots on spotted eagle rays (*Aetobatus narinari*), particularly around the base of the tail, is distinctive for each individual (Gruber, pers. com.). These patterns are similar to human fingerprints in the sense that they are unique and do not change over time. Photo-identification of individual animals, as has been used in cetacea for many years, has recently been employed in elasmobranchs (Gruber, pers. com.). Firchau (pers. com.) has successfully used photo-identification to distinguish between individual chain dogfish (*Scyliorhinus retifer*). The chain-like patterns are characteristic for each individual, with the most distinctive differences occurring in the bands on the dorsal part of the head and the pectoral region (Figure 9.1). Firchau (pers. com.) has posted photos of each chain dogfish above the shark exhibit at the Virginia Aquarium and Marine Science Center, Virginia Beach, USA, as a reference for the aquarists maintaining the sharks.

Fin clipping

Unlike the method of fin clipping employed for teleosts, whereby half or all of a fin is removed (Knauth, 1977; Nielsen, 1992), fin clipping in elasmobranchs only requires a small notch or notches on a fin. For aquarists that regularly dive in the shark tank, notching the dorsal or caudal fin has proven an effective means to identify individuals (Martel-Bourbon, pers. com.). Notches within a dorsal or caudal fin may be obvious to the public and therefore notching of the pectoral or pelvic fins may be preferred, particularly if the aquarists monitor animals from the surface.

Fin notching is usually performed with a sharp knife, shears, or a leather hole punch. It is a fast and unobtrusive surgery that can be performed without anesthetic, although restraint is usually required for a short period. The notch or hole need only be large enough to be visible from the distance that the aquarist monitors the animals on a day-to-day basis. Depending on the type and size of notch, as well as the species, the mark will last from several months to a few years

Figure 9.1. Photographs of the dorsal surface of four chain dogfish (*Scyliorhinus retifer*), showing the individually distinctive chain-like patterning. Top left and right are female specimens and bottom left and right are male specimens. Photos courtesy of: Liz Kopecky.

(Carrier, pers. com.; Correia, pers. com.; Firchau, pers. com.; Wisner, pers. com.).

Branding

Burning, freezing, or chemical techniques have been used to brand fishes. All of these techniques intentionally cause damage to the epidermal skin layers of the branded animal. As the injury heals, scar tissue forms and is visible in the shape of the intended marking or brand.

A certain level of competence is required to ensure that a brand is applied correctly. Heat and freeze brands are particularly difficult to administer, as the potential for the injury of mishandled animals is high. The hot or cold branding tool must be applied firmly, and for sufficient duration, to ensure application of an enduring mark. Brand contact time can range between a few seconds to a minute, depending on the taxa. For this reason contact time can be unintentionally excessive, destroying underlying dermal tissue (Raleigh et al., 1973) and leaving open wounds susceptible to infection (Refstie and Aulstad, 1975; Knauth, 1977). Treatment of an infected brand can be difficult and the resulting open wound may be aesthetically displeasing. Raymond (1974) noted that branding tools applied with excessive pressure can result in cellular damage similar to that resulting from an extended application time.

Both heat and freeze branding involve the use of a heat-conducting branding tool, such as copper, silver, or brass. The brand itself can be a unique symbol applied to a standardized part of the target animal, or the tip of a standard brand (e.g., round rod) applied in a coded manner (see below). To ensure proper contact and an effective brand, it is important to dry the area of skin to be branded. When the tool has acquired the correct heating or cooling temperature, it is applied to the skin of the animal for a specified time. Rays have been branded with a freeze-brand contact time of 10-15 seconds (Dehart, pers. com.). In contrast, to produce an effective brand on sharks, the branding tool needs to be held in place for 30-60 seconds, due to denticles and tough integument (Dehart, pers. com.).

Wisner (pers. com.) has successfully used an electric soldering iron as a heat branding apparatus for blacktip reef sharks (*Carcharhinus melanopterus*). The resulting scars were visible for up to five years following application; nevertheless he halted the practice as he considered it excessively injurious to the animals.

Freeze branding works on the same principal as heat branding, however the branding tool is chilled to low temperatures. Immersion in liquid nitrogen (N_2) (Knight, 1990) or exposure to pressurized carbon dioxide (CO_2), dispensed from a fire extinguisher (Bryant et al., 1990), are the two most common methods for cooling a freeze branding tool.

Chemical branding has been used successfully in many public aquariums. The most commonly used chemical is the cauterizing agent silver nitrate (Firchau, pers. com.; Henningsen, pers. com.; Mohan, pers. com.; Violetta, pers. com.). Silver nitrate-tipped applicator sticks (used in the veterinary field as escharotics or styptics to cauterize bleeding) are the safest and most convenient devices for administration. Wetting the chemically coated end of the applicator activates the silver nitrate.

When branding with a silver nitrate stick, the tip is applied directly to the skin of the elasmobranch and pressure is maintained for approximately 10-15 seconds, for rays, or 30 seconds, for sharks (Davis, pers. com.). Most elasmobranchs must be restrained to allow the proper application of a silver nitrate stick. The area to be branded must be lifted clear of the water, as the chemical cannot be applied underwater. It is recommended to dry the branding area, as excess water on the skin causes silver nitrate to bleed from the applicator and disperse over a larger area of the skin (Violetta, pers. com.). This precaution is especially critical if the brand is to be applied to an area of skin adjacent to the eyes or gills (Firchau, pers. com.). The resulting brand mark is pale or white in color. Immediately after application, the skin should be doused with water to rinse away any remaining silver nitrate. Once the procedure is complete, the animal may be returned to its exhibit.

A small circular brand, up to 10 mm diameter, will usually be sufficient for recognition by the curatorial staff. The longevity of silver nitrate brands varies, depending on the application technique employed and the species marked. The white color of the mark will gradually get darker as the branding site heals, leaving a dark mark that will eventually disappear (Ellis, pers. com.). Firchau (pers. com.) has noted that silver nitrate marks disappear much faster (i.e., ~2 months) on pelagic species (e.g., blacktip sharks) than on more sedentary species (e.g., nurse sharks, *Ginglymostoma cirratum*), where the mark can remain visible for more than a year.

It is common practice to mark female elasmobranchs on the left pectoral fin and males

on the right (Firchau, pers. com.; Henningsen, pers. com.; Romero, pers. com.; Violetta, pers. com.). To distinguish more than one of each sex, the animals are marked with one, two, or more marks. Alternately, a single mark can be placed on a distinctive position of the fins to differentiate between individuals (Mohan, pers. com.). A simple combination of 1-4 marks on a single fin can be used to identify up to 15 individuals. In this case, brands are applied at specific locations, representing the numbers 1, 2, 4, and 8. For example, animal number 5 would be marked with both a 1 and 4 (i.e., 1+4 = 5). Up to eight distinctive positions are available on each fin (Figure 9.2). For larger numbers of animals, one fin can represent the first digit (i.e., the 1s) and another fin can represent the second digit (i.e., the 10s) (Mohan, pers. com.). These coding systems may be equally useful for fin clipping techniques.

Tattooing has been suggested as a means of identifying individual elasmobranchs (Davis, pers. com.) and has been applied successfully in a variety of animals, including some teleosts. However, a recent tattooing attempt with ocellate river stingrays (*Potamotrygon motoro*) resulted in the deaths of the animals. Necropsy showed that all three specimens had embolised the India ink into the gills and some other organs (Raymond et al., 2003). It was suggested that the rich lymphatic system in the subdermal layers facilitated embolisation (Garner, pers. com.).

Tagging

Attaching tags to elasmobranchs has been a regular practice in field research for many years. Tags come in many different shapes, sizes, and designs. Each tag is designed for a specific purpose and researchers can select a tag that most suits their particular requirements (Kohler and Turner, 2001). Only a few tags are considered suitable for use in public aquariums as most are designed to be obvious from a long distance and therefore detract from the natural appearance of the animals.

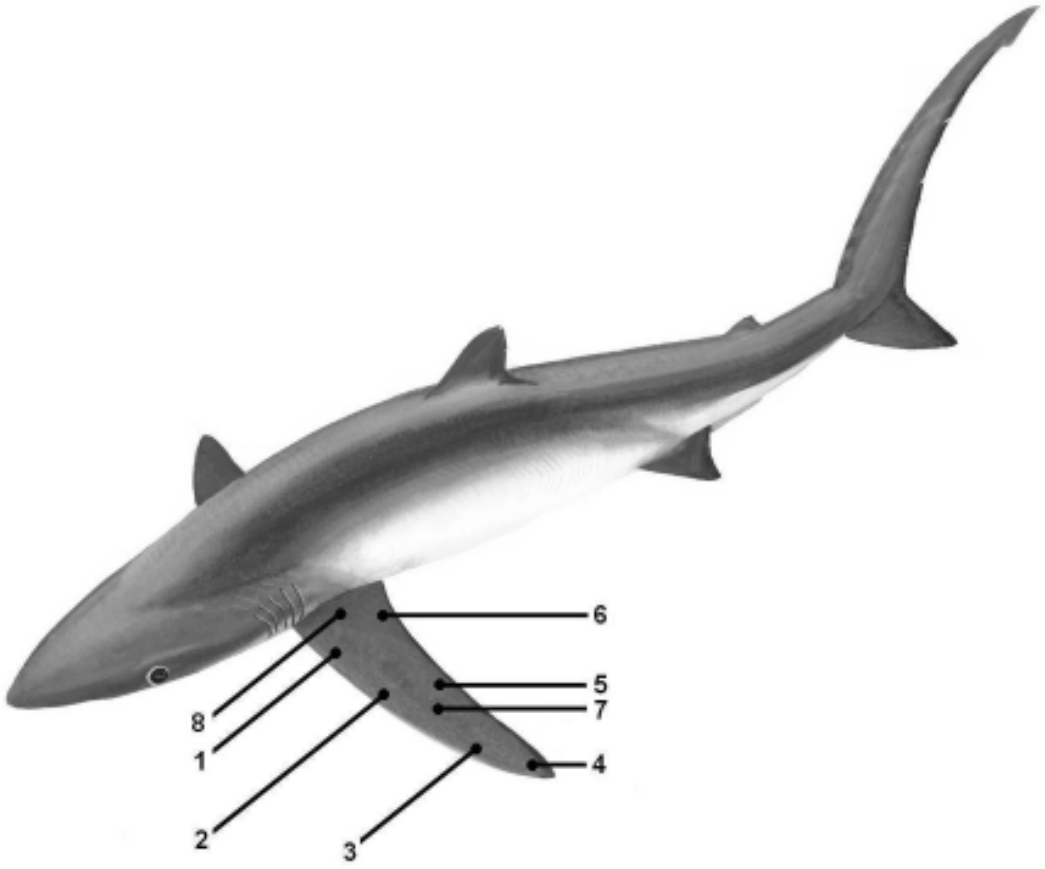


Figure 9.2. Blue shark (*Prionace glauca*) showing the eight distinctive branding positions available on the pectoral fin. 1 - Proximal leading edge, 2 - Median leading edge, 3 - Distal leading edge, 4 - Distal trailing edge, 5 - Median trailing edge, 6 - Proximal trailing edge, 7 - Central, and 8 - Proximal mid-fin.

Tags may be divided into two types: internal and external. Many internal tags are only capable of distinguishing particular groups of animals, such as year classes. Most internal tags, such as coded wire tags require sacrificing the animal to read the tag (Ombredane et al., 1998). Of the internal tags available for individual recognition, public aquariums only use passive integrated transponder (PIT) tags.

PIT tags

PIT tags (AVID Identification Systems, Inc., Norco, USA) consist of a small (~12 mm long x 2 mm diameter), glass encased, electronic chip. When supplied with energy the chip produces a unique, pre-programmed signal in the 40-50 kHz range. A radio receiver picks up the signal and transforms it into a 10-digit alphanumeric code (Prentice et al., 1990). Within the tag is an antenna of copper wire wound around a ferromagnetic core. When the tag enters a magnetic field, produced by an energizing system, an induced electric current within the antenna activates the chip (Nielsen, 1992). Hand-held readers are available that act as both the energizing system and receiver.

Due to their small size, the readable range of PIT tags is restricted to 20-30 cm distance. The reader must therefore be positioned close to the site of the implant in order to get a reading. Until recently, hand-held readers were incapable of being submerged. A new design of reader is now available with a waterproof remote detection device located at the end of a long pole. This new reader enables animals to be identified while underwater and makes PIT tags more practical as an individual recognition device for elasmobranchs. It is possible to attach a submersible reader to the end of a feeding pole and identify individuals as they take food items.

It is recommended that all elasmobranchs within a collection are PIT tagged in a standardized location, to facilitate later identification. Small elasmobranchs often have PIT tags placed in the peritoneal cavity, implanted by making a small incision and inserting the tag through the aperture with forceps (Basavaraju et al., 1998). In larger sharks, PIT tags are usually implanted in the dorsal musculature just below the dorsal fin. Rays are tagged by inserting the PIT tag on the dorsal side of the pectoral flap, midway down the body and lateral to the peritoneal cavity. Elbin (pers. com.) proposed a standard tagging-site protocol for all vertebrates housed at the New York Zoological Park, New York, USA. The protocol recommended using the left side of the body for dorso-muscular implantations, and this standard has been adopted at many institutions in North America. An injecting applicator needle, usually supplied with the tags, is used to implant the PIT tag into the musculature. For intramuscular implantation, it is recommended to insert the applicator needle at a shallow angle to the surface and to push the tag as far as possible away from the entry site. This procedure will reduce the possibility of the tag migrating back through the puncture wound and being shed (Firchau, pers. com.). Nexaband (Veterinary Products Laboratories, Phoenix, USA), a liquid cyanoacrylate tissue adhesive, can be used to close the applicator puncture wound, helping to prevent loss of the injected tag.

Studies have shown PIT tags to have little or no effect on growth, mortality, or behavior, and to have an almost 100% retention rate (Basavaraju et al, 1998). PIT tags provide reliable, positive identification and are small enough to be used on all species of elasmobranchs. Because PIT tags require no batteries, they will function for many years. Despite the initial expense, PIT tags are considered to be an invaluable, reliable identification technique for record keeping.

External tags

Many tagging studies have been performed on a variety of aquatic animal species. As a result of these studies, a vast number of external tag styles have been developed (Kohler and Turner, 2001). There are four basic categories of external tags, classed by the way they attach to the animal (Figure 9.3), and include: (1) trans-body; (2) dart-style; (3) internal-anchor; and (4) tail-loop.

Trans-body tags protrude through both sides of the body (e.g., through the dorsal fin). These tags include disc tags, dangling disc tags, and spaghetti loop tags. Dart-style tags protrude from only one surface of the animal and consist of a training shaft with an anchor on one end. The anchor is inserted into the body of the animal and the trailing end, usually enlarged, details information pertaining to the tagged animal. T-Bar and arrowhead are examples of dart-style tags. Internal-anchor tags are a modification of dart-style tags. Instead of being anchored into muscle tissue, the anchor of an internal-anchor tag is a flat disc that lies against the inside wall of the fish's body cavity (Nielsen, 1992). Tail-loop tags consist

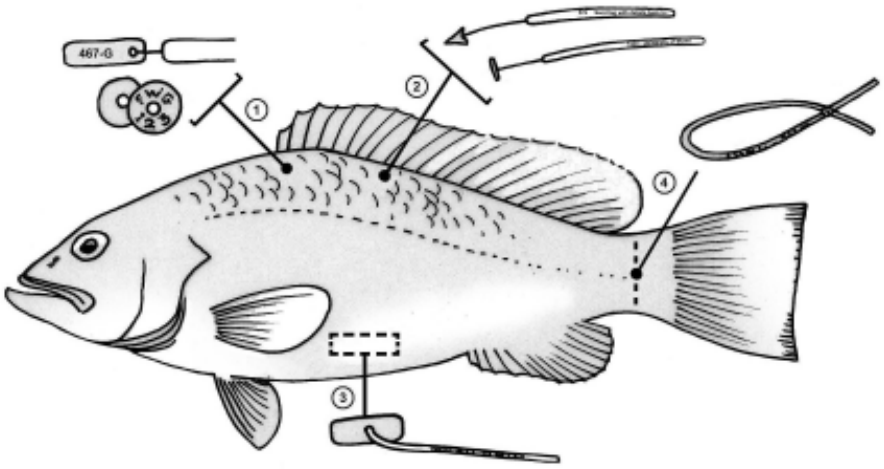


Figure 9.3. The four basic categories of external tags, classed by the way they attach to the animal, including: (1) trans-body; (2) dart-style; (3) internal-anchor; and (4) tail-loop.

of a length of material, or a plastic cable-tie, that is loosely tied around the caudal peduncle of the fish.

Researchers regularly use external tags for animal recognition. Within public aquariums, animals not on display can be tagged temporarily using external tags. Some public aquariums use external tags on display animals and take the opportunity to educate their public about the roles of tagging through written materials and presentations.

Wisner (pers. com.) has used different colored T-bar tags to identify animals within his collection. Instead of using commercial fish marking tags, Wisner used clothing price tags and an applicator gun. The flat end of each tag was colored with a plastic coating (Plasti Dip, PDI Inc., Circle Pines, USA). When the T of the t-bar tag was injected posterior to the first dorsal fin, the colored, trailing end was easily observed. These tags lasted for several years with no reported problems.

For instances where temporary identification is necessary (e.g., sharks kept in holding tanks, etc.) a colored loop may be placed loosely around the caudal peduncle (i.e., a tail-loop tag). The tag may consist of ribbon, rope, string, or plastic cable-ties of various colors (Correia, pers. com.; Firchau, pers. com.; Perego, pers. com.). The tail-loop tag is tied around the animal in such a way as to be loose, but not dangling from the shark. Tail-loop tags provide a readily observable mark and in the short term do not injure the animal. Tail-loop tags should only be used for temporary identification (i.e., not more than two weeks) as constant rubbing of the material against the skin can lead to integument damage and possible infection.

CONCLUSION

Table 9.1 summarizes various identification techniques with respect to the seven ideal criteria of a fish tag. The wide range of options available for positively identifying individual elasmobranchs allows institutions the possibility to use a technique appropriate for their needs. Using natural markings to identify individuals is the least intrusive technique and is preferred, providing that observations can be made reliably, and that unique features are long-lived. Chemical branding of animals has been a reliable technique for many institutions. Chemical branding is relatively easy to accomplish, but it is not permanent and the necessity to restrain animals may cause undue stress to some species. PIT tags are reliable and accurate, with no chance of misidentification, and are usually relied on for accurate record keeping. Because PIT tags are not easily read without special equipment, and potentially restraining animals, they are usually used as a backup identification system to a more simplistic identification technique applied on a day-to-day basis. Further advances in technology may make PIT tags more appropriate for general use.

ACKNOWLEDGEMENTS

Thank you to all those who contributed to this chapter and to the field of elasmobranch identification. Beth Marshall deserves much credit for her critique and typing skills.

Table 9.1. Individual elasmobranch identification techniques, ranked using the seven criteria for an ideal aquarium fish identification mark. Ranking: 1 = very poor, 2 = poor, 3 = average, 4 = good, and 5 = very good.

Method	1 Uniquely identifies individual	2 Remains unaltered for specimen lifetime	3 No effect on specimen health	4 Non-toxic and non-irritating	5 Easy to apply with minimal stress	6 Not obvious to public, but obvious to staff	7 Inexpensive, equipment easy to obtain
Natural Differences							
Markings (spots, mottling)	4	variable	5	4	5	5	5
Markings (scars, fin edges etc.)	4	variable	4	3	5	4	5
Size	3	3	5	5	5	5	5
Behavior	3	variable	3	5	5	4	5
Photo Identification	5	5	5	5	5	5	5
Applied Marks							
Fin clipping	5	4	5	3	3	3	4
Branding (heat)	5	variable	5	2	2	4	4
Branding (freeze)	5	variable	5	2	2	4	3
Branding (chemical)	5	variable	5	2	3	4	4
Tattoo	5	unknown	2	2	3	5	3
Internal tag (coded wire tag)	1	5	4	4	4	1	2
Internal tag (PIT tag)	5	5	5	4	4	5	2
External tag (trans-body)	5	4	3	3	3	1	3
External tag (dart style)	5	4	3	3	3	1	4
External tag (internal anchor)	5	4	3	3	2	1	2
External tag (tail loop)	5	1	2	2	3	1	5

REFERENCES

Basavaraju, Y., B. S. Renuka Devi, G. Mukthayakka, L. Purushotham Reddy, G. C. Mair, E. E. Roderick, and D. J. Penman. 1998. Evaluation of marking and tagging methods for genetic studies in carp. Journal of Biosciences 23(5): 585-593.

Bryant, M. D., C. A. Dolloff, P. E. Porter, and B. E. Wright. 1990. Freeze branding with CO_2: An effective and easy-to-use field method to mark fish. American Fisheries Society Symposium 7: 30-35.

Everhart, W. H., and W. D. Youngs. 1981. Principles of Fishery Science. Cornell University Press, Ithaca, New York, USA. 288 p.

Kelly, W. H. 1967. Marking freshwater and a marine fish by injecting dyes. Transactions of the American Fisheries Society 96: 163-175.

Klimley, A. P. and D. G. Ainley. 1998. Great White Sharks: The Biology of *Carcharodon carcharias*. Academic Press, San Diego, California, USA. 517 p.

Knauth, C. P. 1977. An evaluation of the effects of three marking techniques on the growth, behavior, and mortality of channel catfish, *Ictalurus punctatus* (Rafinesque). Unpublished M.S. Thesis. The University of Tennessee, Knoxville, USA. 39 p.

Knight, A. E. 1990. Cold-branding techniques for estimating Atlantic salmon parr densities. American Fisheries Society Symposium 7: 36-37.

Kohler, N. E. and P. A. Turner. 2001. Shark tagging: A review of conventional methods and studies. Environmental Biology of Fish 60: 191-223.

Nielsen, L. A. 1992. Methods of marking fish and shellfish. American Fisheries Society Special Publication 23. 208 p.

Ombredane, D. J., L. Bagliniere, and F. Marchand. 1998. The effects of Passive Integrated Transponder tags on survival and growth of juvenile brown trout (*Salmo trutta* L.) and their use for studying movements in a small river. Hydrobiologie 371-372: 99-106.

Prentice, E. F., T. A. Flagg, and C. S. McCutcheon. 1990. Feasibility of using implantable Passive Integrated Transponder (PIT) tags in salmonids. American Fisheries Society Symposium 7: 317-322.

Raleigh, R. F., J. B. McLaren, and D. R. Graff. 1973. Effects of topical location, branding techniques, and changes in hue on recognition of cold brands in centrarchid and salmonid fish. Transactions of the American Fisheries Society 102(3): 637-641.

Raymond, H. L. 1974. Marking fishes and invertebrates. I. State of the art of fish branding. Marine Fisheries Review 36(7): 1-6.

Raymond, J. T., Dunker, F. and Garner, M. M. 2003. Ink Embolism in Freshwater Orange Spot Stingrays (*Potamotrygon motoro*) Following Tattooing Procedure. 2003 Proceedings American Association of Zoo Veterinarians. p. 210.

Refstie, T. and D. Aulstad. 1975. Tagging experiments with salmonids. Aquaculture 5: 367-374.

Rounsefell, G. A., and J. L. Kask. 1945. How to mark fish. Transactions of the American Fisheries Society 73: 320-365.

Stott, B. 1971. Marking and tagging. *In:* Methods for the assessment of fish production in freshwaters, p. 82-97. W. E. Ricker (ed.). Blackwell Scientific Publications, Oxford, England.

PERSONAL COMMUNICATIONS

Carrier, J., 2002. Albion College, Albion, Michigan 49224, USA.

Correia, J. P. 2002. Oceanário de Lisboa, Lisboa 1990, Portugal.

Cushing, E. 2002. Maui Ocean Center, Maui, Hawaii 96793, USA.

Davis, R., 2002. SeaWorld Adventure Park, Orlando, Florida 32821, USA.

Dehart, A. 2002. National Aquarium in Baltimore, Baltimore, Maryland 21202, USA

Elbin, S. B. 2002. New York Zoological Park, Bronx, New York, 10460, USA.

Ellis, C. M. 2002. Mystic Aquarium, Mystic, Connecticut 06335, USA.

Firchau, E. 2002. Virginia Aquarium and Marine Science Center, Virginia Beach, Virginia 23451, USA.

Garner, M. M., 2004 Northwest ZooPath, Washington 98290, USA.

Gruber, S. 2002. University of Miami and Bimini Biological Field Station, Miami, Florida 33124, USA.

Henningsen, A. 2002. National Aquarium in Baltimore, Baltimore, Maryland 21202, USA.

Janse, M. 2002. Burger's Zoo, 6816 SH Arnhem, The Netherlands.

Lewand, K. 2002. Aquarium of the Bay, San Francisco, California 94133, USA.

Martel-Bourbon, H. 2002. New England Aquarium, Boston, Massachusetts 02110, USA.

Mohan, P. 2002. Six Flags Worlds of Adventure, Aurora, Ohio 44240, USA.

Perego, C. 2002. Acquario di Genova, Genova 16128, Italy.

Romero, J. 2002. The National Marine Aquarium, Plymouth PL4 0LF United Kingdom

Smith, C. 2002. Newport Aquarium, Newport, Kentucky 41071, USA.

Violetta, G. 2002. SeaWorld Adventure Park, Orlando, Florida 32821, USA.

Wisner, M. 2002. Mauna Lani Resort, Hawaii 96743, USA.

The Elasmobranch Husbandry Manual: Captive Care of Sharks, Rays and their Relatives, pages 143-150.
© 2004 Ohio Biological Survey

Chapter 10
Quarantine and Prophylaxis for Elasmobranchs

RAY DAVIS

Georgia Aquarium, Inc..
225 Baker Street
Atlanta, Georgia 30313, USA.
E-mail: rdavis@georgiaaquarium.org

Abstract: As applied by aquarium facilities quarantine refers to the process of isolating a new or sick specimen for the purpose of treatment and observation, while prophylaxis refers to the process of applying preventative treatments to existing healthy specimens. Treatment protocols frequently overlap between quarantine and prophylaxis. Exemplary water quality is essential to animals surviving the rigors of chemotherapeutic treatments. Both the duration of a protocol and the allowable density of animals may be influenced by the spatial needs of specific species. Throughout quarantine, sterile techniques should be used to ensure that pathogens are not transferred between aquariums or animals. A thorough understanding of the proper handling and use of chemotherapeutics is essential. Veterinary and pathology laboratory services should be retained, to aid in both diagnoses and treatments. The ability to correctly identify pathogenic organisms (e.g., monogeneans, cestodes, nematodes, crustaceans, protozoans, bacteria, etc.), in combination with an understanding of their life history, will lead to informed diagnoses and more effective treatments.

This chapter briefly reviews issues to consider when formulating quarantine and prophylactic protocols for elasmobranchs, including: water treatment considerations, spatial considerations, sterile techniques, an understanding of pathogens, and modes of medication. A summary of typical chemotherapeutics used during quarantine and prophylaxis, based on a review of 22 public aquariums, is presented.

As applied by aquarium facilities quarantine refers to the process of isolating a new or sick specimen for the purpose of treatment and observation, while prophylaxis refers to the process of applying preventative treatments to existing healthy specimens. Treatment protocols frequently overlap between quarantine and prophylaxis.

Where practical, all new animals should go through quarantine for a minimum of 30 days prior to their introduction to a display or experimental aquarium. This time line is based upon our current understanding of the pathogens that affect elasmobranchs.

Prophylaxis is frequently applied while specimens are in display aquariums and is often based on a schedule of expectation (e.g., the appearance of monogeneans at the same time each year). In this situation, the effect of a treatment protocol on other species within the display aquarium needs to be carefully reviewed.

Treatment protocols should be based upon a thorough evaluation of specimens, including an assessment of the following: condition, length and weight, behavior, appetence, skin scrapes, and blood profiles, and, where possible, biopsies, lavages, radiography, and ultrasonography.

TREATMENT PROTOCOLS

Water treatment considerations

Quarantine facilities require the same thorough approach to life support system (LSS) design as display aquariums. Biological, mechanical, and chemical filtration each play an important role. Exemplary water quality is essential to animals surviving the rigors of chemotherapeutic treatments.

The use of ultra-violet sterilizers (UV) and/or ozone and activated carbon, as part of the LSS,

should be considered standard. Total counts of some pathogens can be reduced by the use of UV and/or ozone. In addition, UV and ozone can reduce many treatment chemicals to harmless by-products, which subsequently may be removed by activated carbon.

UV units are typically sized to achieve a specific level of irradiation, expressed as microwatts per second per centimeter squared ($\mu W\ sec^{-1}\ cm^{2-1}$). UV unit manufacturers will supply a chart with suggested irradiation levels required to achieve a given kill ratio of specific pathogens. Suggested exposure rates can be as low as 6,000 $\mu W\ sec^{-1}\ cm^{2-1}$, while others can be as high as 400,000 $\mu W\ sec^{-1}\ cm^{2-1}$. When using UV, a number of issues need to be considered. UV increases the heat load applied to a system. If temperatures are already close to the upper tolerance for elasmobranchs, an increase in chilling capacity may be required. Teflon or quartz sleeves are typically used to separate UV bulbs from the water. Frequent cleaning of these sleeves maintains an effective kill rate. Some UV units do not work well with coldwater aquariums, where condensation can diminish the UV unit's effectiveness. Additionally, organically-rich, turbid water can reduce kill rates. Application of UV is usually via a bypass that allows a portion of aquarium water to be treated. LSS design should be configured to maximize the passage of pathogen-laden water through the UV unit.

Ozone is typically introduced via a venturi to either foam fractionators or contact chambers. When used as a sterilizing agent, ozone is applied at high concentrations to oxidize water-borne parasitic organisms. During this process a number of residual oxidants are produced when used in salt water. Residual oxidants take part in the sterilizing process within the respective reaction chamber. However, if these chemicals persist and are carried into an exhibit, they can present a serious health risk to elasmobranchs. Residual oxidants may be monitored through DPD total oxidant tests, Oxidation Redox Potential, and animal behavior.

Spatial considerations

Elasmobranchs vary widely in their spatial requirements. Serious consideration should be given to these demands when developing quarantine and/or prophylactic protocols. Both the duration of a protocol and the allowable density of animals may be influenced by the spatial needs of a specific species. In some situations it may not be practical to quarantine a particular species of elasmobranch at all.

Pools of inadequate size or shape can be as devastating as pathogens, causing serious health issues to potentially valuable specimens. If pools are not the appropriate dimension and shape, contact lesions can occur on the caudal fin, ventral surface, and rostrum. Reduction in stocking density, modifying swimming patterns (e.g., introducing visual or physical obstacles), changing the lighting, and the addition of a sand substrate can reduce the occurrence of some of these injuries.

In some instances it may be necessary to maintain elasmobranchs in confined conditions (e.g., if protocols require repeated injections, tube feeding, wound care, etc.). In some specific cases, it may be less stressful to use a smaller pool, where it is easier to catch and restrain the elasmobranch, than a larger pool where an animal can swim freely.

Sterile techniques

Throughout quarantine, sterile technique (e.g., sterilization of nets between uses, etc.) needs to be instituted to ensure that pathogens are not transferred between aquariums or animals. With restricted quarantine spaces, sterile technique includes eliminating aerosol transmission via aeration, bio-towers, etc., and splashing caused by elasmobranchs.

The life history of many parasites includes a dormant stage. Thorough cleaning and sterilization of pools and LSSs, after each quarantine cycle, reduce the chance of transferring problems from one quarantine cycle to another. This process entails reseeding of the biological filters before the start of each quarantine cycle.

Safety and Record keeping

Before adopting quarantine and prophylactic protocols, it is important to review any local guidelines and regulations for the use of selected drugs and chemicals. A thorough understanding of the proper handling and use of any product is essential. Material safety data sheets should be studied and appropriate personal protective equipment (PPE) used.

Veterinary and laboratory (i.e., clinical and pathological) services should be retained, to aid in both diagnoses and treatment. In some locations it may be a legal requirement, or a stipulation by professional zoological associations, to retain these services.

Thorough records should be maintained throughout quarantine and/or prophylaxis . These records will help in assessing the efficacy of treatments. All elasmobranch mortalities should be followed by a complete necropsy, and resulting records maintained for future reference.

Pathogen diagnosis

The ability to correctly identify pathogenic organisms (e.g., monogeneans, cestodes, nematodes, crustaceans, protozoans, bacteria, etc.), in combination with an understanding of their life history, will lead workers to make informed diagnoses and implement more effective treatments. In particular, it is important to understand primary and secondary health concerns. For example, it may be determined that an outbreak of monogeneans has been exacerbated by the presence of environmental stressors (e.g., poor water quality, high population density, etc.). Once monogeneans have infested a population of elasmobranchs, a secondary bacterial infection may ensue and ultimately result in specimen mortality. Any treatment regime should thus address the primary infection (i.e., monogeneans), the secondary infection (i.e., bacteria), and importantly, any conditions that have aided the disease process (i.e., poor water quality and/or high population density), for the regime to be effective.

Monogeneans represent the greatest challenge to newly-arrived elasmobranchs. These organisms are difficult to eradicate because of their ability to remain viable, without a host, for extended periods of time. Control of these pathogens, through quarantine, is recommended. If quarantine is impractical, serious consideration should be given to the application of a medicated bath (e.g., praziquantel) before elasmobranchs are moved into their destination aquarium.

If an elasmobranch is suspected to have a specific pathogen, but is asymptomatic and presents no risk to other animals (e.g., in the case of species-specific parasites), it may be deemed appropriate to leave the animal untreated (i.e., forgo prophylaxis). For parasites (e.g., trematodes, cestodes, etc.) requiring an intermediate host that is not present within the system, it is advisable to let the parasite perish naturally. Wherever possible, it is preferred to keep treatments to a minimum. Although chemotherapeutic treatments are obviously intended to aid elasmobranchs, medication will always present an associated stress that could do more damage to the host animal than the intended target pathogen.

Mode of medication

Immersion (bath)

When preparing medicated baths it is critical to accurately assess the volume of treatment water before adding the medication. Water volume can be determined by using a calibrated flow meter, a calibrated container, or by a calculation of vessel volume. For aquariums with irregular dimensions, volume can be calculated by adding a known weight of salt and measuring the change in salinity. Dividing the weight of added salt (grams) by the change in salinity (g l^{-1} = ‰ = ppt) provides the vessel volume in liters. Once the volume of the treatment vessel is known, it is important to accurately calculate the amount of drug or chemical to add to the vessel to achieve the desired dosage. It is highly recommended to have two people perform the calculations independently to ensure accuracy.

An important consideration, when applying medicated baths, is an understanding of the chemical's reaction to LSS components (e.g., some chemicals are destroyed by ozone), and indeed their impact on LSS components (e.g., some antibiotics can damage the beneficial bacteria inside biological filters). Another important consideration is the possibility of synergistic effects—e.g., the presence of nickel at just 2.0 µg l^{-1} will double the effect of a copper treatment (Sorensen, 1991). Thus, a 2.0 mg l^{-1} antiparasitic treatment of copper effectively becomes a 4.0 mg l^{-1} lethal dosage of copper, in the presence of 2.0 µg l^{-1} nickel. In some cases synergy can be used to advantage (e.g., a lower concentration of two treatments—copper and organophosphates—can be used to effectively treat ectoparasites).

Once a bath is complete, medicated water must be safely disposed in accord with domestic and international regulations. This precaution is important not only for the products themselves

(i.e., antibiotics, heavy metals, organophosphates, etc.), but also filter media (e.g., activated carbon) used to remove products from the water.

Oral

When administering oral medications it is important to have an accurate measurement of specimen weight, before calculating dosages. The smaller the animal the more critical it is to have an accurate and precise measurement.

Some oral medications may be rejected by an elasmobranch because of their unusual taste. To disguise the taste, it may be necessary to secrete gel caps, filled with the medication, within a food item.

Parenteral (injectable)

As per oral medications, it is important to have an accurate measurement of specimen weight before calculating the dosage of injectable medications. Most parenteral treatments are administered intramuscularly (IM). Do not sterilize the injection site with alcohol prior to administration as alcohol can damage elasmobranch skin. Intramuscular medications are typically administered via a large muscle mass (e.g., the dorsal saddle) and in some cases multiple injection sites may be required if a large volume of medication is to be administered. Massaging the injection site, during and after administration, can reduce the risks of medications leaking out of the intended site.

Protocol formulation

In addition to the removal of hooks and tags, the treatment of gross lesions and abrasions, and the potential treatment of inappetence, a quarantine protocol for elasmobranchs should address the following problematic organisms: external parasites (i.e., monogeneans, crustaceans, and protozoans), internal parasites (i.e., cestodes, nematodes, and protozoans), and potential secondary bacterial infections.

Table 10.1 presents a summary of some typical chemotherapeutics successfully used during the quarantine and prophylaxis of elasmobranchs. The information contained in Table 10.1, and the discussion that follows, represents a summary of a survey conducted during 2001 of 22 public aquariums. In general, two medications should not be applied simultaneously, although some oral treatments may be given during long-term medicated baths. Extreme caution should be exercised when interpreting these data as they represent very small sample sizes, in some cases only a single individual, and do not have the support of pharmacokinetic studies.

CHEMOTHERAPEUTICS

Amikacin

Amikacin sulphate is a broad-spectrum antibiotic. Amikacin has been administered via IM injection at a dosage of 3.0-5.0 mg kg^{-1} (5.0 mg kg^{-1} in the case of the ocellate river stingray, *Potamotrygon motoro*) every 72 hours for five consecutive treatments.

Ceftazadime

Ceftazadime pentahydrate (Fortaz®, Glaxo-SmithKline Inc., USA) is a broad-spectrum antibiotic. Ceftazadime has been administered via IM injection at a dosage of 30.0 mg kg^{-1} every 72 hours (8 hours in the case of the spotted eagle ray, *Aetobatus narinari*) for five consecutive treatments.

Copper

Copper (citrated and non-citrated) is used as a treatment for external parasites, especially monogeneans, crustaceans, and protozoans. Copper has been administered as a bath at a dosage of 0.15 mg l^{-1} for up to three months (3-4 months in the case of the bat eagle ray, *Myliobatis californica*) and at 0.20 mg l^{-1} for a period of 30 days (0.15 mg l^{-1} for a period of 30 days in the case of the following species: sand tiger shark, *Carcharias taurus*; whitespotted bambooshark, *Chiloscyllium plagiosum*; brownbanded bamboo-shark, *Chiloscyllium punctatum*; nurse shark, *Ginglymostoma cirratum*; epaulette shark, *Hemiscyllium ocellatum*; and the smalltooth sawfish, *Pristis pectinata*). When applying copper baths, activated carbon filtration should be discontinued. Never use copper in the presence of formalin, praziquantel, or trichlorfon.

Table 10.1. Chemotherapeutics used in 22 public aquariums when applying prophylaxis during quarantine (Q) and prophylaxis in exhibit (P). Please refer to body text for details of dosages and treatment conditions for each medication.

Species name	Common name	Amikacin	Ceftazadime	Copper	Enrofloxacin (IM)	Enrofloxacin (PO)	Fenbendazole	Formalin	Hydrogen Peroxide	Ivermectin	Metronidazole	Furanace	Praziquantel (bath)	Praziquantel (PO)	Salinity	Trichlorfon
Aetobatus narinari	spotted eagle ray	P	P		P								Q			Q
Atelomycterus marmoratus	coral catshark							P								
Carcharhinus acronotus	blacknose shark	P			P		P				P		P	P		Q+P
Carcharhinus amblyrhynchos	grey reef shark															Q
Carcharhinus leucas	bull shark	P			P	P		P								Q+P
Carcharhinus limbatus	blacktip shark	P			P								Q+P			Q+P
Carcharhinus plumbeus	sandbar shark	P			P								Q+P			Q+P
Carcharias taurus	sand tiger shark	P		Q+P	P	P	P				P		Q+P	P	Q	Q+P
Cephaloscyllium ventriosum	swellshark							P								
Chiloscyllium plagiosum	whitespotted bambooshark	P		Q+P	P			Q				P	Q+P			Q+P
Chiloscyllium punctatum	brownbanded bambooshark	P		Q+P	P			Q				P	Q+P			Q+P
Dasyatis americana	southern stingray	P		Q+P	P		P				P	P	Q+P	P		Q+P
Dasyatis brevis	whiptail stingray							Q	Q			Q				
Dasyatis sabina	Atlantic stingray	P		Q+P	P		P				P	P	Q+P	P		Q+P
Ginglymostoma cirratum	nurse shark	P		Q+P	P	P	P	Q			P	P	Q+P	P		Q+P
Hemiscyllium ocellatum	epaulette shark	P		Q+P	P			Q				P	Q+P			Q+P
Heterodontus francisci	horn shark	P						Q				Q	Q			Q
Heterodontus portusjacksoni	Port Jackson shark	P	Q										Q			
Himantura fai	pink whipray				P											
Myliobatis californica	bat eagle ray			P				Q	Q			Q				
Negaprion brevirostris	lemon shark	P		Q+P		P	P				P		Q+P	P	Q	Q+P

Table 10.1 (continued). Chemotherapeutics used in 22 public aquariums when applying prophylaxis during quarantine (Q) and prophylaxis in exhibit (P). Please refer to body text for details of dosages and treatment conditions for each medication.

Species name	Common name	Amikacin	Ceftazadime	Copper	Enrofloxacin (IM)	Enrofloxacin (PO)	Fenbendazole	Formalin	Hydrogen Peroxide	Ivermectin	Metronidazole	Furanace	Praziquantel (bath)	Praziquantel (PO)	Salinity	Trichlorfon
Orectolobus japonicus	Japanese wobbegong	P			P			Q					Q+P			Q+P
Orectolobus ornatus	ornate wobbegong	P			P			Q					Q+P			Q+P
Platyrhinoidis triseriata	thornback guitarfish							P								
Potamotrygon motoro	ocellate river stingray	P						P								
Pristis pectinata	smalltooth sawfish	P		Q+P	P		P				P		Q+P	P	Q	Q+P
Pteroplatytrygon violacea	pelagic stingray							Q				Q				
Raja inornata	California ray							P								
Rhinobatos productus	shovelnose guitarfish	P		Q+P	P		P	Q			P	P	Q+P	P	Q	Q+P
Rhinoptera bonasus	cownose ray	P	P	Q+P	P								Q+P			Q+P
Scyliorhinus canicula	smallspotted catshark									Q						Q+P
Scyliorhinus stellaris	nursehound									Q						Q+P
Sphyrna zygaena	smooth hammerhead											P				
Squalus acanthias	spiny dogfish							P								
Squatina californica	Pacific angelshark							P								
Stegostoma fasciatum	zebra shark	P			P		P				P		Q+P	P	Q	Q+P
Triaenodon obesus	whitetip reef shark	P	P		P								Q+P		Q	Q+P
Triakis semifasciata	leopard shark	P		P	P			Q	Q				Q+P			Q+P
Urobatis jamaicensis	yellow stingray	P		Q+P	P		P				P	P	Q+P	P		Q+P
Zapteryx exasperata	banded guitarfish							P								

Enrofloxacin

Enrofloxacin (Baytril®, Bayer Corp., USA) is a broad-spectrum antibiotic. Enrofloxacin has been administered both orally and via IM injection at a dosage of 10.0 mg kg^{-1} every 5-7 days (2 days in the case of the pink whipray, *Himantura fai*, and 3.5 or 7 days in the case of the following species: blacknose shark, *Carcharhinus acronotus*; bull shark, *Carcharhinus leucas*; blacktip shark, *Carcharhinus limbatus*; and the sandbar shark, *Carcharhinus plumbeus*) for three to five consecutive treatments.

Fenbendazole

Fenbendazole (Panacur®, Intervet Inc., USA) is an antihelminthic used for the treatment of internal parasites. Fenbendazole has been used in elasmobranchs to treat nematodes at an oral dosage of 25.0 mg kg body $weight^{-1}$ for 3x each week, over three consecutive weeks of treatment.

Formalin

Formalin is an antibiotic, antihelminthic, crustacicide, and protozoacide. Formalin has been applied as a bath at a dosage of 250 mg l^{-1} for a period of one hour. Formalin has been used in conjunction with hydrogen peroxide when treating the leopard shark (*Triakis semifasciata*), the bat eagle ray, and the whiptail stingray (*Dasyatis brevis*).

Hydrogen peroxide

Hydrogen peroxide is an antibiotic, antihelminthic, crustacicide, and protozoacide. Hydrogen peroxide has been applied as a bath at a dosage of 150.0 mg l^{-1} for a period of one hour.

Ivermectin

Ivermectin (Ivomec®, Merial Inc., USA) is an antihelminthic used for the treatment of internal parasites. Ivermectin has been used in elasmobranchs to treat nematodes and cestodes administered via IM injection at a dosage rate of 200 mg kg^{-1} every 15 days for two treatments.

Metronidazole

Metronidazole (Flagyl®, Rhone-Poulenc Rorer Pharmaceuticals Inc., USA) is a protozoacide and anaerobe antibiotic. Metronidazole has been used in elasmobranchs at an oral dosage of 25.0 mg kg body $weight^{-1}$ for 3 days a week, over three consecutive weeks of treatment.

Furanace

Furanace (Nitrofurazone, Novalek Inc., USA) is broad-spectrum antimicrobial. Furanace has been applied as a bath at a dosage of 20.0 $mg.l^{-1}$ for 2 hours each day of five consecutive days of treatment (10.0 mg l^{-1} for 10 hours each day of five consecutive days of treatment in the case of the smooth hammerhead shark, *Sphyrna zygaena;* 10.0 mg l^{-1} for 8 hours each day of seven consecutive days of treatment in the case of the bat eagle ray; and 10.0 mg l^{-1} for 8 hours each day of five consecutive days of treatment in the case of the following species: the whitespotted bambooshark; the brownbanded bambooshark; the nurse shark; the horn shark, *Heterodontus francisci*; and the epaulette shark). When applying furanace baths, activated carbon filtration, ozone dosing, and UV irradiation should be discontinued.

Praziquantel

Praziquantel (Praziquantel 100%, Professional Pharmacy Services Inc., USA) is an antihelminthic used for the treatment of both internal and external platyhelminthes. Praziquantel has been applied as a bath to treat monogeneans at a dosage of 10.0 mg l^{-1} for a period of two hours and at 2.0 mg l^{-1} for a period of 48 hours (2-20 days in the case of the sandbar shark). When applying praziquantel baths, activated carbon filtration, ozone dosing, and UV irradiation should be discontinued. Never use praziquantel in the presence of copper or trichlorfon. Praziquantel has been used in elasmobranchs to treat trematodes and cestodes at an oral dosage of 50.0 mg kg body $weight^{-1}$ for 3 days a week, over three consecutive weeks of treatment.

Salinity

Reduced salinity can be used as an antihelminthic, crustacicide, and protozoacide. A reduced salinity of 15.0 ‰, maintained for a period

of 14 days, has been used to treat elasmobranchs for external parasites, both as a stand-alone treatment or as a complement to other immersion medications. A 30-minute bath of freshwater has been used to treat lemon sharks (*Negaprion brevirostris*) for external parasites, as has a reduced salinity of 10.0-15.0 ‰ maintained for a period of four weeks.

Trichlorfon

Trichlorfon (Dylox® 80, Bayer Corp., USA) is an antihelminthic and crustacicide. Trichlorfon has been applied as a bath to treat monogeneans and parasitic crustaceans at a dosage of 0.5 mg l^{-1} (0.3 mg l^{-1} in the case of the grey reef shark, *Carcharhinus amblyrhynchos*) for a period of 24 hours, once a week, for a total of four treatments (0.25 mg l^{-1} for a period of 24 hours, once every 10 days, for a total of five treatments in the case of the smallspotted catshark, *Scyliorhinus canicula*, and the nursehound, *Scyliorhinus stellaris*). When applying trichlorfon baths, activated carbon filtration, ozone dosing, and UV irradiation should be discontinued. Never use trichlorfon in the presence of copper, formalin or praziquantel.

ACKNOWLEDGEMENTS

I would like to thank the following institutions for their contributions toward this chapter: Colorado Ocean Journey, Denver, Colorado, USA; The Living Seas Pavilion, Orlando, Florida, USA; Monterey Bay Aquarium, Monterey, California, USA; New England Aquarium, Boston, Massachusetts, USA; Oceanário de Lisboa, Lisboa, Portugal; Sea Life Centre, Benalmedena, Spain; Sea Life Centre Biological Services, Weymouth, England; Sea Life Centre Birmingham, England; Sea Life Centre Blankenberge, Belgium; Sea Life Centre Paris, France; Sea Life Centre Great Yarmouth, England; Sea Life Centre Hunstanton, England; Sea Life Centre Konstanz, Germany; Sea Life Centre Oban, Scotland; Sea Life Centre Scarborough, England; Sea Life Centre Timmendorf, Germany; Sea Life Centre Weymouth, England; Sea World Orlando, Orlando, Florida, USA; Sea World San Antonio, San Antonio, Texas, USA; Sea World San Diego, San Diego, California, USA; Two Oceans Aquarium, Cape Town, South Africa; Virginia Aquarium and Marine Science Center, Virginia Beach, Virginia, USA. I would like to thank Beth Firchau (Virginia Aquarium and Marine Science Center, Virginia Beach, Virginia, USA) for information she supplied from the American Elasmobranch Society's 1999 Captive Elasmo-branch Husbandry Survey.

REFERENCES

Sorensen, E. M. B. 1991. Metal Poisoning in Fish. CRC Press, Boca Raton, Florida, USA. 384 p.

SUGGESTED READING

Herwig, N. 1979. Handbook of Drugs and Chemicals used in the treatment of Fish Diseases. Charles C. Thomas Publishers, Springfield, Illinois, USA. 272 p.

Noga, E. J. 1996. Fish Disease: Diagnosis and Treatment. Mosby, St. Louis, USA. 367 p.

Stoskopf, M. K. (ed.). 1993. Fish Medicine. W. B. Saunders Inc., Philadelphia, Pennsylvania, USA. 882 p.

The Elasmobranch Husbandry Manual: Captive Care of Sharks, Rays and their Relatives, pages 151-162.
© 2004 Ohio Biological Survey

Chapter 11
Elasmobranch Acclimatization and Introduction

SUZANNE M. GENDRON

Ocean Park Hong Kong,
Aberdeen, Hong Kong, SAR CHINA.
E-mail: Suzanne.Gendron@oceanpark.com.hk

STEPHEN MENZIES

Independent Curatorial Consultant,
Perth, Western Australia, Australia.
E-mail: shocaq@yahoo.com.au

Abstract: The long-term success of an elasmobranch acquisition depends not only on how the animal is captured and transported, but also on its careful acclimatization and introduction. Acclimatization is undertaken when moving animals between different environments and involves a process of slowly changing parameters (especially water parameters) in which the animal is held, or transported, to meet the environmental parameters where it will ultimately be living. Acclimatization minimizes the physiological stress inherent in a rapid transition between different environmental parameters. Introduction refers to the process of moving an elasmobranch to its destination environment (e.g., exhibit, experimental tank, etc.), in some cases requiring capture and physical restraint of the animal, and its subsequent careful release. A program of post-introduction monitoring is essential to success, allowing workers to anticipate problems and intervene in the event of complications.

The acclimatization and introduction of an elasmobranch to its destination environment (e.g., exhibit, experimental tank, etc.) represents the final stage of an animal acquisition and must be carefully planned in conjunction with other aspects of a relocation strategy. While the science of elasmobranch husbandry continues to improve, acclimatization and introduction of fishes remains inexact and is often given cursory treatment for many elasmobranch species. It is clear, however, that an animal's expected survivability in captivity depends directly on how well the animal is captured, transported, acclimatized, and introduced.

For the purposes of this chapter, acclimatization refers to the gradual change of environmental parameters, predominantly water quality, to minimize physiological stress imposed on animals moved between different environments (e.g., from a transport vessel to an exhibit, etc.). During acclimatization, prophylactic treatments may be applied, and wounds and abrasions evaluated. Introduction refers to the process of moving an elasmobranch to its destination environment (e.g., exhibit, experimental tank, etc.), in some cases requiring the capture and physical restraint of the animal, and its subsequent careful release.

The basis for what we know about elasmobranch husbandry has been developed predominantly through educated guesses and trial and error. The collection, transportation, and maintenance of many different elasmobranch species was attempted, modified, and attempted again, before success was achieved. Clark (1963), and Gruber and Keyes (1981), published early work on elasmobranch transportation and acclimatization. Since then, many workers have added to the science (Cliff and Thurman, 1984; Hewitt, 1984; Murru, 1990; Smith, 1992; and Lowe, 1996).

In addition to the information reported in the literature, many successful strategies have been developed through accumulated practical experience. It is only through such experience that many of the more subtle indicators of elasmobranch health have been recognized. In many cases, these subtle signs will indicate an animal's status well before quantitative empirical data can confirm it. Changes in ventilation rate, body coloration, attitude in the water, swimming behavior, etc., will all speak to deeper changes at a biochemical and physiological level. Understanding these subtle changes, both within and between species, is crucial to the development of a suitable acclimatization and introduction regime. Responding quickly to negative trends can often be the difference between success or specimen mortality.

Biochemical and physiological changes incurred during capture and transportation, and their impact on survivability, are discussed in detail in Chapter 8 of this manual and repetition of that information will be minimized here. Similarly, quarantine procedures and medical treatments are covered in more detail in Chapters 10 and 29 of this manual, respectively.

ELASMOBRANCH ACCLIMATIZATION

Environmental changes (e.g., a change to water parameters) and the associated physiological stress, directly affect the health of sharks, rays, and their relatives. Elasmobranchs, like other fishes, need time to become accustomed to a change in water chemistry. Acclimatization should therefore be undertaken whenever an animal is moved from one environment, where it has been living for an extended period, to a new environment in which the water chemistry is different.

The importance of acclimatization

Rapid changes in water chemistry or temperature may cause physiological distress to fishes, contribute to disease susceptibility, and even cause death (Stoskopf, 1993). However, as Noga (1996) states: "…many fishes can tolerate stressful conditions if they are introduced to the environment slowly…". Therefore, an excellent axiom for the new aquarist is as follows: poor water quality is bad for fish health, but rapidly changing water quality is even worse.

The goal of acclimatization is to slowly change the water parameters in which an animal is held, or transported, to meet the parameters of the water where the animal will ultimately be living, with a minimum of imposed stress (Spotte, 1992). A complete knowledge of environmental parameters (e.g., temperature, pH, salinity, oxygen concentration, nutrient concentration, lighting regime, etc.), from both the source and destination environment, is therefore essential to best acclimatize a target animal.

In general, acclimatization provides an opportunity to undertake veterinary procedures (e.g., blood sampling, prophylactic treatments, physical inspections, etc.), as the target animal is confined within a small acclimatization vessel and easily accessible. The decision to extend the duration of acclimatization to allow these procedures should be weighed carefully, and only undertaken if the elasmobranch is stable. Over time, an animal will modify the water chemistry within a transport, acclimatization, and/or introduction tank. A balance should be struck between the time it takes to acclimatize and introduce a specimen, and the harmful effects that increasingly changed water chemistry will impose. An unnecessarily delayed introduction may compromise the chances of a successful operation.

Acclimatization and water parameters

Acclimatization can, and frequently should, commence the moment an animal is collected. Parameters can be adjusted gradually throughout transportation, taking into consideration the characteristics of the water at both the collection site and the final destination. Where possible, long transports should be broken into small stages, with corresponding water exchanges, reducing the acclimatization burden on arrival. An elasmobranch will modify the water chemistry of a transport or acclimatization container by consuming oxygen, and excreting nitrogenous wastes, CO_2, and other metabolic toxins. Acclimatization, through water exchanges, addresses each of these aspects of declining water quality, ultimately improving the immediate environment. Many stress-related chemicals are released during the period of initial capture and confinement, so a water exchange relatively early in the transport (e.g., 2-3 hours after confinement) will have immediate beneficial results.

Temperature, pH, and nutrients

In general, temperature, pH, and nutrients can be modified by the exchange of contaminated water with untainted water from the destination environment. Tolerable changes of temperature and pH, and suggested adjustment times, have been estimated from empirical data. Temperature differences of 1.0-2.0 ºC should be equalized in no less than 30 minutes, while pH should not change more than 0.2-0.4 over the same period (Stoskopf, 1993). Where pH levels are not life-threatening, pH should not change by more than 0.2-0.5 each day (Noga, 1996). More rapid parameter changes may cause distress, manifested as blanching, slow or exaggerated swimming and stalling, and difficulty maintaining equilibrium. In the wild, an elasmobranch may swim through temperature gradients greater than 1.0-2.0 ºC with no ill effect, but as a stressor during acclimatization such changes should be minimized. Ensure that pH never drops below 6.0, as this level approaches toxicity for many elasmobranchs.

Nitrite-induced methemoglobin formation reduces the oxygen carrying capacity of the blood (Stoskopf, 1993; Noga, 1996) and should be avoided by maintaining <0.10 mg l^{-1} nitrite ion at all times. Chronic ammonia toxicity, causing kidney damage, should not occur during short-term acclimatization and introduction; however, it may occur during transportation or long-term holding (Thurston and Russo, 1984). Never allow ammonia concentrations to exceed 1.0 mg l^{-1} and where possible, reduce ammonia concentrations to <0.10 mg l^{-1} before moving animals into a new system. In some cases, the use of ammonia sponges may be indicated (e.g., AmQuel®, Novalek Inc., USA), but these chemicals should always be used in conjunction with a pH buffer. Ammonia becomes increasingly toxic as pH increases (Post, 1987) so it is critical to adjust or dilute ammonia concentrations before modifying pH. It should be noted that ammonia toxicity is further affected by temperature and salinity and an understanding of these dynamics is advised (refer to Spotte, 1992).

Dissolved oxygen

Dissolved oxygen (DO) concentrations within an acclimatization and introduction container are vitally important. DO (as percentage saturation) should never fall below 85% and ideally should be maintained at 95-100%. During the acclimatization of highly active, ram-ventilating species DO levels can be as high as 150% without apparent harmful effects. In addition, empirical evidence suggests that hyper-oxygenation may have a mildly sedative effect on most elasmobranch species, a useful side-benefit during transport and acclimatization. The potentially harmful effects of hyper-oxygenation (e.g., respiratory depression and subsequent blood acidosis) must be understood and weighed against the benefits.

When DO needs to be stabilized or raised, it is a simple matter to enhance gas exchange across the water surface by adding air diffusers (Spotte 1973; Noga, 1996). The air bubbles rise to the surface, causing surrounding water to rise as well. Some gas dissolves from the air bubble directly into the water, but this quantity is small compared to the advantage of moving oxygen-poor water to the surface, where most gas exchange takes place. A more effective means of increasing DO is to add pure oxygen bubbles via the intake of a submersible pump (e.g., a 12 or 24 Volt bilge pump) or diffuser. It is important that oxygen is introduced as fine bubbles, promoting oxygen dissolution (Gruber and Keyes, 1981; Smith, 1992; Murru, 1990). Maintaining an oxygen-rich atmosphere immediately above the water surface can be achieved by using a well-fitted lid, which also prevents animals inadvertently exiting the acclimatization vessel.

Acclimatization and specimen origin

If elasmobranchs are acquired from different geographic regions, then water parameters, exhibit topography, and species combinations may be the result of a compromise between different environments. Such compromises required for a multi-species exhibit may affect the long-term health, welfare, and longevity of a given species, and importantly, how that species will be acclimatized and introduced.

It is quite common for facilities to import elasmobranchs from international sources and this may necessitate specimens undergoing a rapid seasonal reversal. Temperature is the most obvious change to water quality and this should be carefully considered when developing transport schedules. It should be recognized that many species will be forced to undergo relatively rapid metabolic changes which will then affect appetite and other aspects of their behavior. Maintaining a homogeneous transport temperature is preferable; however, if a significant temperature

differential is anticipated at the final destination, then a gradual variation can be applied during the transport to reduce acclimatization periods on arrival.

Another consideration, when moving animals between different seasons, is the change in photoperiod. Wherever possible, destination environments should try to match photoperiods encountered at the source.

Acclimatization by elasmobranch type

Elasmobranchs may be categorized into four basic types (essentially the same as those described in Chapter 5 and Table 5.1 of this manual), requiring different acclimatization times and techniques. Factors determining elasmobranch type include species size, ecology, spatial requirements, mode of ventilation, and response to stressors. Table 11.1 presents a review of the four basic elasmobranch types, showing representative species.

Benthic

During acclimatization, source water should be diluted by replacing half the volume over a period of no less than 30 minutes. Use a small diameter siphon hose (i.e., <12 mm diameter) to ensure that the water change is slow. For larger transport containers use a larger diameter hose, but do not complete the dilution in less than 30 minutes. Water from the destination aquarium should be used wherever possible. Since this group generally responds well to confinement, it is preferable to adjust parameters as slowly as possible. Ensure that oxygen concentration remains above 85% and below 100% saturation.

To minimize stress lighting should be dimmed when first opening a transport box, particularly if the lid has been closed throughout transportation. Gradually increase lighting to the lowest level that allows specimen behavior and condition to be monitored. Shortly before specimen introduction (e.g., ~15 minutes), lighting illumination should be slowly raised to levels approximating conditions within the destination environment.

If animals regurgitate, defecate, or produce excessive mucus, remove solid particles immediately and mechanically filter the water if at all possible. Some rays produce copious amounts of mucus that can interfere with oxygen uptake. Ensure that any excess wastes are removed. Transport water should always be sent to waste and not introduced into the destination exhibit. This practice will reduce the chances of introducing disease.

Semi-pelagic

Unless otherwise indicated, follow the guidelines for benthic species. Acclimatization containers should be sized (i.e., ~2.0-4.0 m^3) to allow specimens to swim freely for brief periods. Square or rectangular containers are generally preferred as circular containers may cause the animal to swim along the perimeter, turn constantly, and consequently generate a higher oxygen debt. Although rays and skates typically tolerate longer transport and acclimatization times, they too require room to swim as this facilitates blood circulation and the excretion of metabolic toxins.

Dilute source water by replacing half the volume over 60 minutes. For large volumes, it may be necessary to remove old water from the container before adding new water. If specimens are coping well, an additional dilution (~50%) should be performed to dilute the original transport water by ~75%. This additional dilution should bring water quality parameters within acceptable limits. Dissolved oxygen should be maintained between 85% and 100% saturation, as per benthic animals. However, consider the application of slightly higher oxygen levels when animals are hyperactive (e.g., 90%-105% saturation). In this case, pure oxygen, rather than a supply of air, should be used.

Pelagic (non-obligate ram ventilator)

Non-obligate ram ventilators frequently will be transported in confined containers, due primarily to the space and weight constraints of transport vehicles. Transport containers are usually accompanied by water treatment and oxygenation systems, to provide elevated DO levels. These elasmobranchs should be acclimatized as per semi-pelagic animals, although acclimatization times should not exceed 60 minutes. These animals must be watched closely during acclimatization, as they can easily become distressed. If distress is evident, slowly adjusting water parameters becomes of secondary importance and specimens should be moved into a larger tank or the destination exhibit immediately, allowing them to swim freely.

Table 11.1 Four generalized categories of elasmobranch type, showing issues to consider during specimen acclimatization and introduction.

Category description	Representative species	Typical size	Transportability	Oxygenation	Water circulation	Acclimation duration
1. Benthic						
Sedentary species with low metabolism. Able to actively ventilate. Spend majority of time on bottom without accumulating an oxygen debt. May be attacked by established exhibit residents.	Bamboo sharks (Hemiscylliidae) Cat sharks (Scyliorhinidae) Wobbegong sharks (Orectolobidae) Horned sharks (Heterodontidae) Stingrays (Dasyatidae) Round rays (Urolophidae)	< 1.0 m TL	High	Normal	Preferred	Extended: 0.5-2.0 hours
2. Semi-pelagic						
Free-swimming species. Periodically rests on bottom. Able to actively ventilate. Able to swim in confined areas and negotiate obstacles. May be attacked by established exhibit residents.	Smooth-hound (*Mustelus mustelus*) Spiny dogfish (*Squalus acanthias*) Whitetip reef shark (*Triaenodon obesus*) Leopard shark (*Triakis semifasciata*) Cownose ray (*Rhinoptera bonasus*)	1.0-1.5 m TL	Medium to High	Normal to High	Preferred	Extended: 1.0-2.0 hours
3. Pelagic (non-obligate ram ventilator)						
Large, pelagic species. Some species rest on bottom for limited periods, but normally need to swim to aid respiration and circulate body fluids. Can negotiate obstacles. May be attacked by, or attack, established exhibit residents.	Sand tiger shark (*Carcharias taurus*) Lemon shark (*Negaprion brevirostris*) Spotted eagle ray (*Aetobatus narinari*)	1.5-2.0 m TL	Medium	Normal to Very High	Necessary	Limited: 0.5-1.0 hours
4. Pelagic (obligate ram ventilator)						
Large, pelagic species. Relatively fast metabolism and high oxygen demand. Swim constantly to create hydrodynamic lift, aid respiration, and circulate body fluids. Unable to negotiate tight corners and requires large horizontal distances to allow uninterrupted swimming patterns. Low tolerance to confinement. Should be introduced into a sufficiently large aquarium as quickly as possible for any chance of long-term success. May attack resident animals.	Blacktip shark (*Carcharhinus limbatus*) Caribbean reef shark (*Carcharhinus perezi*) Great white shark (*Carcharodon carcharias*) Sevengill shark (*Notorynchus cepedianus*) Blue shark (*Prionace glauca*) Whale shark (*Rhincodon typus*) Scalloped hammerhead shark (*Sphyrna lewini*) Giant manta (*Manta birostris*) Pelagic stingray (*Pteroplatytrygon violacea*)	1.0-3.0 m TL	Low	Very High	Mandatory	Minimal

Dissolved oxygen should be applied as per semi-pelagic animals. However, if abnormally high ventilation rates are observed, elevated oxygen levels should be applied (i.e., 95%-110% saturation). In this case pure oxygen, introduced via a bilge pump (to maximize dissolution), is recommended.

Pelagic (obligate ram ventilator)

Obligate ram ventilators typically have high oxygen requirements and need to swim constantly. If an obligate ram ventilator has been in transit for >3.0 hours, it may not tolerate further confinement for acclimatization purposes. On arrival, a quick assessment of the animal's condition should be made to determine if normal acclimatization protocols should be bypassed. If it has been deemed appropriate to acclimatize an obligate ram ventilator then transport water should be rapidly diluted by 75% over a period of 20-30 minutes. If the animal becomes distressed it should be moved into its destination exhibit immediately. Dissolved oxygen should be applied at elevated levels (i.e., 95%-150% saturation).

SPECIMEN ASSESSMENT

It is important to carefully assess the condition of an elasmobranch throughout acclimatization to detect possible deterioration. Any potential negative trends should be counteracted immediately, as delay can result in specimen mortality. A detailed report of capture techniques, transport times and conditions, and water quality parameters will be invaluable in assessing an animal's condition and formulating an effective acclimatization regime.

The clinical assessment of an animal during acclimatization is often quite subjective given that the animal's history may be largely unknown. Where possible, blood should be drawn and biochemistry analysed. This information is invaluable when formulating long-term medical treatments, future transports, and acclimatization regimes. Blood-gas monitors give immediate results and provide an opportunity to apply informed corrective therapies as and when they are required.

Acclimatization provides an ideal opportunity to inspect specimens, since they are accessible and usually docile. If no further isolation is anticipated prior to specimen release, then acclimatization represents the final opportunity to check for abrasions, lacerations, external parasites, and other unusual or life-threatening conditions. During this time, short-term clinical procedures may be undertaken and can include:

1. Application of topical medications;
2. Application of injectable antibiotics or other therapies;
3. Acquisition of blood samples;
4. Removal of capture tags or fishing hooks;
5. Measurement of baseline husbandry data (e.g., length, weight, etc.);
6. Removal of external parasites (manually, or through the use of medicated baths);
7. Confirmation of sex and reproductive status (possibly influencing the method and timing of specimen introduction); and,
8. Application of sutures.

Many profound internal problems, that may threaten the life of an elasmobranch (e.g., hypoxia, acidosis, hyperkalemia, etc.), present few or no external signs but may be suspected if there is a significant deviation from the normal aspect and behavior of healthy conspecifics. Familiarity with a species will allow recognition of unusual behaviors or changes in appearance that may indicate a problem during acclimatization and introduction. Key areas to consider include, but are not limited to, the following:

1. Changes to ventilation rate (both elevated or depressed);
2. Changes to body coloration (blanching can indicate shock, but may also be normal—e.g., many rays mimic container coloration, in this case a positive sign);
3. Swimming behavior (e.g., hyperactivity can indicate distress and exaggerated swimming can indicate increasing exhaustion);
4. Body attitude and orientation, especially while swimming; and
5. Responses to stimuli.

It is essential to assess specimens quickly, on arrival, and at regular intervals, to guide the most appropriate acclimatization and introduction strategy. If negative trends are observed, steps must be taken to mitigate trends and, in some extreme cases, discontinue acclimatization or treatments and release specimens into destination environments immediately.

ELASMOBRANCH INTRODUCTION

Ideally, all new animals should be quarantined, to control disease and allow for observation of

behavior (e.g., feeding, swimming, etc.), before introduction into their final environment. Unfortunately, this requirement is often impractical for larger specimens. In this case, it is important to carefully observe new specimens, following introduction, to ensure both a healthy status and normal feeding behavior.

Wherever possible, it is preferable to maintain some level of control over the animal until it is clearly healthy and apparently able to survive in its new environment. If at all possible, exhibit design should include a large, smooth-walled isolation pool, directly linked to the main filtration system and exhibit.

Careful thought should be given to the introduction of multiple specimens and, if possible, plan arrivals to minimize compatibility problems. It is better to habituate potential prey species to a system before predators are introduced. Many species will benefit from being introduced as a group (e.g., Myliobatids), and it may be better to hold small numbers of specimens until a larger group can be released simultaneously.

Lighting should be at lowered levels when animals are first introduced and it is usually best to maintain some light throughout the first few nights, minimizing predation and assisting in orientation. Full-strength lighting may increase stress levels and should be avoided. The ideal intensity and diurnal variation of lighting is species-dependent and therefore it is often best to have a variety of “night light” intensities in different parts of the aquarium. This accomodation allows animals to choose an intensity under which they are most comfortable.

Elasmobranchs may be introduced into a new exhibit using four basic methods:

1. Lifted into the exhibit by stretcher and immediately released;
2. Lifted into the exhibit by stretcher, restrained near the surface within a well-oxygenated current for a short period, and then released;
3. Lowered into the exhibit within a restraining vessel (e.g., the vessel used to transport the animal) and gently released; and
4. Maintained in an adjoining holding pool, or a floating cage within the exhibit, for an extended period (e.g., a week) and then subsequently released.

A basic summarized checklist of equipment and logistics required for an elasmobranch acclimatization and introduction has been provided in Table 11.2.

Handling and restraint of elasmobranchs

Personnel handling elasmobranchs should always wear protective clothing/wetsuits and sterile latex gloves to protect the skin of the specimen. When handling or restraining elasmobranchs, avoid using unprotected nets as they may abrade the skin and lead to secondary infection. Heavy plastic bags, or stretchers made of canvas or plastic tarpaulin, are typically used to restrain or move elasmobranchs. It is best to have a selection of stretcher sizes and designs to allow the greatest flexibility of use. A large, 1.0 mm thick, plastic bag has been used successfully to capture and restrain a 4.0 m TL tiger shark (*Galeocerdo cuvier*). The bag was submerged inside the shark’s holding pool and the animal encouraged to swim inside. Once the shark was caught, staff were able to safely enter the water and maneuver the shark onto a stretcher. To remove the shark from the plastic bag, staff cut along a predetermined sacrificial seam. Minimal abrasions were incurred to both shark and personnel (Long, pers. com.). Self-draining stretchers with poles have the advantage of allowing more people to assist with lifting and restraint. Usually the most difficult specimens to lift out of the water are rays, because they are slippery and relatively heavy for their size. A self-draining, circular, or “dish-shaped”, stretcher is often better suited to moving rays.

When handling sharks, consideration should be given to covering the mouth of the animal, and it is best if the system used is inherent in the design of the carrying device. Preventing significant lateral movement of the head and body is advantageous. Once released, if an animal does not immediately swim out of its restraining bag or stretcher, gently tracing a hand along the lateral line can often stimulate the animal to start swimming (Gruber and Keyes, 1981).

Holding pools and floating cages

Where possible, holding pools linked to the main system, or floating cages, should be used to habituate elasmobranchs to their new environment, allowing them to normalize their swimming and feeding behavior. This strategy reduces the chances of new specimens becoming prey when they are finally released into an exhibit, as the behavior of newly-arrived and stressed animals will frequently stimulate resident animals to attack (Lai, pers. com.). Floating cages can be made from heavy and perforated clear plastic

Table 11.2. Summarized checklist of equipment and logistics required for elasmobranch acclimatization and introduction.

Consideration	Critical Information and Equipment	Strategies and Tips
Transport regime	Specimen condition. Journey duration. Water quality.	Undertake water exchanges at strategic locations. Brief all personnel in advance. Minimize delays at customs in advance. Ensure that all transport vehicles are reliable. Healthy animals respond better to acclimatization and introduction.
Cargo handling	Container dimensions. Container volume. Container weight.	Remove portion of water to make handling easier. Forklifts represent a good alternate unloading strategy.
Access	Doorway dimensions. System to access upper floors.	Move specimen to acclimatization site in the transport container. Ensure tank at acclimatization site is suitably sized for extended holding. Design specimen holding spaces with plenty of access space. Design specimen holding spaces with lifting systems.
Water circulation	Hoses for siphons. System for waste water disposal. Power outlets (for pumps).	Siphons are failsafe and avoid the need for electricity. Hose size will determine water flow rate. Ensure suitable head pressure to drive siphons. If pumps are required, use 12-Volt batteries and bilge pumps. Introducing water with buckets is not recommended for large specimens.
Lighting	Power outlets (for lights). Reliable and adjustable lights.	Use low lighting to minimize specimen stress. Lighting should be variable to allow specimen inspection and safe handling. Underwater torches are useful.
Oxygenation	Oxygen bottles. Oxygen regulators. Oxygen airstones/diffusors. Weights for airstones/diffusors.	An excess of oxygen bottles is better than running out at a critical moment. If oxygen is unavailable, air is essential. Dive cylinders can be used as an air supply in an emergency.
Test equipment	Testing equipment for water parameters.	Testing equipment for oxygen, temperature, pH, salinity, and ammonia. Testing equipment for blood-gas, lactate, etc., if required. Equipment should be waterproof, easy to use, reliable, and give rapid results. Assign someone to record all data and give updates.
Other equipment	Stretcher. Scales and weighing slings. Flexible tape measures. Hand tools (for hook or tag removal).	

sheeting, heavy plastic mesh, or rigid plastic bars (e.g., PVC pipes). Floating cages should always incorporate perforations or mesh to allow a free exchange of water with the destination exhibit, as water quality can deteriorate quickly in the relatively small volume of a cage.

Water quality in destination environments

Any enclosure designed for a new elasmobranch must have a fully functioning water treatment system, mature biological filters, and optimal water parameters. Adding elasmobranchs to an exhibit implies an increased biological load and nutrient concentrations (e.g., ammonia, nitrite, and nitrate) must be closely monitored to ensure that they are stable and do not reach toxic levels. Poor water quality will almost certainly reduce the chances of a specimen adapting to its new environment, exacerbate post-transport trauma, promote the proliferation of disease, and eventually may result in mortality. Despite this cautionary approach, it may not always be practical to introduce new specimens into an optimal environment, and exposure to immature filtration systems and compromised water quality may result. The purpose of acclimatization must remain the process of gradually adjusting specimens to destination water quality, despite the less-than-ideal environment. Most elasmobranchs can tolerate a wide range of water quality parameters as long as they are given sufficient time to adapt. As a new system matures the water quality should improve gradually and the animals will correspondingly adapt to this gradual change.

In some circumstances, it may be necessary to manipulate water quality within a new system to reduce the impact of a specific water parameter (e.g., lowering pH to reduce the toxicity of ammonia). Many facilities using synthetic seawater maintain a salinity lower than seawater to control disease or save on costs associated with sea salt acquisition. With appropriate acclimation many elasmobranchs can tolerate

salinity levels slightly lower than normal seawater. It should be emphasized, however, that some species will not tolerate low salinity when other environmental stressors have lowered overall tolerance to water quality challenges.

Obstructions in destination environments

When an elasmobranch is released, it should be prevented from hitting the walls or other obstructions within the pool. The most likely time for an animal to hit the walls is when it is darting away as it is first released. It is therefore important to release the animal so that it is unlikely to immediately encounter an obstruction (e.g., orientated in the direction of the largest horizontal dimension of the exhibit, or possibly parallel to a long wall). In some cases it may be necessary to station personnel around the edge of an exhibit to ward animals away from the walls. If an animal approaches a wall, personnel wave a conspicuous PVC pole (e.g., wrapped candy-cane style with colored tape) in front of the animal to ward it away. If pelagic animals are expected to hit walls repeatedly within an exhibit, it is possible to line the pool with a curtain of heavy plastic or tarpaulin mounted 30-50 cm away from the walls. This barrier provides a cushion to animals that swim toward the walls and will often ward them away before they strike the solid surface of the wall itself. A barrier of this type should be installed before new animals are introduced, and staff should be aware that animals could conceivably get caught between the curtain and the walls.

Introduction by elasmobranch type

Benthic

Benthic elasmobranchs can usually be released directly into an exhibit as they are unlikely to crash into a wall by rapidly swimming away. Resident animals should be fed 3-4 hours beforehand and a regular feeding schedule maintained while introduced animals adjust to their new environment. If the predation of newcomers by existing inhabitants is a concern, the exhibit should remain illuminated for at least 24 hours as this will discourage aggression and facilitate monitoring.

Semi-pelagic

When possible, semi-pelagic elasmobranchs should be introduced via a holding pool or floating cage, following a 4-7 day staging period. This duration may be reduced for more active sharks if it is felt they won't tolerate the confined space for an extended period. If an animal exhibits signs of stress, the causes should be investigated and immediately rectified. In some cases this may necessitate the release of the animal into the exhibit. Resident animals should be fed 3-4 hours before new animals are released and illumination should be maintained for at least 24 hours thereafter to reduce the risk of predation.

Pelagic (non-obligate ram ventilator)

Ideally, non-obligate ram ventilators should be maintained in a sufficiently large holding pool or floating cage, for a 1-2 day staging period, before final release into their new environment. This requirement may overstretch the resources of most institutions. If this is the case, non-obligate ram ventilators can be gently lifted into an exhibit using a stretcher and released directly into the water. Resident animals should be fed 3-4 hours before new animals are released and illumination should be maintained for 48-72 hours, to reduce the risk of predation. A 24-hour watch should be maintained for at least one day to monitor the status of new animals.

Sand tiger sharks (*Carcharias taurus*) maintain a small amount of air in their gut to achieve neutral buoyancy. This air may be lost during handling or transport. Some commercial collectors intentionally remove air from sharks to encourage negative buoyancy within the transport container, effectively immobilizing the specimen. Once introduced into its new environment, it is not unusual to observe a sand tiger shark swimming rapidly to surface and swallowing or "gulping" air. This behavior is quite normal, and indeed is desired. If a sand tiger shark does not achieve optimum buoyancy within the first few days of release, it may be necessary to intervene and artificially introduce air into the gut using a flexible tube.

Pelagic (obligate ram ventilator)

The needs of pelagic obligate ram ventilators are not well understood and are still under investigation. However, anecdotal information from various aquariums, about specific species, can lead us to some general conclusions.

Bonnethead sharks (*Sphyrna tiburo*) have become relatively common in public aquariums

over the past 20 years, with many reproducing in captivity. In general, this species should be transported and acclimatized as per semi-pelagic elasmobranchs. Transport and acclimatization tanks should have rounded corners and lids. This species has been known to jump out of small tanks. Transport times for sharks >1 year of age (i.e., >85 cm TL) should be kept to a minimum, and dissolved oxygen levels should be maintained at >95% at all times. Acclimatization times will depend on animal size and overall post-transport condition. Larger specimens (>1.5 m TL) are easily stressed during transport and may not respond well to acclimatization in a small container. If signs of distress are evident, specimens should be moved immediately to the final exhibit or a large holding pool. Where possible, bonnethead sharks should be introduced via a holding pool or a floating cage. Bonnethead sharks are readily preyed on by large elasmobranchs and teleosts, so take great care choosing your initial species list and carefully monitor new specimens during introduction.

Scalloped hammerhead sharks (*Sphyrna lewini*), close relatives of the bonnethead shark, are less common in public aquariums but have been successfully displayed in Asia, Europe, and the USA. Young, small (<1.0 m TL) sharks are the best candidates for transportation, and high dissolved oxygen levels (~85-120% saturation) are critical throughout (Young et al., 2002). If sharks are able to swim freely within the transport container, a 30-minute acclimatization period is possible on arrival. The skin of scalloped hammerheads is easily bruised and the location of their eyes, at the extremities of the "hammer" (i.e., cephalofoil), make them vulnerable to damage. Thus, no nets can be used and handlers must wear sterile latex gloves. In some cases, a soft wet protective cloth may be placed over the eyes during handling. Hammerheads should be moved using a rigid stretcher (e.g., a net stretched tightly over a PVC frame and covered with soft plastic). Introduction via a floating cage for specimens <1 meter TL is recommended. Animals should only remain in the floating cage for a sufficient period to allow their swimming patterns to normalize, before final release.

Oceanic whitetip sharks (*Carcharhinus longimanus*) have been maintained successfully in aquariums for up to two years (Hamilton, pers. com.; Uchida, pers. com.). Acclimatization for this species is secondary to the demands of its physiology and the requirement to swim unimpeded. The key factor for success with oceanic whitetip sharks is to minimize transport times and introduce specimens into their final exhibit immediately on arrival.

The great white shark (*Carcharodon carcharias*) is yet to survive in captivity for more than 17 days. Great care and minimal handling during capture and transport are critical to success. The shortest possible transport times are recommended, and once again, acclimatization is secondary to the animal's requirement to swim freely. It has been observed that this species does not respond well to physical obstructions within an exhibit, so personnel will be required to ward a new specimen away from the walls during the first few days. It is recommended that displaying great white sharks should not be attempted without adequate research, resources, and experience. The same general recommendation can be made for mako (*Isurus* spp.) and thresher (*Alopias* spp.) sharks. Although there have been some recent positive attempts at maintaining these species, no long-term successes have been recorded.

In general, feeding and lighting regimes during the introduction of obligate ram ventilators should be same as for non-obligate ram ventilators. These elasmobranchs require high concentrations of dissolved oxygen all the time. Constant monitoring and adjustment of oxygen concentrations should remain a priority throughout transport, acclimatization, and introduction.

"Walking" distressed elasmobranchs

If pelagic sharks have been excessively hyperactive or traumatized during transport, acclimatization, or introduction, they may rest on the bottom of the tank when released into the final enclosure. This is generally considered to be a warning sign that the animal is distressed and may be at risk of permanent or even fatal physiological changes. In particular, obligate ram ventilating animals cannot remain in this condition for any extended period, as they need to remain swimming to facilitate gas exchange and systemic circulation. If a pelagic animal is observed "resting" on the bottom, following introduction, it should be gently encouraged to swim as per its natural behavior. If the shark continues to stall and fall to the bottom, two courses of action are available. The first course of action is to "walk" the shark. This procedure requires a diver to walk

or swim beside the shark, while supporting it under the belly, and move it forward into the prevailing current. This process aids gas exchange, helps void metabolic toxins, and ultimately encourages the shark to start swimming by itself. There is some concern that the metabolic activity induced by "walking" elasmobranchs may compound post-transport trauma, in particular lactic acid accumulation (Stoskopf, 1993). However, the authors have found that "walking" an animal for 5-10 minutes, immediately after it has been removed from the confines of a transport container, can have a beneficial influence. In one case, a distressed lemon shark (*Negaprion brevirostris*), having blanched skin and an immobile trunk, was walked for over an hour with positive results. Should "walking" an animal yield no immediate reaction, it may be deemed appropriate to place the animal in front of an oxygen-rich (i.e., ~100-120% oxygen) stream of water and not disturb the animal further. This procedure will partially simulate ram ventilation and aid the animal in overcoming incurred acidosis.

It is quite common for sand tiger sharks to "rest" on the bottom after an arduous transport and this should not cause immediate concern, especially if color is normal (i.e., dark and homogeneous) and respiration is regular. This species is quite capable of buccal respiration and seems to benefit from a brief period of post-transport inactivity. In addition, if a sand tiger shark lies on the bottom it does not necessarily indicate buoyancy problems, unless a prolonged period of labored, non-horizontal swimming (i.e., with the tail down) has been observed.

Post-introduction monitoring

Once an elasmobranch has been introduced into a new exhibit, it should be carefully monitored for at least 24 hours. Signs of physiological complication (e.g., abnormal ventilation rates, unusual swimming behavior, etc.) should be assessed and corrective measures undertaken where deemed appropriate. It may be necessary to augment dissolved oxygen concentrations within the new environment for the first few days.

Close attention should be paid to resident animals to allow intervention should aggression or risk of predation become evident. In multi-species tanks this may be difficult or may even occur before preventative action can be implemented. In some cases it may be necessary to move other animals to a separate holding tank, allowing time for new animals to adjust to the exhibit without the complication of aggressive residents. An alternative option can be to divide an exhibit in half using a net or perforated plastic sheeting, which should be taut to prevent animals becoming entangled. Of course, it is preferable to avoid obvious compatibility problems during exhibit planning and by introducing animals in an appropriate sequence.

Personnel and SCUBA equipment should be prepared for direct intervention before new animals are introduced. Consideration should be given to the availability of alternative holding systems, should a compromised animal need to be removed. It is usually much easier to introduce an animal to a large exhibit than to subsequently remove it, so plan ahead in anticipation of having to safely remove a specimen where necessary.

In addition to medical considerations, newly acquired animals may need to acclimatize to new foods. Where possible, animals should be given the same foods they were eating before transportation began, and these foods should then gradually shift to match their long-term diet. A healthy appetite is a good indicator of a successful introduction. Animals conditioned to take food from aquarists are less likely to prey on other inhabitants, although this is no guarantee, and controlled feeding provides the opportunity for administering oral medications.

CONCLUSIONS

An aquarist intending to keep sharks, skates, or rays must be familiar with an elasmobranch's physiology and natural history. Only in this way can they accurately assess how well an animal is responding to its new environment. Observation and recognition of problems is critical. It is, of course, preferable to use this knowledge during exhibit design to facilitate species selection, habitat design, and formulate suitable transport, acclimatization, and introduction regimes. In a typical multi-species aquarium there will be many compromises, but a fundamental understanding of acclimatization and introduction strategies, will mitigate many of these negative effects. Acclimatization and introduction strategies however, should never be viewed as a solution to inadequate planning or inappropriate species selection.

ACKNOWLEDGMENTS

The authors wish to thank the following individuals for their contributions and support: Trevor Long, Jim McBain, David Powell, Andrew Routh, John Rupp, and Derek Spielman.

REFERENCES

Clark, E. 1963. The maintenance of sharks in captivity, with a report on their instrumental conditioning. *In:* Sharks and Survival, p. 115-149. P. W. Gilbert (ed.). D.C. Heath and Company, Boston, Massachusetts, USA.

Cliff, G., and G. D. Thurman. 1984. Pathological and physiological effects of stress during capture and transport in the juvenile dusky shark, *Carcharhinus obscurus*. Comparative Biochemistry and Physiology 78: 167-173.

Gruber, S. H., and R. A. Keyes. 1981. Keeping sharks for research. *In:* Aquarium Systems, p. 373-402. A. D. Hawkins (ed.). Academic Press, New York, USA.

Hewitt, J. C. 1984. The great white in captivity: A history and prognosis. *In:* AAZPA Annual Conference Proceedings, September 9-13, Miami, Florida, p. 317-324. American Association of Zoological Parks and Aquariums (American Zoo and Aquarium Association), Silver Spring, Maryland, USA.

Lowe, C. G. 1996. Kinematics and critical swimming speed of juvenile scalloped hammerhead sharks. The Journal of Experimental Biology 199: 2605-2610.

Murru, F. L. 1990. The care and maintenance of elasmobranchs in controlled environments. *In:* Elasmobranchs as Living Resources: Advances in Biology, Ecology, Systematics, and the Status of Fisheries, p. 203-209. H. R. Pratt, S. H. Gruber, and T. Taniuchi (eds.). U.S. Department of Commerce, NOAA Technical Report 90.

Noga, E. J. 1996. Fish disease: Diagnosis and treatment., Mosby-Year book Inc., St. Louis, Missouri, USA. 367 p.

Post, G. 1987. Textbook of Fish Health. T. F. H. Publications, Neptune City, New Jersey, USA. 288 p.

Smith, M. F. L. 1992. Capture and transportation of elasmobranchs, with emphasis on the grey nurse shark (*Carcharias taurus*). Australian Journal of Marine and Freshwater Research (Sharks: Biology and Fisheries) 43: 325-343.

Spotte, S. 1973. Marine Aquarium Keeping, The Science, Animals, and Art. John Wiley & Sons, New York, USA. 171 p.

Spotte, S. 1992. Captive Sea Water Fishes, Science and Technology. John Wiley & Sons, Inc. New York, USA. 942 p.

Stoskopf, M. K. 1993. Environmental requirements and diseases of sharks. *In:* Fish Medicine, p. 758-763. M. K. Stoskopf (ed.). W. B. Saunders Company, Harcourt Brace Jovanovich, Inc., Philadelphia, Pennsylvania, USA.

Thurston, R. V., and R. C. C. Russo. 1984. Chronic toxicity of ammonia to rainbow trout. Transactions of the American Fisheries Society 113: 56-73.

Young, F. A., S. M. Kajiura, G. J. Visser, J. P. S. Correia, and M. F. L. Smith. 2002. Notes on the long-term transportation of the scalloped hammerhead shark, *Sphyrna lewini*. Zoo Biology 21(3): 242-251.

PERSONAL COMMUNICATIONS

Hamilton, R. 2000. Monterey Bay Aquarium, Monterey Bay, CA 93940, USA.

Lai, D. 1999. Ocean Park, Hong Kong SAR, China

Long, T. 2003. Sea World Australia, Surfers Paradise, Queensland 4218, Australia.

Uchida, S. 1995. Okinawa Expo Aquarium, Okinawa 905-03, Japan.

The Elasmobranch Husbandry Manual: Captive Care of Sharks, Rays and their Relatives, pages 163-167.
© 2004 Ohio Biological Survey

Chapter 12
Diving with Elasmobranchs: Safety Protocols

VALLORIE HODGES

Oregon Coast Aquarium,
2820 SE Ferry Slip Road,
Newport, Oregon 97365, USA.
E-mail: edofleini@yahoo.com

JUAN SABALONES

Bass Pro Shops
2500 E. Kearney,
Springfield , MO 65898, USA.
E-mail: jasabalones@basspro.com

Abstract: Many aquariums displaying large elasmobranchs use SCUBA (or surface supply) as a means to perform certain essential husbandry tasks. If diving is to be employed, provisions must be made by the institution to ensure the health and safety of divers, support staff, and animals. These measures must include establishing and maintaining a dive program that is compliant with all appropriate government regulations and industry standards, and developing and implementing an institutional policy and safety protocols for diving with large elasmobranchs.

Diving with large elasmobranchs in an enclosed aquarium exhibit or holding tank, as compared to diving with them in the wild, presents a variety of logistical and safety challenges. In addition, the practice of diving, especially if it is done as a part of one's employment, is regulated by governmental, professional, and/or industry organizations. This chapter provides a brief overview of diving regulations, safety protocols, diving techniques, and other considerations for diving with elasmobranchs, especially large sharks.

DIVING REGULATIONS AND STANDARDS

The first step in establishing a diving safety program, under which diving with large elasmobranchs will occur, is to be fully compliant with any relevant regional, national, and international diving regulations. Since regulations can vary widely from country to country, it is incumbent upon administrators of diving safety programs to be fully conversant with any diving regulations for their region.

DIVING SAFETY PROTOCOLS AND TECHNIQUES

Although strict and safety-oriented, occupational diving regulations do not address the risks of diving with potentially dangerous animals. Provided dives comply with applicable regulations, safety protocols related to diving with large sharks should be established at an institutional level by developing a written diving safety policy and case-specific protocols. Diving safety policies should be formulated to reflect the specific requirements and conditions of the institution in question, should comply with accepted industry standards, and should be followed carefully once established. Protocols should be based on the individual needs of the institution and at a minimum, include an overview of activities while diving with large elasmobranchs, emergency care and evacuation procedures, and exhibit-specific requirements (e.g., window cleaning, general exhibit maintenance, staff training, specialized equipment use, record keeping, etc).

During the development of protocols, methods employed to ensure diver safety should be established and detailed. Choices range from protective cages or other types of rigid barriers, to barrier net systems, to teams of safety divers, or indeed, to no special precautions at all. The appropriate safety system will depend on tasks to be accomplished, the aggressiveness of shark species, the physical features of the exhibit, and other local considerations.

Protective cage

The use of a protective diver cage might be an appropriate choice for educational presentations or feeding of large sharks, where a single diver may not be able to focus on all animals. Cage systems, however, can be cumbersome to deploy, require significant above-tank supportive structures, are expensive, and may not allow complete access to the entire exhibit.

Barrier net

Barrier nets allow relatively unobstructed access to an exhibit, while separating divers from large elasmobranchs. Heavy, knotless, nylon netting (3-6 mm mesh size) is best suited to this application. Nets should be constructed to allow ~6.0 m of excess net, beyond the length or width of the exhibit, depending on the axis of deployment. The barrier net is usually fitted with a heavy lead line and double floatation line. An excellent example of a barrier net system is that employed at SeaWorld San Diego's (California, USA) Shark Encounter (Keyes, 1979). For more information about the use of barrier nets in large shark exhibits please refer to Chapter 20 of this manual.

One of the main disadvantages of a barrier net is the additional personnel required to prevent sharks escaping through gaps at the bottom and sides of the net, and to prevent animals from becoming trapped in loose folds of the net. Great care must be taken when using this method to ensure that animals do not become cornered and subject to unnecessary stress. The use of large barrier nets can present a significant safety hazard to divers, requiring personnel to take extra care and attention during net deployment and use. Another disadvantage of barrier nets is their tendency to get caught on, and possibly damage, exhibit décor (e.g., plastic replicas of corals, etc.).

Safety divers

Safety divers have been used, in various forms, by aquariums around the world. In general, one or more safety divers are positioned next to one or two working divers. The safety divers use "shark wands" to alert the sharks to diver presence. Shark wands are typically made from pieces of PVC pipe (1.0-1.5 m long x 25 mm in diameter). The ends of the pipe are smoothed (or partially covered with vinyl tubing) to minimize injury to animals, in the event of accidental contact. Shark wands are typically uncapped, allowing water to flow through the pipe and thus facilitate their movement through the water. In addition, shark wands are wrapped, in a spiral pattern, with a contrasting colored tape. Shark wands are presented to approaching sharks, warding them away from working divers and thus providing a safe working zone. Experienced and attentive safety divers should be able to readily guide sharks using this technique. The use of safety divers can be labor intensive for the divers, supervisory staff, and training staff. In addition, this system implies a considerable learning curve. However, the safety diver system is flexible, cheap, effective, and allows unimpeded access to the entire exhibit.

In addition to the primary safety systems described above, some aquariums insist on secondary safety systems. Examples include the use of thick full-body wetsuits (Violetta, pers. com.), or chain mail suits or gloves (Jewell, pers. com.), when diving with sharks.

DIVING SAFETY AND EXHIBIT DESIGN

Although the safety systems described above may be necessary for diving with large captive elasmobranchs, diving safety can be greatly enhanced by proper exhibit and holding tank design, and careful choice of exhibit species.

Species composition

Some elasmobranch species can pose a greater risk to divers than other exhibit animals—e.g., bull sharks (*Carcharhinus leucas*), oceanic whitetip sharks (*Carcharhinus longimanus*), tiger sharks (*Galeocerdo cuvier*), lemon sharks (*Negaprion brevirostris*), and hammerhead sharks (*Sphyrna* spp.). Relative risks should be carefully assessed by the institution prior to acquiring animals, if divers will be entering the exhibit and feeding dives are anticipated.

Exhibit depth, profile, and décor

A deep exhibit can pose potential safety problems if it exceeds 10 meters (i.e., two atmospheres) and divers perform repetitive dives throughout the course of their working day, as divers could potentially exceed their no-decompression limit. Exhibits or enclosures with depths >10 meters should have specific protocols encompassing repetitive diving safety considerations.

Constrictions within an exhibit, allowing limited diver access, should be avoided. If such conditions are created, specialized safety protocols should be developed and implemented. Facilities intending to use barrier nets for diver safety should design low-profile exhibit décor, minimizing potential net and shark entanglements. Net attachment points, to secure the barrier net during diving activities, should be considered.

Diver access

Diver access to an exhibit (i.e., entrance and egress) should be considered during exhibit design, for both regular and emergency access. Stairs or ladders leading directly into the exhibit, designed for divers wearing cumbersome and heavy gear, are preferred. Diver access may be through adjoining holding or isolation pools, with access points themed to disguise them from visitor view. Access, for emergency personnel, diver extraction, and ambulance proximity, must be included.

Emergency equipment

Emergency safety equipment, for use in the event of a diving accident or injury, must be provided (e.g., a diver alarm accessible from the water, telephones within the immediate vicinity, oxygen administration equipment, shepherds hooks, stretchers, etc.).

TYPICAL DIVING ACTIVITIES

When diving with sharks, divers are engaging in behavioral modification and are thus effectively training the sharks (for more information about training please refer to Chapter 13 of this manual). For this reason it is important that diver behavior within the exhibit is consistent wherever possible. In all cases, caution must be exercised when diving with sharks as these animals have a high degree of maneuverability and, in some cases, can inflict serious injuries to the unwary diver. Diving activities in elasmobranch exhibits generally fall into one of the following categories: (1) feeding, (2) exhibit maintenance, (3) repair, (4) veterinary, (5) educational, and (6) guest. Diving safety plans should incorporate an overview of any activities that will be performed while diving, and in all cases, an institutionally-appointed diving safety officer must ensure that appropriate documentation, planning, and safety protocols are employed.

Feeding dives

There are different opinions as to the wisdom and safety of divers feeding large elasmobranchs. The principal concern is that sharks may associate divers with food, increasing the level of risk to divers at non-feeding times. There is some validity to this concern; however, many institutions have used divers to feed their large sharks without incident. The decision to feed sharks while diving should be carefully considered (based on species behavior, number and size of specimens, exhibit design, etc.) and a sound institutional policy adopted.

If feeding of elasmobranchs is to be performed by divers, the aquarist responsible for the exhibit should prepare an overall feeding plan for the dive team, taking into account changing shark behavior, desired nutritional content and amount of food for each specimen, etc. If possible, a separate observer should be used to monitor feeding sessions, recording food consumed, shark behavior, etc. Divers should always be prepared to adjust to changing conditions and terminate a feeding dive as necessary.

A minimum of two divers should be used, one feeder and one observer/safety diver, when feeding shark populations considered to be of low risk. Additional diving personnel may be considered necessary as feeding sessions become more intense or complex. Two separate feeding stations (each with one feeder and one observer/safety diver) may ease pressure on a single feeding station, depending on the size of the exhibit, the number of sharks, and the species involved. Setting up feeding stations on the bottom of an exhibit (as opposed to mid-water) decreases the number of blind spots, enabling divers to better monitor approaching sharks. Using a wall or other vertical feature will similarly improve diver security. It may be helpful to feed from the surface

before divers enter an exhibit, or alternatively once they have reached a feeding station, to reduce pressure on the station.

Food containers typically used by divers while feeding sharks include plastic Ziploc® bags (SC Johnson and Sons, Racine, Wisconsin, USA), mesh cloth bags, and clear plastic cylinders. The important feature of each is that divers can see how much food is left, animals cannot easily get to food if the container is left unattended, and the container is not buoyant. A popular feeding container consists of a clear acrylic cylinder with two thin sheets of neoprene stretched over an open end. Both sheets of neoprene cover a little over half of the opening, creating a flap through which the divers may push their hand, but preventing food from drifting out.

Gloves are recommended while feeding sharks and are often required for insurance purposes. Regular neoprene dive gloves seem to work best. Heavy leather gloves, or chain mail boning gloves, may be used, but impair the ability to feel food items. Feeding tongs or poles may be indicated in situations where animals are too cautious to approach divers. Feeding poles can provide a higher degree of safety, distancing overzealous sharks from divers.

Exhibit maintenance dives

Exhibit maintenance dives are routinely performed for the purposes of maintaining a healthy environment for captive animals and an aesthetically pleasing environment for visitors. The type and frequency of exhibit maintenance dives vary between exhibits, depending on exhibit size, biomass, water sources, water temperature, life support systems, types of lighting, etc. Examples of maintenance tasks include, the removal of undesirable algal species from exhibit surfaces, siphoning or blowing debris off exhibit décor, cleaning detritus off the substrate, etc. As a general rule, maintenance hoses should be weighted so they remain on the bottom of the exhibit. This precaution reduces the risk of entangling pelagic elasmobranchs. Of course care must be employed when moving weighted equipment around exhibit décor. Regardless of the type of maintenance task, it is important to monitor elasmobranchs for adverse reactions to equipment and take preventative measures should such reactions be observed.

Repair dives

Repair dives refer to specialized activities undertaken while diving (e.g., replacing or repairing broken décor, polishing scratches in acrylic windows, etc.). While this typically involves the use of simple tools, it may be necessary to use specialized equipment or materials. It is important to review the nature of these dives, and the tasks and tools to be used, and determine whether they should be considered commercial diving activities and/or whether additional training may be required. Some underwater repairs (e.g., underwater repair of life support systems, underwater repair of leaks, etc.) may fall outside the scope of scientific diving regulations.

Veterinary dives

On occasion, large elasmobranchs have to be restrained for veterinary purposes. These procedures need to be accomplished with a high degree of safety and minimum of stress to both animals and staff. Some exhibits are designed with a system of surface controlled nets or gates, used to herd sharks into holding or isolation pools (Keyes, 1979). In this case, divers are only required to handle sharks once they are in the holding pool, and later to guide specimens back into the exhibit. In other cases, divers may be required to guide sharks from the exhibit into the holding pool. Herding “boards,” fabricated from PVC pipe frames covered with plastic mesh, may be used by two or more divers to guide sharks into the holding pool.

Staff at Sea World Australia (Surfers Paradise, Queensland, Australia) have successfully used a shark-shaped clear acrylic box (and/or a clear vinyl bag) to directly capture and handle large sharks (e.g., sand tiger sharks, *Carcharias taurus*) underwater (Long, pers. com.). Once caught, animals can be readily moved to any part of the exhibit or even a holding pool. For more information about restraining large elasmobranchs please refer to Chapter 20 of this manual.

In some rare cases, it may be necessary for divers to administer medications (e.g., pills in food, intramuscular injections, etc.) to free-swimming sharks. Such activities should only be conducted by trained personnel, and under the guidance of an experienced veterinarian.

Educational dives

Educational dives are conducted primarily to provide an educational or interpretive experience for aquarium visitors, and may include a shark feeding display. In some cases divers interact with the public by making a presentation using underwater communication equipment, which may be hard-wired or wireless. Generally speaking, hard-wired systems provide superior sound reproduction, while wireless systems don't require a tether to the surface. The diving safety officer should be involved in the selection and implementation of any underwater communication equipment. The reaction of elasmobranchs to communication systems should be closely monitored and their use discontinued if stress is evident. In lieu of voice communication, it is possible to make presentations using underwater slates or hand signals, with the help of staff in the public space. Divers may be asked to field questions from the public. It is important to consider and allow for the safety implications of this distraction, from the activity of nearby sharks.

Guest dives

There are occasions when it serves the interest of the institution to issue temporary dive permits to external personnel (e.g., underwater cinematographers, celebrity VIPs, etc.). Regardless of the activity, it is important to establish a written policy, complying with accepted industry standards, and then strictly adhere to the policy. Some regional standards, for example, provide specifically for this eventuality having a *temporary diver permit* category. Temporary diver permit holders must be provided with an adequate pre-dive briefing, including, standards of behavior when diving with large elasmobranchs, emergency procedures, and any other safety issues. A checkout dive, to ascertain the guest diver's competency, is strongly advised. Regular diving staff should provide assistance, as needed, for in-water supervision.

Film crews tend to use hot lights and a lot of electrical equipment, creating potentially dangerous situations. Safety, as always, is paramount. Film crews will expect spotless windows, crystal-clear water, demonstrations of typical elasmobranch behavior, large densities of animals "in-shot," extended filming times, repeated takes, and sometimes, expect animals to appear on cue. Responsible staff should be polite and clear about the limitations of these expectations.

ACKNOWLEDGEMENTS

The authors wish to thank the following for their extensive support, contributions, and assistance: Frank Bulman (Ripley Aquariums), Joe Choromanski (Ripley Aquariums), João P. S. Correia (Oceanario de Lisboa), Michael Farquhar (Two Oceans Aquarium), Andreas Fisher (Underwater World), Oliver Franklin, Suzanne Gendron (Ocean Park Hong Kong), Jeff Gibula (Newport Aquarium), Claudia Gili (Acquario di Genova), Heather Hall (Zoological Society of London), Tim Handsel (Ripley Aquariums), John Hewitt (Aquarium of the Americas), Bruno Lanman (SCUBA Unlimited), M. Manqué, B. T. Moose, Murray Phillips, Jen Rehberger (SCUBA Unlimited), Malcolm Smale (Port Elizabeth Museum), Les Thomas (Squid, Inc.), Lori Thomassen (SeaWorld Orlando), and Matt Walker (Blue Planet Aquarium).

REFERENCES

Keyes, R. S. 1979. Description of live shark exhibit and research center, Sea World San Diego. San Diego Research Center Report 79(1). 48 p.

PERSONAL COMMUNICATIONS

Fischer, A. 2000. Underwater World, Mooloolaba, Queensland, 4557, Australia.

Jewell, J. 2004. Shark Reef at Mandalay Bay, Las Vegas, NV 89119, USA.

Long, T. 2004. Sea World Australia, Surfers Paradise, Queensland 4218, Australia.

Violetta, G. 2004. Sea World, Orlando, FL 32821, USA

The Elasmobranch Husbandry Manual: Captive Care of Sharks, Rays and their Relatives, pages 169-182.
© 2004 Ohio Biological Survey

Chapter 13
Learning and Behavioral Enrichment in Elasmobranchs

JUAN SABALONES

Bass Pro Shops,
2500 E. Kearney,
Springfield , MO 65898, USA.
E-mail: jasabalones@basspro.com

HANS WALTERS

Wildlife Conservation Society,
New York Aquarium,
Boardwalk at West 8th Street,
Brooklyn, New York 11224, USA.
E-mail: hwalters@wcs.org

CARLOS ALBERTO BOHORQUEZ RUEDA

Oceanario Islas del Rosario,
P. O. Box 7877, Cartagena, Colombia.
E-mail: oceanario@ctgred.net.co

Abstract: Professionals who care for sharks, skates, and rays in controlled environments must become knowledgeable about the biology and behavior of the animals within their care and develop a familiarity with the behavior of individual specimens. Natural behaviors can be encouraged by the implementation of behavioral enrichment programs, including animal training through operant conditioning. Training programs can reduce stress in situations where animals must be moved or handled, assist with basic husbandry procedures, and enhance public and educational displays. Since these factors contribute to improving the overall health and welfare of animals in controlled environments, animal training through operant conditioning is an important enrichment tool for facilities that exhibit elasmobranchs.

It was long believed that the behavior of sharks and rays was driven solely by basic, instinctive responses. However, there is a great deal of scientific evidence to suggest that this view is an oversimplification. As a group, elasmobranchs exhibit relatively complex behaviors. It is known that elasmobranchs communicate and interact with conspecifics and other species (Bres, 1993) and some elasmobranchs demonstrate social behaviors, including dominance hierarchies.

There is great educational benefit to be gained by presenting the rich behavioral repertoire of elasmobranchs. One of the primary aims of modern zoos and aquariums is to present species as they would be seen in nature, with as many natural behaviors as possible. In addition, one of the common gauges for measuring the success of an animal's acclimatization to controlled environments is its continued display of natural behaviors. However, aquarium exhibits are not an exact reproduction of the natural environment and it is to be expected that the behavior of captive animals will differ from their wild counterparts. The degree to which the behavior of these two groups diverges can be reduced by the implementation of an environmental and behavioral enrichment program. The intent of such programs is to provide

animals with opportunities to engage in and display natural behaviors, thereby positively affecting the overall health and welfare of the animals.

ENRICHMENT

Sheperdson et al., (1998) defines environmental enrichment as "...an animal husbandry principle that seeks to enhance the quality of captive animal care by identifying and providing the environmental stimuli necessary for optimal psychological and physiological well-being. In practice, this covers a multitude of innovative, imaginative, and ingenious techniques, devices, and practices aimed at keeping captive animals occupied, increasing the range and diversity of behavioral opportunities, and providing more stimulating and responsive environments..."

Success in animal care and exhibition is commonly measured by parameters such as breeding, longevity, and observations that the animal is demonstrating normal or natural behaviors. In this context, it is in the best interests of the animals if exhibit design enables and encourages as many natural behaviors as possible. In an elasmobranch exhibit, examples of such techniques may include varying temperature gradients, moving underwater structures, hiding pieces of food in different parts of the tank, changing water flow and/or adding currents, and adding live prey or providing food to benthic animals at the bottom of the tank (e.g., via an underwater feeding device) rather than introducing food at the surface. For many elasmobranchs, the natural environment is often quite dynamic. Therefore, the addition of novel objects, such as those frequently provided to marine mammals in controlled environments, can have an enrichment value. While sharks are unlikely to exhibit playing behavior (as observed in marine mammals), novel objects—either floating at the surface, within the water column, or lying on the bottom—can disrupt stereotypic swimming patterns. Besides exhibit design, other factors (e.g., feeding techniques, lighting regimes, etc.) can impact captive behavior and therefore offer opportunities for enrichment.

Providing enrichment for elasmobranchs and achieving or increasing the incidence of natural behaviors can be a challenge. What precisely constitutes natural behavior for elasmobranchs is often subjective and open to debate. Their elusive nature and the constraints imposed by the ocean environment make elasmobranchs difficult to observe under natural conditions. Prior to initiating a training program, aquarists should learn as much as possible about the natural history, biology, and behavior of the animals within their care. Consistent observation of the animals will facilitate this understanding. One of the most important starting points in evaluating behavior, and therefore in developing a good enrichment program for any species, is to understand the sensory systems of the animals in question.

ELASMOBRANCH SENSORY SYSTEMS

The highly developed sensory systems of sharks and rays have an important influence on their behavior and learning capability. Elasmobranchs have the same senses as humans (i.e., vision, smell and taste or chemoreception, and hearing and touch or mechanoreception) as well as an additional sense (electroreception).

Vision

Sharks have highly developed vision (Gruber and Cohen, 1978) capable of perceiving a wide range of wavelengths and tolerating different light intensities (Hueter and Gilbert, 1990). The rod-packed retina of elasmobranchs indicates a high sensitivity to light stimuli, a valuable adaptation to low-light conditions (Hueter and Gilbert, 1990). An abundance of retinal cones in several species suggests the existence of color vision, although this has not yet been proven (Gruber and Cohen, 1978). Behavioral studies have concluded that sharks can differentiate between contrasting colors (i.e., between a light and dark color) (Greenberg et al., 1981).

Chemoreception

Elasmobranchs have a particularly acute sense of smell (Tester, 1963). For example, lemon sharks (*Negaprion brevirostris*) and nurse sharks (*Ginglymostoma cirratum*) are sensitive to electrolytes, amino acids, and amines, capable of detection at concentrations of ~1 mg l^{-1} (= 1 ppm). In addition, these sharks can readily distinguish waters of different salinities (Hodgson and Mathewson, 1978). Taste buds have been described in the mouth and throat of some species of elasmobranchs, and food preferences observed (Bunting, 1979).

Mechanoreception

Rheotaxis (i.e., movement of an organism in response to a current), predator avoidance, schooling behavior, social communication, prey detection, water movement detection, and hydrodynamic imaging are mediated by the mechano-sensory system of elasmobranchs, or the lateral line (Roberts, 1972; Popper and Fay, 1977; Roberts, 1978; Corwin, 1981; Tricas, 2001). The system consists of two specialized nerve terminals, the sensory hair cells of the lateral line, a number of free neuromasts or pit organs scattered over the head and body, and the inner ear.

Acoustic stimuli have an important influence on elasmobranch behavior. The inner ear of elasmobranchs has a structure much like that of bony fishes, except for an enlarged macula neglecta in species that actively hunt. Since sedentary, benthic feeders possess a relatively small macula neglecta, this structure is probably involved in detecting and localizing distant sound sources (Bleckmann and Hofmann, 1999). The hearing range of sharks is 10-800 Hz (cycles per second), while the hearing range of an adult human is 25-16,000 Hz. Sharks can thus detect lower frequencies than humans, while humans can detect higher frequencies than sharks (Myrberg, 1978). Low-frequency vibrations, such as those produced by a struggling or distressed fish, can be attractive to nearby sharks. Most naturally-occurring sounds attractive to elasmobranchs have a detection threshold of ~25-30 m. Loud, artificially-produced sounds may attract or repel sharks from beyond that limit. Sharks can learn to ignore a sudden increase in sound intensity (Eibl-Eibesfeldt and Hass, 1959; Myrberg et al., 1978; Klimley and Myrberg, 1979).

Electroreception

Sharks can detect bio-electrical fields emanating from prey with voltage gradients as low as 0.01 V cm^{-1} (Kalmijn, 1966; Kalmijn, 1974; Kalmijn, 1981). The ampullae of Lorenzini, located on the head of elasmobranchs, constitute the receptors for this remarkable detection system (Dijkgraaf and Kalmijn, 1963; Murray, 1974). Field experiments with dusky smoothhounds (*Mustelus canis*), blue sharks (*Prionace glauca*), and swell sharks, (*Cephaloscyllium ventriosum*) have demonstrated that all these animals can detect prey using electroreception (Kalmijn, 1978, Heyer et al., 1981; Tricas, 1982). Electroreception is only effective for prey detection at close range, the magnitude of bio-electrical fields falling off steeply, to below threshold sensitivity, beyond a meter.

An elasmobranch swimming at ≧ 2.0 cm $second^{-1}$ through the earth's magnetic field will induce a detectable voltage gradient of 0.05-0.5 V cm^{-1}, dependent on the direction of movement. This phenomenon endows elasmobranchs with an internal geomagnetic compass and may aid animals during long-range migrations (Kohler et al., 1998). Haller's round ray (*Urolophus halleri*) is capable of geomagnetic orientation (Kalmijn, 1978; Kalmijn, 1984).

LEARNING IN ELASMOBRANCHS

In addition to highly developed senses, it has been demonstrated that the brain size of many elasmobranchs is comparable to that of birds and mammals (Northcutt, 1977; Northcutt, 1978). This finding is at odds with the claim that elasmobranchs have comparatively small brains, a misconception that stems from early studies of relatively primitive species—i.e., the spiny dogfish (*Squalus acanthias*) and the smallspotted catshark (*Scyliorhinus canicula*).

In the wild, reef sharks have learned to associate the noise of boat engines and divers entering the water with food (Jensen, pers. com.). Elasmobranchs in captivity quickly learn the location of feeding stations and the timing of regular feeding sessions. Rays and skates may learn faster than most bony fishes, at a rate comparable to white rats and pigeons (Alston et al., 1987).

In early studies involving the operant conditioning of nurse sharks and lemon sharks, specimens were successfully trained to press a target in return for food (Clark, 1959; Clark, 1963). Additional studies have demonstrated the capacity for visual and acoustic discrimination in lemon sharks (Clark, 1961; Banner, 1967; Nelson, 1967) and visual discrimination in nurse sharks (Graeber and Ebbesson, 1972), including light-dark discrimination in juveniles (Aronson et al., 1967). More recently it has been shown that some sharks can be taught to distinguish patterns presented on targets (Greenberg et al., 1981). Gruber and Schneiderman (1975) used classical conditioning to elicit a response in the nictitating membranes of young lemon sharks.

BEHAVIORAL MODIFICATION THROUGH TRAINING

Many benefits can be achieved through the implementation of animal training programs, including: (1) enrichment, or the physical and mental stimulation of trained animals; (2) better control and monitoring of animals during feeding sessions, particularly when many animals are involved; (3) reduced stress to animals while handling during transports, veterinary examinations, and in-house research; (4) the implementation of advanced husbandry techniques; (5) enhanced educational presentations; and (6) the development of a positive association between animals and caretakers, facilitating acclimatization to new environments (Baker, 1991).

It has been known for many years that sharks and rays are responsive to conditioning techniques. It is therefore surprising that many facilities exhibiting elasmobranchs have not made more use of this tool. It is possible that elasmobranchs were regarded as un-trainable and that behavioral modification techniques were not taught to aquarists. Thus, the current lack of formal training is probably a result of ill-defined objectives, unstructured programs, and insufficiently-trained personnel, rather than un-trainable animals. Indeed, aquarists regularly train elasmobranchs without specific intent. Pole-feeding sharks and rays is a simple example of conditioning. Increased elasmobranch activity at a feeding station, prior to regular feeding times, is another conditioned response.

Behavior modification is the process by which a subject's responses to a stimulus are altered or changed by successively reinforcing certain aspects of a targeted behavior (Scardina-Ludwig and Messinger, 2001). Operant conditioning (= instrumental conditioning) is a form of behavior modification in which behaviors are altered primarily by regulating the consequences that follow them. Thus, operant conditioning is a learning process by which a subject produces a behavior (called a response) in the presence of some kind of cue (called a stimulus) on the condition of achieving desirable (or avoiding undesirable) consequences. Appendix 13.1 summarizes the basic terms and concepts of operant conditioning.

Animal training, through the use of operant conditioning, has been a crucial component of the husbandry of marine mammals (e.g., cetaceans, pinnipeds, etc.) for a number of years. More recently, modern zoological institutions have established operant conditioning protocols for birds, reptiles, invertebrates (e.g., octopus), and terrestrial mammals as part of their behavioral enrichment programs. These programs rely primarily on the positive reinforcement of successive approximations to a behavioral goal or goals. The basic concepts of operant conditioning remain the same whether applied to marine mammals, elasmobranchs, or any other animal. The implementation of an effective program requires both a good understanding of the principles of operant conditioning and a thorough knowledge of the natural history, sensory capabilities, and behavior of the species to be trained. Appendix 13.2 provides a list of resources that will assist the reader in learning more about the basics of operant conditioning, and the biology and behavior of elasmobranchs.

Behavior modification is both an art and a science. A little knowledge in inexperienced hands can be counterproductive, so those wishing to start an operant conditioning program should communicate with experienced personnel (Appendix 13.3). Trained behavior requires constant and continued reinforcement. It is therefore imperative that institutional commitment and support are ensured before a training program is implemented.

DEVELOPING A TRAINING PROGRAM

The American Zoo and Aquarium Association (AZA) recommends a six-step process for establishing an animal training program. This process was first developed by Mellen and MacPhee (www1) and can be remembered by the acronym SPIDER:

1. ***S: Set goals***. Review and assess the natural history of the species, the history of the individual (e.g., medical, breeding, etc.), and possible exhibit constraints. The stimulus used to elicit a behavior is most effective when the capabilities and limitations of the subject have been considered. An animal with compromised vision is a poor subject for visual stimuli; auditory or tactile stimuli may be more effective. Assess the potential benefits of training to the animal, husbandry staff, and the facility itself. Be specific and realistic about the results you want to achieve (e.g., when targeting a shark, do you want the shark to: (1) remain near the target; (2) touch the target; or (3) hold on target until cued to release).

2. ***P: Plan.*** Carefully plan the training of the behavior. Break the behavior into small steps or approximations. Approximations should not be too large and thus frustrate the animal, nor too small and waste time. Establish a schedule and set dates to achieve each approximation. Dates can be adjusted during training, but there should be an initial plan. Define everyone's role (e.g., primary trainer, backup trainer, supervisor, etc.) and ensure that all personnel know their responsibilities (i.e., who will do what and when, etc.). Consider required resources (e.g., facilities staff to build training platforms, additional staff to distract/feed other animals during training sessions) and ensure they are in place before training commences. Set dates for all participants and make sure they are in agreement.

3. ***I: Implement the plan.*** Execute the plan. Keep things as simple as possible and use common sense. Be patient. Training animals through the use of operant conditioning takes time and dedication.

4. ***D: Document results.*** Keep records of training progress. Record keeping is an area that is frequently neglected, or alternatively, an area where time and effort are wasted on excessively elaborate records. Neglected records are usually the result of a limited schedule. Over-elaborate records, where a trainer has painstakingly recorded every detail, are time-consuming, tend to be written in subjective terms, and, paradoxically, are often difficult to interpret. The use of pre-designed training record sheets will increase efficiency and produce standardized data, comprehensible to backup trainers and supervisors.

5. ***E: Evaluate results regularly.*** Determine in advance how frequently results should be evaluated (i.e., weekly, bi-weekly, etc.). Adhere to your evaluation schedule. Failure to evaluate training progress will impede training effectiveness. If a team of individuals is training an animal, regular scheduled meetings are essential.

6. ***R: Re-adjust.*** Make changes to plans based on successes, or lack thereof. Training requires a great deal of patience; however, it is pointless to stick with a plan when it is not working. Stay flexible and be prepared to modify the plan.

CASE STUDIES

While the training of sharks and rays has been somewhat limited, a number of workers have achieved impressive results. These activities merit further discussion and are representative of training options for facilities exhibiting elasmobranchs. Training terminology is used frequently throughout these case studies. For clarification of these terms, the reader is directed to Appendix 13.1.

1. Target training a sand tiger shark

Workers (Colleen Bronstead, Elizabeth Fincher, and John Rupp) at the Pacific Rim Center for elasmobranch studies, Point Defiance Zoo and Aquarium, USA, used operant conditioning to train a sand tiger shark (*Carcharias taurus*) to make snout contact with a target. Prior to the commencement of training, Rupp (pers. com.) conducted a two-year observational study to determine the baseline behavior of the target animal: an immature (203 cm TL) female sand tiger shark housed in a controlled environment for 10 years. Swimming speed, direction, and depth, along with attitude in the water, ventilation patterns, and interactions with other animals (both in holding and on exhibit) were carefully documented. Water quality parameters were recorded (e.g., temperature, salinity, etc.).

The target consisted of a Masonite board (30 cm x 30 cm x 3 mm thick), half black and half white, attached to a 1.5 m long x 12.5 mm diameter PVC pipe. Trainers desensitized the shark to the presence of the target, by presenting it at a distance of 1.5-2.5 m, as she swam in her normal counterclockwise pattern around the tank. Behavior consistent with the shark attempting to avoid the target was not observed during desensitization trials. Target presentation was then paired with a primary reinforcer (i.e., a food reward). Each training session lasted ~10 minutes. The sand tiger shark was positively reinforced for successive approximations toward the desired behavior (i.e., recognition of and approach toward, and finally snout contact with, the target). Training sessions took place during non-feeding times (for the rest of the collection) to avoid confusion and interference with the other animals. A total of 38 training sessions were conducted during a period of ~3 months.

The training team met with limited success when only using a primary reinforcer. When they raised their criteria, by reinforcing the shark only when

she made direct snout-contact with the target, the shark began avoiding the target area. After consulting a behavioral expert, they found that the frequency of correct responses might be increased by the addition of a bridging stimulus (or bridge) in the form of an auditory cue. The chosen bridge was a 6.0 Volt door buzzer, encased in a plastic cylinder. A hydrophone placed near the buzzer enabled the trainers to accurately monitor the delivery of the bridge. For two weeks the buzzer was paired with food, during normal feeding times, without the target present, resulting in the buzzer becoming a secondary reinforcer for the shark. The target was then reintroduced into the training program and the shark bridged and fed for successive approximations toward snout-contact with the target. Trials occurred over the course of one month, ending when the shark showed a 100% success at bumping the target. The target had become the discriminative stimulus (SD), and the targeting behavior under stimulus control.

Following a 5-week interval, when no trials were conducted, the target was reintroduced to the sand tiger shark during normal feeding times. The shark was bridged and fed for appropriate responses. Interference from other animals was minimized by feeding them at different stations, and only when the target was not present. The sand tiger shark displayed an 85% success rate during the trials conducted after the 5-week interval, suggesting that the sand tiger shark was capable of retaining learned behavior for several weeks (Rupp, pers. com.).

In accord with SPIDER, the training team set clear goals; after making careful observations, developed a cohesive training plan, which was carefully implemented; and, documented their results. When encountering a setback, the team consulted a behavioral expert, evaluated their results, and re-adjusted their training plan accordingly.

2. Guiding a sandbar shark

A worker (Larry Rutherford) at the Living Seas Pavilion, Walt Disney World, USA, used operant training techniques to condition a sandbar shark (*Carcharhinus plumbeus*) to come to a target and receive food, and ultimately, to be led to preferred parts of the exhibit (Rutherford, pers. com.).

Routine and repetition are important when training any behavior. Rutherford (pers. com.) stationed the shark at the same time and same place, and used the target during every feeding session. The target was made from a 1.2 m length of 10 cm diameter PVC pipe. At the beginning of each feeding session, the target was suspended in the water, at a specific location, where it became the visual stimulus or SD. The shark was reinforced, using food fish delivered with aluminum tongs, if and when it swam toward the target. If the shark did not approach the target within 10 minutes, predominantly because the animal was elsewhere in the exhibit, Rutherford would rap the pipe with a wooden pole and the gonging sound would usually elicit a positive response in the target shark. Once the behavior was learned, it became possible to move the target and lead the animal to a different part of the exhibit. Rutherford's (pers. com.) ultimate goal was to use this technique to draw the sandbar shark to an area where it could be readily captured for physical examinations.

3. Training sharks to use a feeding station

Rutherford (pers. com.) conditioned a bonnethead shark (*Sphyrna tiburo*) and blacknose shark (*Carcharhinus acronotus*) to feed at specific stations within an exhibit at the Living Seas Pavilion. The sheer size of the exhibit, and the voracious nature of the collection, made it difficult to locate small sharks and ensure they received their daily food ration. For this reason the bonnethead and blacknose sharks were trained to come to specific feeding stations as per the sandbar shark mentioned in case study 2.

Targets consisted of 1.2 m lengths of 10 cm diameter PVC pipe, painted with black vertical stripes. Stationing was established in quarantine, before the animals were introduced into the main exhibit, and was retained by the sharks following their transition into the new environment. The sharks were coming to the targets, during feeding sessions, within a week of transfer. Shortly thereafter, the sharks would approach a target as soon as it was lowered into the water, even pushing it around to receive their reward. Rutherford (pers. com.) attributes his success to working with the animals shortly after they were acquired, while they were still in quarantine. Rutherford (pers. com.) believes that almost any type of target would suffice, although PVC pipe was inexpensive, low tech, and worked well.

4. Acclimating lemon sharks to tactile stimuli

A worker (Robert Siders) at the Theater of the Sea, Islamorada, USA, conditioned a group of four

lemon sharks to feed at a specific station, and ultimately, accept tactile stimulation without adverse reaction (Siders, pers. com.).

Prior to training, the sharks were broadcast fed, but it was difficult to monitor food intake for each animal and Siders wanted to find a way to feed the animals individually. By observing the animals carefully it was noted that the sharks would swim single-file, in a clockwise circular pattern around the perimeter of the tank and past the feeding dock, when public entered the area for feeding demonstrations. It was believed that vibrations caused by the arrival of the public cued the sharks.

To condition the sharks to feed at the dock an olfactory stimulus was used. A hollow plastic ball, with two holes, was attached to the end of a ~45 cm long stick, the holes filled with fish, and the ball placed in the water. Sharks were rewarded with food when they oriented toward the ball. By approximations, the ball was moved toward the desired feeding station and the sharks conditioned to eat from the end of the dock. Each shark could then be fed individually and its food intake monitored and recorded.

Once trained to station, Siders conditioned the lemon sharks to accept tactile stimulation with the intent of reducing stress during handling. The sharks were acclimated to human presence through a number of steps, including: (1) food dropped in the water; (2) food held using long, blunt, forceps; (3) food presented by hand; (4) hand-feeding from the dock; (5) hand-feeding with legs dangling in the water (when a shark swam too close, it was nudged away with a stick); and (6) hand-feeding while standing in shallow water beside the dock. The solid dock prevented sharks from turning around and approaching the person in the water from their blind side. As sharks swam by and accepted food, they were briefly and gently touched. Over time, touching was steadily increased in duration and intensity (i.e., rubbing and scratching, etc.) until it was possible to gently hold the sharks by the tail and temporarily halt swimming. Sharks would resume normal swimming when released. Siders (pers. com.) reported that one female shark would swim to him between feeding sessions, to be scratched and rubbed, without food reinforcement. It is possible that the shark accepted touch as a secondary reinforcer, possibly facilitated by the fact that lemon sharks are accustomed to rubbing their bodies on the bottom to dislodge parasites.

5. Enhancing a display of nurse sharks

One of the authors (Rueda), at the Oceanario Islas del Rosario, Colombia, developed a public exhibition of trained nurse sharks. Many of the sharks had been at the facility for over 15 years before the training program was initiated. All of the sharks were well acclimated to their controlled environment, growing and reproducing normally without health problems.

The following guidelines (in accord with SPIDER) were used to establish the training program: (1) work with individuals that are easy to recognize; (2) identify dominance hierarchies and/or other social behaviors; (3) select target animals and determine their typical behavior(s); (4) establish and maintain a stable environment within which to train; (5) clearly define desired behavior; (6) break the desired behavior into a series of discrete steps; and (7) define a clear training schedule.

Many of the sharks were easily recognizable, having distinct marks on their dorsal fins, pieces of dorsal fin missing, etc. Those sharks that were more difficult to identify by size, or unique marking, were marked with a plastic tag on the second dorsal fin.

The nurse sharks were social. They frequently segregated by age, older animals gathering under a small platform in a deeper part of the exhibit, often on top of one another. The juveniles rested together in a shallower part of the exhibit, but always side-by-side and not as close to one another as the older individuals.

The largest male shark was probably dominant, always reaching the feeding platform first and using his strength to keep the other sharks behind and underneath him. Initially, this shark was considered to be a poor subject for training as his sheer bulk made it difficult to balance the floating platform where training would take place. However, the large male was persistently curious, extremely cooperative, tolerant of handling, and very food-motivated. All of these qualities made him a good subject for training.

Prior to formal training, shark feeding and educational presentations were performed from a floating platform at 08:00 and 14:30. It was considered the most unambiguous strategy to make these presentations training sessions as well. A public address system was used to signal

the beginning of presentations, but was never used during the actual sessions themselves. The sharks were cued with hand signals and the SD delivered exclusively by the right hand of the trainer. At all times the presenter and trainer remained in a crouched position.

One of the educational messages communicated during the presentation was the abrasive nature of shark skin. This was demonstrated by having a shark come out of the water onto the training platform and remain on the platform for 90 seconds while the presenter rubbed a piece of fish on the shark's back. When complete, a hand signal (SD) would cue the shark to re-enter the water.

The large male nurse shark was chosen as the animal to be trained. As the large male usually fed near the platform, it was easy to condition an association between the platform and food. Once again, the behavior was trained through approximations. Food was initially presented adjacent to the platform, then at the top edge of the platform, and ultimately, on the top of platform itself. Eventually, the shark was taking food, on the platform, with a large proportion of his body out of the water (Figure 13.1). Initially, the large male was fed as soon as he reached the desired position on the platform. However, when stationing consistently, the trainer began to extend the time period before the shark received his fish reward. Once the shark consistently stationed on the platform for the desired duration, the trainer desensitized the shark to tactile stimulation (in preparation for the fish rubbing demonstration). For the final step, the trainer would gently push the shark back into the water using his left hand, while giving the desired hand signal with his right hand. By degrees, the presenter softened the push and eventually only a hand signal was required for the shark to re-enter the water.

On training days, between three and seven training sessions were conducted. Training sessions were short, usually lasting 3-10 minutes each. Days without dedicated training sessions did not negatively influence trained behaviors, as public presentations functioned as training sessions and maintained the criteria of trained behaviors.

6. Acclimating sharks to clinical procedures

Workers from International Zoological Applications (IZA) successfully trained a group of nurse sharks, southern stingrays (*Dasyatis americana*), and chupare stingrays (*Dasyatis schmardae*) in a variety of husbandry behaviors at Parque Nizuc, Cancun, Mexico. These behaviors both enhanced public display of the animals and acclimated them

Figure 13.1. Nurse sharks (*Ginglymostoma cirratum*) stationed on a training platform at the Oceanario Islas del Rosario, Colombia.

to a number of advanced husbandry and veterinary procedures (Jensen, pers. com.).

IZA staff trained the elasmobranchs to use a feeding station while they were in a holding facility. This behavior was trained by pairing an auditory signal (i.e., striking a metal triangle) with a consistent feeding time and location. Within weeks staff noted an increased level of activity in the designated area when the triangle was struck. Thereafter, IZA staff conditioned the animals to hand-feed at the surface. This behavior was initiated by splashing the surface of the pool while waving a fish underwater. All the animals were hand-feeding within a short period. Once the sharks and rays were moved into their final exhibit, the metal triangle was used to establish feeding times and a feeding station. Hand-feeding was once again trained using successive approximations.

For the training of more sophisticated behaviors, a bridge was conditioned using an Acme pea-less whistle (J. Hudson and Co. Ltd., UK). Food fish were cut into smaller pieces to enable longer training sessions, while maintaining food motivation. For example, the optimal amount of food for one of the nurse sharks was 0.45-0.70 kg of fish, cut to enable 30-40 rewards per training session (Jensen, pers. com.). The team adopted a continuous reinforcement schedule, bridging and feeding the animals after each correct response.

The elasmobranch exhibit was an immersive (or swim-through) experience for guests. Animals were therefore target-trained, using a white float on the end of a pole, and subsequently fed, to reduce any chance that sharks would associate guests in the water with food. Guests were never permitted in the water during feeding times, and a strict no-touch policy was in force for swim-through participants.

The target was initially introduced by touching it to an animal's snout as it entered the feeding area. The animal was bridged and rewarded with a piece of fish. Trainers hand-fed the animals, as opposed to dropping food in the water, encouraging them to remain in position rather than moving away in search of the food item. Target-training required one to two sessions per day for a period of six to seven weeks. The start of each training session was signaled with the metal triangle. Sharks began to voluntarily touch the target after only a few sessions. Training staff were thus able to move the target and lead the sharks short distances, ultimately leading them to designated areas within the pool (Figure 13.2). Sharks were easily led using the target, while rays responded better to tactile cues (i.e., tapping the edge of the animal's disc) to lead the animal in a desired direction.

Target-training enabled staff to shape several useful behaviors. For example, by successive approximations it was possible to stretcher-train an animal (i.e., have an animal swim into a stretcher). Once a feeding station was established, staff introduced a stretcher to desensitize the animals to its presence. One side of the stretcher was suspended above the water-line, and the rest allowed to drape over the inner wall and floor of the pool. Sharks were fed next to, and over, the stretcher. After a few days, staff raised both sides of the stretcher and fed animals over the resulting "bag" as it hung in the water. Trainers gradually increased the length of time sharks remained at station within the stretcher and ultimately lifted the stretcher slightly, desensitizing animals to the upward pressure and movement of the stretcher. Stretcher-training reduced stress

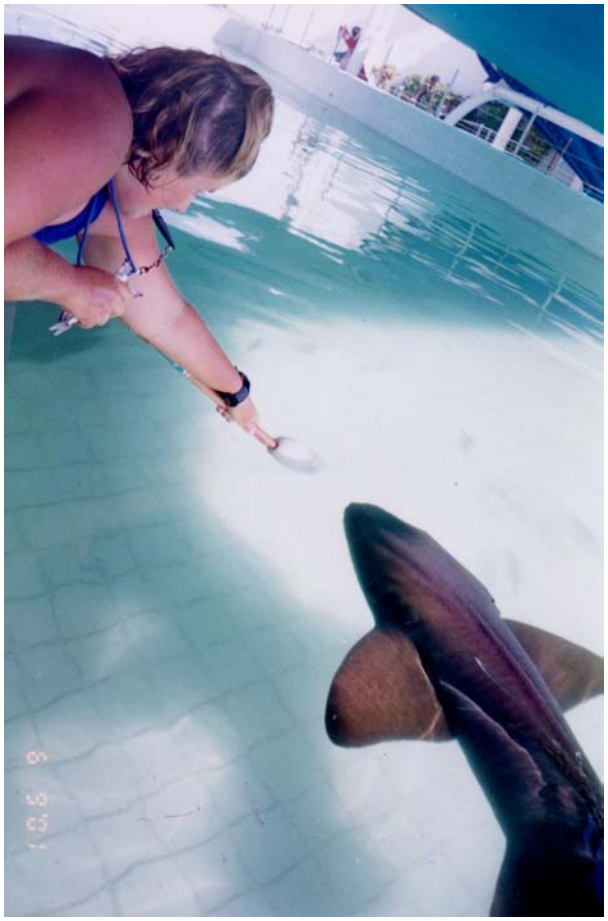


Figure 13.2. Nurse shark (*Ginglymostoma cirratum*) being led using a target at the Parque Nizuc, Cancun, Mexico.

during animal handling and enabled staff to take measurements readily, to perform visual examinations, and to treat superficial wounds.

Using targets, sharks and rays were trained to lie on PVC tables submerged ~35 cm below the water-surface (Jensen, pers. com.). Animals would remain in position until bridged. This behavior enabled animals to be subjected to ultrasonography or other procedures, while under stimulus control on the table. In addition, visitors were able to touch sharks and rays in a controlled and safe manner during public exhibitions, facilitating the education and conservation aims of the facility. During desensitization to clinical procedures one trainer reinforced the animal to remain stationed while another trainer manipulated the animal, acclimating animals to handling.

During training, staff observed a lack of motivation in one of the sharks, probably as a result of breeding activity. No training sessions were conducted during this period, avoiding unproductive sessions and maintaining a positive training environment for both animals and trainers.

IZA staff maintain that target-training is an essential requirement before moving on to behaviors that involve the handling of elasmobranchs. They stress the importance of consistency, small approximations, numerous repetitions, adaptability to changing conditions, teamwork, and the development of a good plan when establishing a training program.

CONCLUSIONS

It is imperative that professionals caring for elasmobranchs are well-versed in the natural history, sensory biology, and behavior of species maintained at their facilities. Each animal should be regularly observed and a familiarity with its behavior developed. Facilities can use an understanding of elasmobranch sensory biology and natural history, through well-planned enrichment programs, to reinforce useful natural behaviors within their elasmobranch collections. Enrichment programs will benefit from operant conditioning techniques. At present, formal training and enrichment programs for elasmobranchs are rare in public aquariums. Existing programs serve to reduce stress in elasmobranchs during handling, improve basic husbandry, enable more advanced husbandry techniques, and enhance public presentations. As institutions seek more effective ways to display elasmobranchs, exhibit design will become more complex and innovative. Exhibit design will affect husbandry procedures, just as future exhibits will be influenced by improved husbandry techniques. Enrichment programs are particularly valuable within this context.

ACKNOWLEDGEMENTS

The authors wish to thank the following: Jay and Elizabeth Jensen, for use of detailed information about the shark and ray training program at Parque Nizuc (Cancun, Mexico); John Rupp, for permission to include the operant research conducted by the team at the Pacific Rim Center for Elasmobranch Studies (Point Defiance Zoo and Aquarium, USA); Larry Rutherford of the Living Seas Pavilion (Walt Disney World USA,) and Robert Siders of the Theater of the Sea (Islamorada, USA) for sharing their elasmobranch training experiences; Alejandro Perez and Angel Ibarra, presenters of the nurse shark demonstrations at the Oceanario Islas del Rosario (Colombia); and Rafael Vieira, Pam Schaller, Sue Hunter, B. T. Moose, P. V. Nikki, F. K. Angell, Mark Nocito, Michael Stevens, Pam Lyons, Ed J. Samson, Dr. Ray Jones, and Dr. Robin Sherman for their help and support in preparing this chapter. Finally, the authors extend sincere gratitude to Martha Hiatt of the Wildlife Conservation Society Animal Enrichment Program for contributing her animal training and enrichment expertise in editing this manuscript.

APPENDIX 13.1

The terminology of operant conditioning

Bridging stimulus

A bridging stimulus is a cue used to signal to an animal that it has performed a requested behavior correctly. It is called a bridge because it bridges the gap between an animal's correct response and subsequent reinforcement (e.g., food). A bridge therefore acts as both a stimulus and a secondary reinforcer. As a secondary reinforcer, a bridge is one of the first behaviors an animal learns. A bridge is conditioned over time to be positive and is initially paired with food so the animal learns to associate the bridge with a primary reinforcer. A bridge can be auditory (e.g., a spoken word or the sound of a whistle), visual, or tactile. It may seem redundant to signal to the

animal that it has delivered a correct response, however, the bridge is valuable as it pinpoints the exact moment a correct behavior has been executed and is thus more accurate than any other reinforcer. Unless an animal is right in front of you, there will always be a time delay before the animal receives the food reinforcer, especially true of aquatic animals that may be beneath the surface and not easily accessible to the keeper. The bridging stimulus must therefore be precise and brief to be effective (Ramirez, 1999).

Continuous reinforcement schedule

A continuous reinforcement schedule is a protocol for determining the timing of the delivery of reinforcers to an animal in which each correct response is reinforced. Either primary or secondary reinforcers may be used, but the schedule never varies (Ramirez, 1999).

Criteria

Criteria, in a trained behavior, is the set of standards an animal must meet in order to receive reinforcement (Hiatt et al., 2003).

Desensitization

Desensitization is a basic form of learning whereby an animal's reaction to a novel stimulus decreases through repeated exposure to that stimulus. There are two types of desensitization:

1. *habituation*: desensitization occurs after increased or repeated exposure to a stimulus; and

2. *counter-conditioning*: desensitization occurs by modifying an animal's response to a stimulus by associating it with a positive reinforcement (Hiatt et al., 2003).

Discriminative stimulus (SD)

A discriminative stimulus is a cue given to an animal to invoke a specific behavior and can be visual (e.g., a motion of the hand or posture of the body, a light, or the presentation of a target), auditory (e.g., a word or tone), or tactile (e.g., touching a specific body part). Consistent pairing of a signal with a behavior conditions a specific association, making the signal the SD for that behavior. Each SD must be clear and easily distinguished from any other signal, and delivered in a consistent manner, avoiding confusion and preventing animals from giving inconsistent responses (Ramirez, 1999).

Operant conditioning

Operant (or instrumental) conditioning is a learning process whereby a subject produces a behavior in the presence of a cue (called a stimulus), on the condition of achieving desirable, or avoiding undesirable, consequences. Operant conditioning differs from classical conditioning in that the subject has an active role in the process. Classical conditioning (also Pavlovian conditioning or associative learning) is passive, as it involves the conditioning of reflexive behaviors (Ramirez, 1999).

Reinforcement

Reinforcement is an event that follows, and is a consequence of, a behavior, that strengthens the behavior and increases the likelihood that it will be repeated in the future. Modern operant conditioning protocols prefer to rely on positive reinforcement.

Positive reinforcement

Positive reinforcement is the consequence added to a subject's environment following the correct execution of a specified behavior, serving to increase the likelihood or frequency of that behavior. There are two types of reinforcement:

1. *Primary reinforcers*: reinforcers that are intrinsically valuable to the subject (e.g., food).

2. *Secondary reinforcers*: reinforcers whose value is conditioned over time. Secondary reinforcers (e.g., tactile) are initially paired with primary reinforcers (e.g., food), the subject eventually forming an association between the two and giving the secondary reinforcers value. Secondary reinforcers provide variety in training sessions and become particularly useful when an animal is not food-motivated (e.g., in the case of illness or when an animal has already eaten its normal food ration) (Ramirez, 1999).

Shaping

Shaping is a method of modifying a behavior, through successive approximations, to achieve a specifically desired behavior (Scardina-Ludwig and Messinger, 2001).

Stimulus control

Stimulus control is the process by which a desirable behavior is paired with a specific signal or cue (or stimulus) so that the animal performs the behavior immediately after the stimulus is given. The desired behavior should never occur in the presence of a different stimulus (Ramirez, 1999).

Successive approximation

Successive approximation is the process by which desirable behaviors are broken down into a series of steps, or approximations, with each step building on skills learned in the previous step and preparing the animal for the following step. By breaking a behavior into small steps, learning is simplified (Ramirez, 1999). Successive approximation is an efficient training tool, especially for difficult or complicated behaviors, because each step offers multiple opportunities for reinforcement and sets the animal up for success. Successive approximation fosters a positive learning environment and can strengthen the relationship between the animal and its trainer.

Target

A target is a prop that can act as a visual stimulus, pinpointing a specific location for an animal to touch during training (Scardina-Ludwig and Messinger, 2001).

APPENDIX 13.2

Further reading

1. Clark, E. 1981. Sharks—magnificent and misunderstood. National Geographic 160:138-187.
2. Hamlett, W. C. 1999. Sharks, Skates, and Rays: The Biology of Elasmobranch Fishes (Ed.). Baltimore, Maryland: Johns Hopkins University Press, 525 pp.
3. Hodgson, E. S. and Mathewson, R. F. 1978. Sensory Biology of Sharks, Skates, and Rays (Eds.). Arlington, Virginia: Office of Naval Research, Department of the Navy, 278 pp.
4. Markowitz, H., Schmidt, M. J., and Moody, A. 1978. Behavioral engineering and animal health in the zoo. International Zoo Yearbook 18: 190-19.
5. Mellen, J. and MacPhee, M. S. 2001. Philosophy of environmental enrichment: past, present, and future. Zoo Biology, 20(3): 211-226.
6. Myrberg, A. A., Jr. 1987. Understanding Shark Behavior. *In* Sharks, An inquiry into biology, behavior, fisheries and use (Ed. Cook, S.). Proceedings of a Conference. Oregon State University Extension Service, 41-83 pp.
7. Pratt, H. and Carrier, J. 2001. A review of elasmobranch reproductive behavior with a case of study on the nurse shark, *Ginglymostoma cirratum*. Environmental Biology of Fishes 60: 157-188.
8. Priest, G. M. 1991. The Use of Operant Conditioning in Training Husbandry Behaviors with Captive Exotic Animals. Proceedings of the National AAZK [American Association of Zoo Keepers] Conference 16: 94-108.
9. Pryor, K. 1983. Don't shoot the dog! The new art of teaching and training. New York: Simon and Schuster, 224 pp.
10. Stevens, V. J. 1978. Basic operant research in the zoo. *In* The Behavior of Captive Wild Animals (Eds. Markowitz, H. and Stevens, V. J.). Chicago, Illinois: Nelson Hall, 209-246 pp.

APPENDIX 13.3

Animal training and enrichment resources

The following list of Internet resources was taken from the Wildlife Conservation Society's animal training manual (Hiatt, et al., 2003):

1. The American Zoo and Aquarium Association (AZA) has a list server dedicated to animal training (www.aza.org).

2. The Animal Behavior Society operates a website and produces a journal: Animal Behavior (www.animalbehavior.org).

3. The Animal Behavior Management Alliance is a comparatively new association committed to behavioral husbandry (www.theabma.org).

4. The International Marine Animal Trainers Association (IMATA) is a group of trainers who work with a wide variety of animals. IMATA holds annual conferences and publishes a quarterly magazine (www.imata.org).

5. Walt Disney World has websites dedicated to animal enrichment (www.animal-enrichment.org) and animal training (www.animaltraining.org).

REFERENCES

Alston, A., I. Close, J. Pepperell, P. Gilbert, R. Pullan, O. Ruhen, V. Taylor, R. Taylor, P. Goadby, S. Turner, S. Johnson, N. Kemp, and N. Otway. 1987. Sharks, Silent Hunters of the Deep. Reader's Digest Services, Sydney, Australia. 207 p.

Aronson, L. R., F. R. Aronson, and E. Clark. 1967. Instrumental conditioning and light-dark discrimination in young nurse sharks. Bulletin of Marine Science 17: 249-256.

Baker, A. 1991. Training as a management tool: Creating the climate and maintaining the momentum. *In:* AAZPA Annual Conference Proceedings, September 15-19, 1991, San Diego, CA, p. 563-568. American Association of Zoos, Park, and Aquariums (American Zoo and Aquarium Association), Silver Spring, Maryland, USA.

Banner, A. 1967. Evidence of sensitivity to acoustic displacements in the lemon shark, *Negaprion brevirostris* (Poey). *In:* Lateral Line Detectors, p. 265-273. P. Cahn (ed.). Indiana University Press, Bloomington, Indiana, USA.

Bleckmann, H. and M. H. Hofmann. 1999. Special senses. *In:* Sharks, Skates, and Rays: The Biology of Elasmobranch Fishes, p. 300-328. W. C. Hamlett (ed.). Johns Hopkins University Press, Baltimore, Maryland, USA.

Bres, M. 1993. The behavior of sharks. Reviews in Fish Biology and Fisheries 3: 133-159.

Bunting, E. 1979. The Sea World Book of Sharks. Harcourt Brace Jovanovich Publishers, San Diego, California, USA. 79 p.

Clark, E. 1959. Instrumental conditioning in lemon sharks. Science 136: 217-218.

Clark, E. 1961. Visual discrimination in lemon sharks. *In*: Tenth Pacific Science Congress, August 21-September 2, 1961, Honolulu, Hawaii. p. 175-176. J. Barrau (ed). Bernice P. Bishop Museum Press, Honolulu, Hawaii, USA.

Clark, E. 1963. Maintenance of sharks in captivity with a report on their instrumental conditioning. *In:* Sharks and Survival, p. 115-149. P. W. Gilbert (ed.). D. C. Heath and Co., Boston, Massachusetts, USA.

Corwin, J. T. 1981. Audition in elasmobranchs. *In:* Hearing and Sound Communication in Fishes, p. 81-102. W. N. Tavolga, A. N. Popper, and R. R. Fay (eds.). Springer-Verlag, New York, USA.

Dijkgraaf, S. and A. J. Kalmijn. 1963. Untersuchungen uber die funktion der lorenzinischen ampullen an haifischen. Zeitschrift fuer Vergleichende Physiologie 47: 438-456.

Eibl-Eibesfeldt I. and H. Hass. 1959. Erfahrungen mit Haien. Zeitschrift fuer Tierpsychologie 16: 733-746.

Graeber, R. C. and S. O. E. Ebbesson. 1972. Visual discrimination learning in normal and tectal-ablated nurse sharks *Ginglymostoma cirratum*. Comparative Biochemical Physiology 42A:131-139.

Greenberg, J., I. Greenberg, and J. Greenberg. 1981. Sharks and Other Dangerous Sea Creatures. Seahawk Press, Miami, Florida. 62 p.

Gruber, S. R. and J. L. Cohen. 1978. Visual system of the elasmobranchs - State of the art: 1960-1975. *In:* Sensory Biology of Sharks, Skates, and Rays, p. 11-106. E. S. Hodgson and R. F. Mathewson (eds.). Office of Naval Research, Department of the Navy, Arlington, Virginia, USA.

Gruber, S. R. and N. Schneiderman. 1975. Classical conditioning of the nictitating membrane response of the lemon shark, *Negaprion brevirostris*. Behavioral Research Methods and Instrumentation 7: 430-434.

Hueter, R. E. and P. W. Gilbert. 1990. The sensory world of sharks. *In:* Discovering Sharks, p. 48-55. S. H. Gruber (ed.). American Littoral Society, Highlands, New Jersey, USA.

Heyer, G. W., M. C. Fields, R. D. Fields, and A. J. Kalmijn. 1982. Field experiments on electrically evoked feeding responses in the pelagic blue shark, *Prionace glauca*. Biological Bulletin 161: 344-345.

Hiatt, M., K. Walsh, D. Reiss, D. Moore, C. McCann, and B. Mangold. 2003. The WCS Training Manual. Wildlife Conservation Society, New York, USA. 90 p.

Hodgson, E. S. and R. F. Mathewson. 1978. Electrophysiological studies of chemoreception in elasmobranchs. *In:* Sensory Biology of Sharks, Skates, and Rays, p. 227-267. E. S. Hodgson and R. F. Mathewson (eds.). Office of Naval Research, Department of the Navy, Arlington, Virginia, USA.

Kalmijn, A. J. 1966. Electro-perception in sharks and rays. Nature 212: 1232-1233.

Kalmijn, A. J. 1974. The detection of electric fields from inanimate and animate sources other than electric organs. *In:* Handbook of Sensory Physiology, Volume III, p. 147-200. A. Fessard (ed.). Springer-Verlag, New York, USA.

Kalmijn, A. J. 1978. Electric and magnetic sensory world of sharks, skates and rays. *In:* Sensory Biology of Sharks, Skates, and Rays, p. 507-528. E. S. Hodgson and R. F. Mathewson (eds.). Office of Naval Research, Department of the Navy, Arlington, Virginia, USA.

Kalmijn, A. J. 1981. Biophysics of geomagnetic field detection. International Society of Electrical and Electronic Engineers Transactions of Magnetics 17: 1113-1124.

Kalmijn, A. J. 1984. Theory of electromagnetic orientation: a further analysis. *In:* Comparative Physiology of Sensory Systems, p. 525-560. L. Bolis, R. D. Keynes, and S. H. P. Maddrell (eds.). Cambridge University Press, Cambridge, England.

Klimley, A. P. and A. A. Myrberg, Jr. 1979. Acoustic stimuli underlying withdrawal from a sound source by adult lemon sharks, *Negaprion brevirostris*. Bulletin of Marine Science 29: 447-458.

Kohler, N. E., J. G. Casey and P. A. Turner. 1998. NMFS cooperative shark tagging program, 1962-1993: an atlas of shark tag and recapture data. Marine Fisheries Review 60(2): 1-87.

Murray, R. W. 1974. The ampullae of Lorenzini. *In:* Handbook of Sensory Physiology, Volume III, p. 125-146. A. Fessard (ed.). Springer-Verlag, New York, USA.

Myrberg, A. A., Jr. 1978. Underwater sound - its effect on the behavior of sharks. *In:* Sensory Biology of Sharks, Skates, and Rays, p. 391-417. E. S. Hodgson and R. F. Mathewson (eds.). Office of Naval Research, Department of the Navy, Arlington, Virginia, USA.

Myrberg, A. A., Jr., C. Gordon, and A. P. Klimley. 1978. Rapid withdrawal from a sound source by open-ocean sharks. Journal of the Acoustical Society of America 64: 1289-1297.

Nelson, D. R. 1967. Hearing thresholds. Frequency discriminations and acoustic orientation in the lemon shark. *Negaprion brevirostris* (Poey). Bulletin of Marine Science 17: 741-768.

Northcutt, R. G. 1977. Elasmobranch central nervous system organization and its possible evolutionary significance. American Zoologist 17: 411-429.

Northcutt, R. G. 1978. Brain organization in the cartilaginous fishes. *In:* Sensory Biology of Sharks, Skates, and Rays, p. 117-193. E. S. Hodgson and R. F. Mathewson (eds.). Office of Naval Research, Department of the Navy, Arlington, Virginia, USA.

Popper, A. N. and R. R. Fay. 1977. Structure and function of the elasmobranch auditory system. American Zoologist 17: 443-452.

Ramirez, K. 1999. Animal Training: Successful Management Through Positive Reinforcement. John G. Shedd Aquarium, Chicago, Illinois, USA. 578 p.

Roberts, B. L. 1972. Activity of lateral line sense organs in swimming dogfish. Journal of Experimental Biology 56: 105-118.

Roberts, B. L. 1978. Mechanoreceptors and the behavior of elasmobranch fishes with special reference to the acoustico-lateralis system. *In:* Sensory Biology of Sharks, Skates, and Rays, p. 331-390. E. S. Hodgson and R. F. Mathewson (eds.). Office of Naval Research, Department of the Navy, Arlington, Virginia, USA.

Scardina-Ludwig, J. and C. Messinger, 2001. Training and Behavioral Terms Glossary. International Marine Trainers Association, Chicago, Illinois, USA. 21 p.

Sheperdson, D. J., J. D. Mellon, and M. Hutchins (eds.). 1998. Second Nature: Environmental Enrichment for Captive Animals. Smithsonian Institute, Washington, D. C. 332 p.

Tester, A. L. 1963. Olfaction, gustation, and the common chemical sense in sharks. *In:* Sharks and Survival, p. 255-282. P. W. Gilbert (ed.). D. C. Heath and Co., Boston, Massachusetts, USA.

Tricas, T. C. 1982. Bioelectric-mediated predation by swell sharks, *Cephaloscyllium ventriosum*. Copeia 1982: 948-952.

Tricas, T. C. 2001. The neuroecology of the elasmobranch electrosensory world: Why peripheral morphology shapes behavior. Environmental Biology of Fishes 60: 77-92.

PERSONAL COMMUNICATIONS

Jenson, E. 2002. Parque Nizuc, Cancun, Quintana Roo, 77500, Mexico.

Rutherford, L. 2002. The Living Seas Pavilion, Lake Buena Vista, FL 32830, USA.

Rupp, J. 2002. Point Defiance Zoo and Aquarium, Tacoma, WA 98407, USA.

Siders, R. 2002. Theater of the Sea, Islamorada, FL 33036, USA.

INTERNET RESOURCES

www1 www.animalenrichment.org

The Elasmobranch Husbandry Manual: Captive Care of Sharks, Rays and their Relatives, pages 183-200.
© 2004 Ohio Biological Survey

Chapter 14
Elasmobranch Nutrition, Food Handling, and Feeding Techniques

MAX JANSE

Burgers' Zoo,
Antoon van Hooffplein 1,
6816 SH, Arnhem, The Netherlands.
E-mail: m.janse@burgerszoo.nl

BETH FIRCHAU

Virginia Aquarium and Marine Science Center,
717 General Booth Boulevard,
Virginia Beach, VA 23451, USA.
E-mail: bfirchau@vbgov.com

PETER J. MOHAN

5802 Thorndale Drive,
Kent, OH 44240, USA.
E-mail: petemohan@aol.com

Abstract: The correct nutrition of elasmobranchs in captivity is of fundamental importance to their health and survival. The optimal diet for a captive elasmobranch is a copy of its diet in the wild, both in quantity and quality. These parameters change seasonally, within species and between species. It is incumbent upon the aquarist to determine the appropriate diet for a given age class and species of elasmobranch. Feeding of elasmobranchs in captivity is mostly done with pre-frozen food, to eliminate the possibility of parasitic infection and to ensure the continuity of food availability. Loss of vitamins and minerals due to food transport, storage, and preparation make vitamin and mineral supplementation necessary. Of equal importance to nutritional content and food quantity, is the manner in which the food is stored and thawed, its hygienic preparation, and the techniques used for delivering the food to specific elasmobranchs. Target-feeding individual specimens is preferred, as this allows an accurate assessment of the status of each animal and facilitates the administration of medications. As a last resort, tube-feeding may be employed to prevent dehydration and excess loss of body weight in inappetant elasmobranchs.

The correct nutrition of elasmobranchs in captivity is of critical importance to their health and survival. The optimal diet for a captive elasmobranch is a copy of its diet in the wild, in both quantity and quality. Often, this diet is not well defined in the scientific literature, making it difficult for aquarists to include all the necessary dietary ingredients. The information contained in this chapter should be used as a starting point to develop appropriate dietary regimes, food preparation routines, and feeding techniques for captive elasmobranch collections. Specifically, this chapter examines nutrition, dietary composition, feeding frequency, daily ration, vitamin and mineral supplementation, food sourcing, food storage, food preparation, feeding, and tube-feeding inappetant animals.

DIETARY REQUIREMENT

Protein

Bowen (1987) gives an overview of the dietary protein requirements of fishes. Protein is the major source of energy for fishes and the basic component of animal tissue (De Silva and Anderson, 1995). Protein is an essential nutrient for both maintenance and growth. Proteins are composed of amino acids, which can be classified as essential, semi-essential, and non-essential. An animal cannot synthesize essential amino acids, while non-essential amino acids can be synthesized from carbon and nitrogen precursors. Most animal proteins contain essential amino acids. Proteins are lost from food during storage. Omotosho and Olu (1995) found a decrease in protein content following the frozen storage of different African freshwater fishes.

Carbohydrates

Carbohydrates, such as sugar, starch, gum, and cellulose, are the main biomass constituents of plants. Carbohydrates are only present in minute quantities in animals, taking the form of glycogen, sugars, and their derivatives. The natural diet of carnivorous fishes, such as elasmobranchs, contains relatively few carbohydrates.

Lipids

Lipids in fishes function both as high-energy storage molecules and as components of cellular membranes. There are five major classes of lipids: fatty acids, triglycerides, phospholipids, sterols, and sphingolipids. The first two types of lipids are generally used for storage of metabolic energy, while the last three types of lipids are generally used as components of cellular membranes. Animals can synthesize triglycerides, phospholipids, sterols, and sphingolipids (De Silva and Anderson, 1995).

Fatty acids are classified according to the degree to which they are saturated with hydrogen. Marine fishes contain large amounts of *n*-3 highly-unsaturated or poly-unsaturated fatty acids (HUFA or PUFA) (Sargent et al., 1999; Lytle and Lytle, 1994). Two types, docosahexaenoic acid (22:6*n*-3) and eicosapentaenoic acid (20:5*n*-3), are common in the phospholipids of the cellular membranes. These types cannot be synthesized by marine fishes and are therefore essential fatty acids (Sargent et al., 1999).

Lytle and Lytle (1994) found small lipid variations in temperate sharks and rays within and between seasons, indicating that food requirements are most likely met without need to store these nutrients for future requirement. Lipid concentration among rays and sharks varies little between animals from cold and warm waters. Excess lipid consumption in captive fishes is known to cause health problems. Fatty liver syndrome has been described for salmonids when feeding high-fat diets (Post, 1993).

Chemical changes during food storage are attributed to the breakdown of fatty acids. In general, PUFA and pure lipids are more prone to oxidation. Omotosho and Olu (1995) found a decrease in total lipid, after frozen storage, for different African freshwater fishes.

Vitamins

Vitamins can be divided into fat-soluble (A, D, E, and K) and water-soluble vitamins. Table 14.1 gives an overview of the function of different vitamins, including the vitamin requirement of marine teleosts. The figures quoted in Table 14.1 will, of course, only give an approximation of the requirement for elasmobranchs. More research is needed in this field.

A common practice in most aquariums is to use pre-frozen food. Chemicals produced during the degeneration of stored foods may reduce amino acid and vitamin availability; vitamin C being particularly susceptible (De Silva and Anderson, 1995). The presence of thiaminase in stored food steadily reduces the concentration of thiamine. During the thawing and feeding of pre-frozen food, water-soluble vitamins are easily lost. It is therefore necessary to add extra quantities of these vitamins to food immediately before feeding.

In contrast to the water-soluble vitamins, fishes accumulate fat-soluble vitamins in both muscle and liver tissue under conditions where dietary intake exceeds metabolic demand. Under certain circumstances excess accumulation produces a toxic condition (hypervitaminosis) (Tacon, 1992).

Minerals

Mineral requirements are difficult to study because minerals are required in trace amounts, while others are absorbed from the water through the gills (Pike et al., 1993). Some minerals are lost in closed aquarium systems due to the formation of chemical

Table 14.1. Overview of the function of vitamins in marine teleosts and associated vitamin requirements. Except where otherwise noted, all units are quoted in mg kg dry weight^{-1}. Sources for data: [a] Anon. (1983); [b] Halver (1979); [c] Anon. (1981); [d] Halver (1989); [e] Post (1993); [f] Boonyaratpalin (1997).

Vitamin	Function	Requirement (mg.kg dry weight^{-1})
Thiamine (Vitamin B1)	Coenzyme for reactions involving carbohydrate metabolism; role in conversion of pyruvate to acetyl CoA.	1^{a}-$10^{b,c,d,e}$
Riboflavin (Vitamin B2)	Component of flavin adenine dinucleotide (FAD), which has an important roles in degradation of pyruvate, fatty acids, and amino acids; electron transport.	4^{a}-$20^{b,c,d,e}$
Nicotinic acid (Vitamin B3) (niacin)	Part of NAD and NADP, which are important in many metabolic reactions.	20^{a}-$150^{b,c,d,e}$-163^{f}-200^{b}
Panthothenic acid (coenzyme A - Vitamin B5)	Involved in reactions in fatty acid oxidation, fatty acid synthesis, pyruvate oxidation and acetylations; carrier of various carbohydrate groups.	12^{a}-15^{g}-$40^{b,c,d,e}$-50^{b}
Pyridoxine (Vitamin B6)	Coenzyme in transamination reactions (degradation of amino acids).	3^{a}-5^{h}-$10^{c,e,h}$-14^{f}-$15^{b,d}$-$20^{b,d}$
Biotin	Cofactor to transfer CO_2 to another molecule; involved in lipid synthesis.	0.1^{a}-$1^{b,c,d,e,f}$-$1.5^{b,d}$
Folic acid	Cofactor in the transfer of single entities to other molecules; involved in blood cell formation.	$5^{b,c,e}$-$6^{b,d}$-7^{f}-$10^{b,d}$ (µg)
Cyanocobalamin (Vitamin B12)	Essential for normal maturation and development; part of synthesis of choline.	0.01^{a}-$0.02^{b,c,d,e,f}$
Ascorbic acid (Vitamin C)	Role in cartilage synthesis; involved in carnitine synthesis and in the detoxification of pesticides; antioxidant; involved in maturation of erythrocytes.	$100^{b,c,d,e}$-$150^{b,d}$-500^{f}-1100^{f}
Inositol	Important in cell membrane synthesis; homeostasis of lipid metabolism.	$300^{a,b,d}$-375^{f}-$400^{b,c,d,e}$
Choline	Methyl donor in a number of metabolic reactions.	$600^{b,d}$-$800^{b,d}$-1000^{a}-1850^{f}-$3000^{e,c}$
Retinoic acid (Vitamin A)	Important as a component of the protein rhodopsin, a light-absorbing pigment found in the retina of the eye; antioxidant.	2000^{d}-$2500^{a,c,d,e,f}$ (IU)
Vitamin D3	Precursor to 1,2,5-dihydroxycholecalciferol, a hormone important in regulating calcium and phosphate levels in the serum.	$2400^{a,c,d,e,f}$ (IU)
Vitamin E (tocopherol)	Antioxidant, particularly protecting polyunsaturated fatty acids.	$30^{c,d,e}$-38^{f}-40^{b}-$50^{a,b}$
Vitamin K	Important in the synthesis of prothrombin, a protein important in blood clotting.	5^{e}-$10^{a,c,d,e,f}$

complexes. Calcium reacts with phosphates, forming calcium phosphate. Iodine is possibly lost due to complex-formation or extraction via the foam discharge of protein skimmers.

Minerals are required as essential factors in the metabolism and growth of fishes (Table 14.2). Care must be taken when directly applying the mineral requirement of teleosts to elasmobranchs. Copper is toxic for elasmobranchs at a concentration of 2.0 mg kg dry weight^{-1} (Stoskopf, 1993a), while teleosts have 1.0-4.0 mg kg dry weight^{-1} dietary requirement of copper (Chow and Schell, 1980; Cowey et al., 1985).

Table 14.2. Overview of the function of minerals in marine teleosts and associated mineral requirements. Figures quoted as requirement per kilogram of dry diet. Sources of data: [a] De Silva and Anderson (1995); [b] Hepher (1988); [c] Chow and Schell (1980); [d] Cowey et al. (1985); [e] Hilton et al. (1980).

Mineral	Function	Requirement per kg dry diet
Calcium	Bone, teeth, and cartilage formation; blood clotting; muscle contraction; nervous impulses.[a,b]	5[c] g
Phosphorus	Bone formation; high energy phosphate esters (like ATP); other organo-phosphorus compounds.[a,b]	7[c] g
Magnesium	Enzyme co-factor extensively involved in the metabolism of fats, carbohydrates and proteins; important in maintaining muscle tone.[a]	0.5[c] g
Sodium	Primary monovalent cation of intercellular fluid; involved in acid-base balance and osmoregulation.[a]	1-3[c] g
Potassium	Primary monovalent cation of intracellular fluid; involved in nerve action and osmoregulation.[a]	1-3[c] g
Sulphur	Integral part of sulphur amino acids and collagen; involved in detoxification of aromatic compounds.[b]	3-5[c] g
Chlorine	Primary monovalent anion in cellular fluids; component of digestive juice (HCl); acid-base balance.[a]	1-5[c] g
Iron	Essential constituent of heme in hemoglobin and the cytochromes, proteins that are important in oxidative phosphorylation.[a,b]	50[c]-80[d]-100[c,d] mg
Copper	Cofactor in thyrosinase and ascorbic acid oxidase.[a]	1[c]-3[d]-4[c] g
Selenium	Imparts a protective effect against heavy metal toxicity.[a]	0.15-0.38[e] mg
Manganese	Cofactor for arginase and certain other metabolic enzymes; involved in bone formation, erythrocyte regeneration and maintaining proper function in nerve cells.[a]	20-50[c] mg
Cobalt	Metal component of cyanocobalamin (B12); prevents anaemia; involved in C1 and C3 metabolism.[a,b]	5-10[c] mg
Zinc	Essential for insulin structure and function; co-factor of carbonic anhydrase.[a]	15[f]-30[c,f]-100[c] mg
Iodine	Constituent of thyroxine.[a]	100-300[c] mg
Chromium	Involved in collagen formation and regulation of the rate of glucose metabolism.[a]	trace[c]

Table 14.3. Overview of diet composition for elasmobranchs in the wild. Sources for diet composition: [1] Compagno (1984); [2] Compagno et al. (1989); [3] Jones and Geen (1977); [4] Stevens and McLoughlin (1991); [5] Castro (2000); [6] Gray et al. (1997); [7] Henningsen (1996).

Family	Common name	Diet composition
Callorhinchidae	elephantfishes	Echinoids, bivalves, crustaceans, and small teleosts[2]
Carcharhinidae	requiem sharks	Large and small teleosts, crustaceans, cephalopods, and elasmobranchs[1]
Dasyatidae	whiptail stingrays	Crustaceans, teleosts, bivalves, polychaetes, and cephalopods[2]
Ginglymostomatidae	nurse sharks	Small teleosts (88%[5]), cephalopods (14%[5]), crustaceans (8%[5]), bivalves, and echinoderms [1]
Gymnuridae	butterflyrays	Crustaceans, teleosts, cephalopods, gastropods, and polychaetes[2, 7]
Hemiscylliidae	bamboo sharks	Bivalves, crustaceans, small teleosts, cephalopods, and gastropods[1]
Heterodontidae	horn sharks	Echinoids, crustaceans, mollusks, polychaetes, and small teleosts[1]
Mobulidae	devilrays	Plankton and small teleosts[2]
Myliobatidae	eaglerays	Bivalves (40%[6]), crustaceans (40%[6]), polychaetes (10%[6]), tunicates, cephalopods, and small teleosts[2]
Odontaspididae	ragged-tooth sharks	Large and small teleosts, small sharks, crustaceans, and cephalopods[1]
Orectolobidae	wobbegongs	Bivalves, echinoids, crustaceans, cephalopods, and small teleosts[1]
Pristidae	sawfishes	Small teleosts, shellfish, and crabs[2]
Pristiophoridae	saw sharks	Small teleosts, crustaceans, and squid[1]
Rajidae	skates	Teleosts, small sharks, crustaceans, bivalves, cephalopods, and polychaetes[2]
Rhincodontidae	whale sharks	Plankton and small teleosts[1]
Rhinidae	sharkfin guitarfish	Crabs, shrimp, bivalves, and small teleosts[2]
Rhinobatidae	guitarfish	Crustaceans, bivalves, polychaetes, and small teleosts[2]
Rhinopteridae	cownose rays	Bivalves[2]
Scyliorhinidae	catsharks	Bivalves, crustaceans, echinoderms, cephalopods, and small teleosts[1]
Sphyrnidae	hammerheads	Large and small teleosts, elasmobranchs, gastropods, echinoderms, crustaceans, and cephalopods[1]
Squalidae	dogfish sharks	Small teleosts (55%)[3], crustaceans (35%)[3], cephalopods (5%)[3], polychaetes, sea cucumbers, and jellyfish[1]
Squatinidae	angelsharks	Small teleosts, crustaceans, cephalopods, gastropods, and bivalves[1]
Stegastomatidae	zebra sharks	Bivalves, crustaceans, and small teleosts[1]
Torpedinidae	electric rays	Teleosts, cuttlefish, and small sharks[2]
Triakidae	houndsharks	Small teleosts, crustaceans, and cephalopods[1]

DIET COMPOSITION IN THE WILD

Examining the stomach contents of wild elasmobranchs (e.g., Cortés and Gruber, 1990, Stevens and McLoughlin, 1991; Stillwell and Kohler, 1993; Castro, 1996) has provided an indication of food preferences, food availability, and nutritional requirements in natural habitats. Table 14.3 gives an overview of the diet composition of different species of wild elasmobranchs.

Stillwell and Kohler (1993) conducted a feeding habit survey on sandbar sharks (*Carcharhinus plumbeus*) off the northeastern coast of the United States. The stomach content of sub-adult and adult sharks (n=321; mean fork length or FL=138 cm; and mean bodyweight or BW=34 kg) consisted of 43% teleosts, 16% elasmobranchs, and 3% cephalo-pods. The size of the prey ingested appeared to be an important factor and the majority of prey items observed were small enough to be swallowed in one piece.

Generally, it can be said that the main food item for sandbar sharks is teleosts for adult sharks and crustaceans for juvenile sharks. Other adult carcharhinids include teleosts as the largest part of their diets. Stevens and McLoughlin (1991) found large quantities of fishes (>76% for n>35) in the stomachs of the graceful (*Carcharhinus amblyrhynchoides*), pigeye (*Carcharhinus amboinensis*), spinner (*Carcharhinus brevipinna*), whitecheek (*Carcharhinus dussumieri*), and hardnose (*Carcharhinus macloti*) sharks. Castro (1996) found that the stomachs of blacktip sharks (*Carcharhinus limbatus*) taken from the southeastern U.S. coastline contained 76% fish and 9% crustacean remains. In 96% of the stomachs only one type of prey was found.

Even within a species there are dietary differences between distinct populations. A survey done on 116 specimens of Australian sandbar sharks showed that 88% of the animals had recently eaten teleosts, 22% had eaten cephalopods, 8% had eaten crustaceans, 1% had eaten mollusks (other than cephalopods), and 2% had eaten miscellaneous material (Stevens and McLoughlin, 1991).

Diet can differ according to the age of a shark. Stillwell and Kohler (1993) found crustaceans (82%) and teleosts (14%) in the stomach of pup and juvenile sandbar sharks (n=94; FL=55 cm; BW=1.7 kg). Crustaceans were represented primarily (75%) by soft blue crabs (*Callinectes sapidus*). Fish prey consisted of small flounder, anchovy, Atlantic silversides, and mullet. Castro (1989) found similar results for the smalleye hammerhead (*Sphyrna tudes*), whereby 90% of the juveniles had eaten shrimp and 18% had eaten fish remains, while 89% of adults had eaten teleosts and 18% of adults had eaten shrimp.

Seasonal changes in diet may be important. Jones and Geen (1977) found that spiny dogfish (*Squalus acanthias*) predominantly ate teleosts in the winter and invertebrates in the summer.

DIET COMPOSITION IN CAPTIVITY

Different studies on the diet of wild elasmobranchs demonstrate that a large variety of prey is consumed (Table 14.3). This variety should be the basis of a dietary composition for elasmobranchs in captivity. In addition, it is important to consider changes in diet that may occur during the life history of a particular species. For example, juvenile sandbar sharks should be fed mainly on crustaceans, while adults should be given teleosts.

An overview of food items regularly fed to elasmobranchs in captivity has been provided in Table 14.4. Some of the ranges described are due to seasonal variations. For example, a study of Atlantic mackerel (*Scomber scomber*) revealed a fat content of 10 g 100 g^{-1} during the fall and 17 g 100 g^{-1} during the winter (Karakoltsidis et al., 1995).

Both lean (i.e., <2 g 100 g fat^{-1}) and fatty fish, as well as crustaceans, cephalopods, and possibly some bivalves or gastropods, should be included in a diet for elasmobranchs. Feeding different items with varying nutritive values will increase the probability that most of the essential elements are ingested. Variation in the diet is easy to coordinate when multiple feedings occur each week. Sharks may prefer certain food items over others. By feeding one type of food per feeding session, animals will be encouraged to eat less appealing items and thus vary their diet.

The choice of food types depends on availability, quality, supply consistency, and price. Diets should be selected to reflect the natural diet components as closely as possible. A local source for food items is obviously preferable to an exotic source, but potential nutritional limitations should always be considered for exotic sharks. If elasmobranch collections are from local waters, and supplies are available, seasonal changes in wild diets should be reflected in captive diets. Likewise, if seasonal diet changes are known for exotic species, they should be catered to where possible. To maintain the right quality of food fishes, it is recommended that all foods be

supplied by certified companies providing seafood for human consumption.

GASTRIC EVACUATION AND FEEDING FREQUENCY

Feeding frequency depends on many factors (e.g., metabolism, age class, hormonal status, food availability, etc.). Of course feeding frequency in captivity depends on the number of feeding sessions per week and the amount of food given during each session, and there is a demonstrated variety of opinions as to what is considered appropriate. Janse (2003) found that European aquariums (n=15) fed a variety of dermersal sharks between 1-7 times a week. Similar species were fed 2-4 times a week in public aquariums throughout the USA (Branstetter, 1987b; Van Dykhuizen and Mollet, 1992; Demetrios and Denardo, 1995; Sabalones, 1995; Mohan, 1996; Schmid and Murru, 1990). This disparity highlights the need to use biological parameters (e.g., gastric evacuation rates) to help determine feeding frequencies for captive populations of elasmobranchs.

A number of studies determining the stomach status (i.e., full or empty) of wild sharks have been

Table 14.4. Protein, fat, and energy content of the edible portion of some food items used to feed elasmobranchs in captivity. Sources for nutritional content: [a] Lall and Parazo (1995); [b] Sidwell et al. (1974); [c] Sidwell (1981); [d] Scherz and Senser (1994); [e] Ackman (1995): [f] Ensminger et al. (1995); [g] Schmid and Murru (1994); [h] Karakoltsidis et al. (1995).

Food type (Species name)	Food type (Common name)	Water (g 100 g^{-1})	Protein (g 100 g^{-1})	Fat (g 100 g^{-1})	Energy (kcal 100 g^{-1})
Invertebrates					
Crangon crangon	shrimp	78[d]	19[d,h]	1[d]	87[d]
Euphausia superba	krill	78[d]	15[d]	3[d]	91[d]
Mytilus edulis	mussel	80[c]	8[h]-10[d]-12[c]-13[h]	1[d,h]-2[h,i]	51[d]-80[c]
Loligo sp.	squid	79[c,h]	13[h]-15[a,b,h]-16[f]-17[c]	0.2[h]-1[a,b,c,f,h]	84[f]-85[c]
Octopus vulgaris	octopus	84[h]	11[h]-15[f,h]	0.2[h]-1[e,f]	73[f]
Peaneus duorarum	gamba	78[c]	20[c]	1[c]	87[c]
Sepia sp.	sepia	80[h]-81[d]	16[d,f]-17[h]	1[d,f]	73[d]-81[f]
Callinectes sapidus	blue crab	80[c]	16[c]	1[c]	78[c]
Cerastoderma edule	cockle	-	17[f]	1[f]	81[f]
Fishes					
Caranyx chrysos	blue runner	75[c]	22[c]	2[c]	96[c]-163[g]
Clupea harengus	Atlantic herring	65[d]-74[c]	18[c,d]	10[c,d]-19[d]	160[c]-223[g]-233[d]
Engraulis encrasicholus	anchovy	74[h]-75[d]	18-21[h]	0.6-4[h]	-
Gadus morhua	Atlantic cod	81[c]	18[c,d]	1[d]	73[c]-77[d]
Merluccius merluccius	European hake	80[d]-81[h]	16[h]-17[d]-18[h]	0.4[h]-2[h]-3[d]	92[d]
Micropogonias undulatus	Atlantic croaker	78[h]	18[f]-20[h]	1[f,h]	84[f]
Pleuronectus flesus	flounder	80[d]	17[d]	1[d]	86[d]
Pollachius virens	saithe	79[c]-80[d]	18[d]-19[c]	1[c,d]	81[d]-84[c]
Pomatomus saltatrix	bluefish	75[c]	21[c]	4[c]	96[c]
Salmo trutta	trout	65[d]	20[d]	3[d]	103[d]
Sarda sarda	Atlantic bonito	70[h]	19-25[h]	3-8[h]	97[g]
Scomber scombrus	Atlantic mackerel	62[c]-68[d]-70[h]	18[h]-19[d]-20[c]	10[h]-12[d]-17[h]	182[d]-198[c]
Scomber japonicus	Pacific mackerel	71[c]	21[c]-25[h]	4[h]-7[c]	142[c]-152[g]
Sprattus sprattus	sprat	66[c]	17[d]	17[d]	217[d]
Trachurus trachurus	scat	75[d]-79[h]	18[h]-20[d]	1[h]-4[d]	114[d]

undertaken. A summary of this work is given in Table 14.5. Fishing techniques that used bait probably attracted actively hunting sharks and therefore specimens having empty stomachs. This artifact may have resulted in an artificially high proportion of sharks with empty stomachs; actively hunting sharks being expected to have less food in their stomach and a higher feeding motivation than recently satiated animals (Branstetter, 1987a). All species in Table 14.5, except the dusky smoothhound (*Mustelus canis*), were frequently landed with empty stomachs. In general, species that are more active yield a higher percentage of empty stomachs, possibly due to increased digestion rates, increased gastric evacuation rates, and/or decreased feeding frequencies. Since no empty stomachs were observed in the dusky smoothhound, it was concluded that this species eats more frequently and that gastric evacuation is relatively slow in relation to feeding frequency (Gelsleichter et al., 1999).

Mean gastric evacuation time (or retention time) for young sandbar sharks (n=17; FL=56 cm; BW=1.8 kg), to eliminate 98% of a meal, was 92 and 71 hours, when fed menhaden (*Brevoortia tyrannus*) and soft blue crabs, respectively (Medved, 1985; Medved et al., 1988). Medved et al. (1988) used the proportion of empty stomachs and average evacuation time to calculate a feeding frequency for juvenile sandbar sharks of 95.2 hours (i.e., 1.76 times per week). Wetherbee and Gruber (1990) conducted a study on the gastric evacuation of young lemon sharks (*Negaprion brevirostris*) (BW=1.6-2.1 kg) at different feeding ratios (i.e., 1.7-4.3% BW day^{-1}). Food was eliminated from the digestive tract within 69-109 hours and transit time increased with the food intake. Larger meals were processed at the same rate as smaller meals, but required a longer period of time to be eliminated (Wetherbee and Gruber, 1990). Similar results were found in another study of lemon sharks, where total retention time ranged between 68-82 hours (Wetherbee et al., 1987). Extrapolating retention times to a captive situation yields a feeding frequency of 2-3 times per week for both sandbar sharks and lemon sharks.

Studies have shown that the number of empty stomachs in adult sharks (Table 14.5) is higher than for pups and juveniles (Jones and Geen, 1977; Stillwell and Kohler, 1993). Juvenile sharks may eat more often than adults as a result of their higher metabolic rates. At SeaWorld Ohio, USA, neonate sand tiger sharks (*Carcharias taurus*) were fed daily, 1.0-1.5 m TL juveniles were fed three times per week, and adult sharks were fed twice a week.

Not much research has been done on the feeding frequency of rays. On average, their feeding

Table 14.5. Percentage of empty stomachs for different shark species in the wild. Where: [a] = pups and juveniles, and [b] = adults.

Species name	Common name	% of empty stomachs	n	Reference
Carcharhinus amblyrhynchoides	graceful shark	32	186	Stevens and McLoughlin (1991)
Carcharhinus amboinensis	pigeye shark	49	35	Stevens and McLoughlin (1991)
Carcharhinus falciformis	silky shark	97	114	Branstetter (1987a)
		79	24	Stevens and McLoughlin (1991)
Carcharhinus isodon	finetooth shark	39	80	Castro (1993)
Carcharhinus limbatus	blacktip shark	49	174	Castro (1996)
Carcharhinus obscurus	dusky shark	38	59	Gelsleichter et al. (1999)
Carcharhinus plumbeus	sandbar shark	36	181	Stevens and McLoughlin (1991)
		20[a]	94	Stillwell and Kohler (1993)
		51[b]	321	Stillwell and Kohler (1993)
Carcharias taurus	sand tiger shark	22	42	Gelsleichter et al. (1999)
Galeocerdo cuvier	tiger shark	21	98	Stevens and McLoughlin (1991)
Ginglymostoma cirratum	nurse shark	55	91	Castro (2000)
Hemigaleus microstoma	sicklefin weasel shark	26	446	Stevens and McLoughlin (1991)
Mustelus canis	dusky smooth-hound	0	64	Gelsleichter et al. (1999)
Negaprion brevirostris	lemon shark	26	86	Cortés and Gruber (1990)
Rhizoprionodon acutes	milk shark	48	315	Stevens and McLoughlin (1991)
Rhizoprionodon terraenovae	Atlantic sharpnose shark	61	129	Gelsleichter et al. (1999)
Sphyrna lewini	scalloped hammerhead shark	83	59	Branstetter (1987a)
Squalus acanthias	spiny dogfish	31[a]	4155	Jones and Geen (1977)
		38[b]	10641	Jones and Geen (1977)

schedule should probably be similar to that described for sharks. Gray et al. (1997) found only 7 % (n=503) of bat eagle rays (*Myliobatis californica*) to have empty stomachs. This finding suggests that bat eagle rays eat on a regular basis. The team at Burger's Zoo, Arnhem, The Netherlands, feed their spotted eagle rays (*Aetobatus narinari*) 12 times per week over six of seven days.

ESTIMATION OF FEEDING RATION

Several models have been used for measuring food consumption in teleosts (Gerking, 1994) and elasmobranchs (Medved et al., 1988; Cortés and Gruber, 1990). Linear and exponential evacuation models examine the mean weight of food in the stomach, over a 24-hour period, and the hours required for complete gastric digestion. For elasmobranchs, a number of studies have measured stomach weights (Stillwell and Kohler, 1982; Medved et al., 1988; Cortés and Gruber, 1990). The bioenergetics model of estimating food consumption employs a balanced energy equation (Winberg, 1956; Gerking, 1994), where consumption is expressed as the sum of energy used for growth, reproduction, metabolism, and fecal and other excretory wastes. The food values of prey items are expressed as energy, in kcal (Table 14.4). Ultimately, comparisons between the weights of similar-sized wild and captive sharks will give a good indication of whether feeding rations are correct (Mohan, 1996). These techniques are further described in Chapter 15 of this manual.

A literature review of feeding rations for elasmobranchs has been summarized in Table 14.6. Even within a species large differences for feeding ration are observed, making it difficult to predict precisely the ration for a particular species. Feeding ration will depend on the type of food available, the age of the animal, health and hormone cycle, size and shape of the tank, and water temperature and quality.

When using daily feeding rate to calculate weekly ration, the number of days a shark is fed must be taken into consideration. For example, sharks fed at a daily rate of 1% BW day^{-1} three times a week are receiving 3% BW week^{-1} and not 7% BW week^{-1}. Since sharks held at different institutions may be fed at different frequencies, useful comparisons of feeding ration can only be made if intake is expressed as percent body weight per week (i.e., % BW week^{-1}).

To ensure meaningful adjustments of the feeding ration, it is important to observe the collection closely. If possible, monitor gut distension and evacuation to determine how rapidly food is moving through an animal. These observations will give a better understanding of the effect of captive conditions and dietary regime on metabolism. In addition, monitor overall growth, body width behind the head, and un-gorged gut size. These parameters will give an indication of the need to adjust feeding rations.

Food type, and therefore energy content, will of course influence feeding ration. Atlantic herring (*Clupea harengus*) have a much higher food energy, of 223 kcal 100 g^{-1}, than the leaner flounder (*Pleuronectus flesus*), with a food energy of 86 kcal 100 g^{-1} (Table 14.4).

Gruber and Keyes (1981) suggest feeding less to lemon sharks when kept in a larger tank, as unhindered swimming promotes higher metabolic efficiency. In general, juvenile sharks require a higher feeding ration than adults, using the excess energy for growth.

Since feeding ration is dependent on many factors, it is impossible to give an exhaustive list of rations for all elasmobranchs. However, as a starting point, the following feeding rations may be applied to adult animals of the following taxa: (1) bottom dwelling sharks (e.g., Hemiscylliidae and Stegostomatidae) 4-6% BW week^{-1}; (2) slow-swimming ram ventilating sharks (e.g., Odontaspididae) 1-2.5% BW week^{-1}; (3) fast-swimming ram ventilating sharks (e.g., Carcharhinidae) 3-4% BW week^{-1}; (4) bottom dwelling rays (e.g., Dasyatidae) 4-6% BW week^{-1}; and, (5) ram ventilating rays (e.g., Myliobatidae) 4-6% BW week^{-1}. Endotherms may require much higher daily rations than those described above (refer to Chapter 15 of this manual). When feeding juveniles, the ration should be multiplied by a factor of 1.5-3.0. These feeding rations should be considered guidelines only, as they may not be applicable under all circumstances. In particular, feeding rations should be adjusted according to growth data or body weight comparisons with wild sharks (refer to Chapter 15 of this manual), overall appearance, common sense, and where possible, blood analyses. Intentional overfeeding might appear to be a simple and obvious solution to developing a suitable feeding ration. However, this practice should be avoided as rapid growth and body weight gains can lead to permanent physical deformity and poor health.

Table 14.6. Feeding rations for different species of elasmobranchs. Where: [1] refers to data converted from daily to weekly rates (i.e., multiplied by 7). Unless otherwise indicated by (W), for wild caught, all specimens were captive.

Species name	Common name	Temperature (°C)	Age	Feeding ration (% BW week^{-1})	Reference
Carcharhinus leucas	bull shark	23-25	Juveniles-subadults	3.4	Schmid and Murru (1994)
			Adult	2.7	Schmid and Murru (1994)
Carcharhinus limbatus	blacktip shark	18-27	Juveniles	10.0-15.0	Branstetter (1987b)
		25	Juveniles	7.0	Janse (2003)
Carcharhinus melanopterus	blacktip reef shark	25	Juveniles	10-12	Crow et al. (1991)
Carcharhinus plumbeus	sandbar shark	25	Juveniles	6.5-9.1[1] (W)	Medved et al. (1988)
		18-27	Juveniles	10.0-15.0	Branstetter (1987b)
		22-26	Juveniles	22.0	Janse (2003)
		25	Juveniles	6.0	Mohan (1996)
		24-26	Adult	7.0	Janse (2003)
		19-21	Adult	2.6	Janse (2003)
		19-21	Adult	1.2	Janse (2003)
		23	Adult	1.2	Janse (2003)
		21-27	Adult	4.3	Janse (2003)
Carcharias taurus	sand tiger shark	23-24	Adult	1.1-1.3	Mohan (1996)
		24-26	Adult	2.0	Janse (2003)
		19-21	Adult	2.6	Janse (2003)
		19-21	Adult	1.2	Janse (2003)
		21-27	Adult	4.3	Janse (2003)
Isurus oxyrinchus	shortfin mako shark	19	Mix	17.0-21.0 (W)	Stillwell and Kohler (1982)
Negaprion brevirostris	lemon shark	31	Mix	10.5-14.7[1] (W)	Cortés and Gruber (1990)
		25	Adult	8.0	Gruber and Keyes (1981)
		?	?	4.0-7.5	Pike et al. (1993)
Notorynchus cepedianus	broadnose sevengill shark	?	Pups	14.0	Van Dykhuizen and Mollet (1992)
		?	Juveniles	4.2	Van Dykhuizen and Mollet (1992)
		?	Adults	1.4	Van Dykhuizen and Mollet (1992)
Rhizoprionodon terraenovae	Atlantic sharpnose shark	18-27	Juveniles	10.0-15.0	Branstetter (1987b)
Squalus acanthias	spiny dogfish	10	?	9.1 (W)	Brett and Blackburn (1978)
Gymnura altavela	spiny butterfly ray	23	Adults	4.2-8.8	Henningsen (1996)

NUTRITIONAL DEFICIENCY

Nutritional deficiency problems have been infrequently described for elasmobranchs. Most baseline conclusions are derived from teleosts, as reviewed by Tacon (1992). In general, a variety of foods will ensure that most essential nutrients and trace elements are present in the diet.

Controlling feeding rations is an easy way to avoid excessive fats in the diet. Of course, when caring for a multi-species collection, or aggressive elasmobranchs that prey on tank-mates, the control of feeding rations may be more of a challenge. Storage of fat in the liver of elasmobranchs assists with buoyancy control. It is unknown if excess fatty food leads to fatty infiltration of the liver, as has been described for salmonids (Post, 1993) and cod, but such symptoms may be expected. High levels of polyunsaturated fatty acids in the food will increase vitamin E requirements, which acts as an anti-oxidant. Rancid fats may be present in food, through auto-oxidation during storage, and can result in lipid degeneration of the liver, microcytic anemia, and steatitis.

Anemia was observed in horn sharks (*Heterodontus francisci*) at SeaWorld Ohio and was probably caused by the loss of vitamin-rich organs from food fishes during their preparation for feeding. When food was supplemented with liquid vitamins, particularly folic acid and other vitamins used specifically to combat anemia, the problem disappeared. At the same aquarium, blood parameters of sandbar sharks and sand tiger sharks were analyzed twice a year and any vitamin supplementation adjusted according to hematology results.

Poor growth can be a result of either low feeding ration or a vitamin deficiency. Deficiencies in vitamins A, D, E, thiamin, choline, inositol, folic acid, and riboflavin all have an adverse effect on growth (Halver, 1989).

Spinal deformations have been described in the rainbow trout (*Oncorhynchus mykiss*) by Madsen and Dalsgaard (1999) and in some elasmobranchs (Heupel et al., 1999). This phenomenon is possibly caused by vitamin C deficiency, bacterial infections, rapid growth rates, or spinal compromise during capture. More study on the exact cause of this condition is needed.

To date, the only known mineral-related health condition is goiter or thyroid hyperplasia, resulting from iodine deficiency (Gruber and Keyes, 1981; Uchida and Abe, 1987; Pike et al., 1993; Lloyd, 1995; Sondervan, 1997; Van der Veek et al., 2001; Janse, 2003). The symptoms of goiter include a ball-like swelling under the lower jaw which may prevent the shark from eating and ultimately lead to death. An oral treatment (PO) of 10 mg KI kg BW^{-1} has prevented the appearance of goiter in sandbar sharks (Stoskopf, 1993b) (refer Chapter 28 of this manual for more information about goiter).

VITAMIN AND MINERAL SUPPLEMENTATION

Feeding of captive elasmobranchs is mostly done with pre-frozen food, to eliminate the possibility of parasitic infection and ensure the continuity of food availability. Loss of vitamins and minerals due to food transport, storage, and preparation may make vitamin and mineral supplementation necessary. Fennema (1978) has examined vitamin loss from animal tissues during storage. After six months storage at -18°C, oysters lost 22% B1, 0% B2, 35% niacin, and 46% pyridoxine. No figures were given for other vitamins. Water-soluble vitamins can be readily lost from food, through leaching, prior to ingestion. No fat-soluble vitamins will be lost during this process.

It is important to thaw food fishes in air rather than water. Cuzon et al. (1982) noted a 45-98% loss of water-soluble vitamins from pelleted shrimp food after one hour immersion in seawater. In general, the smaller the food particle, and the longer the food remains uneaten in the water, the greater the loss of water-soluble vitamins (Tacon, 1992).

The vitamin and mineral budget of a standard diet can be compared with known vitamin (Table 14.1) and mineral requirements (Table 14.2) to give some insight as to required supplementation. However, be aware that it is not always easy to accurately ascertain the nutritional content of different food items, due to differences in quality of food fishes throughout the year, loss of vitamins during storage and thawing, etc.

A number of workers (Gruber and Keyes, 1981; Branstetter, 1987b; Murru, 1990) and commercial nutrition companies have developed specialized vitamin supplements for elasmobranchs (e.g., Mazuri® Vita-Zu Shark/ray tablets, PMI Nutrition International, Missouri, USA; and Aquavit® tablets, International Zoo Veterinary Group, Keighley, UK).

Vitamin and mineral supplements are mostly added to the food. Vitamins can be added in different forms: powder, liquid, or tablets. When using powder, the

vitamins can be sprinkled over the food, though the vitamins can easily be lost prior to ingestion and the taste of the food may change. A better technique is to inject food items with liquefied vitamin mixtures. During medical procedures, vitamins can be injected intramuscularly (IM) directly into the animals themselves. Tablets offer the most common and successful route for vitamin supplementation. The size of the tablet should be adjusted to suit the size of the food item and the target animal. Tablets can be inserted in the mouth cavity or abdominal cavity of food items or via a small incision under the skin of the food item. The mantle of food squid provides another good avenue for tablet administration. Care must be taken to ensure that the elasmobranch does not discard the tablet during feeding. Use of small food items (that can easily be swallowed whole), and the careful insertion of tablets will reduce the chances of vitamins being lost during ingestion. Finally, vitamins can easily be added to a gelatin-based food mixture, which should consist of food items from the elasmobranch's customary regime so the taste is familiar.

Mineral shortages can be caused by depletion of minerals within the water through complex formation and precipitation (e.g., iodine), or direct loss via a protein skimmer (e.g., calcium). Mineral supple-mentation can be achieved via both food and water. From a study of 15 European public aquariums, only iodine (n=9) and calcium (n=3) were added to the diet of sharks (Janse, 2003). Iodine is the most supplemented mineral, either in food (Gruber and Keyes, 1981; Uchida and Abe, 1987; Pike et al., 1993; Van der Veek et al., 2001; Janse, 2003) or in the water (Sondervan, 1997). Please refer to Chapter 28 of this manual for more information about iodine supplementation.

FOOD STORAGE

It is recommended that all fresh seafood be thoroughly frozen, as soon as possible, for at least 36 hours before thawing and distribution to elasmobranchs. This process reduces the chances of transferring parasitic infections through the diet. Although health problems associated with parasites contracted through the feeding of fresh, unfrozen food have not been described, many parasitic infections do occur within elasmobranchs. Regardless, some workers have fed elasmobranchs fresh fishes without observable health problems related to parasites (Sabalones, 1995; Janse, 2003). The Kattegat-centret, Grena, Denmark, fed fresh food to their sharks, including sand tiger sharks, sandbar sharks, and blacktip reef sharks (*Carcharhinus melanop-terus*), without observable problems (Janse, 2003). It should be noted that there is no diagnostic test which can be used to screen fish on a large scale for infective stages of metazoan parasites.

Ko (1999) mentions that a large number of marine and freshwater fishes can serve as a source of medically important parasitic zoonoses, including trematodiasis, cestodiasis, and nematodiasis. Fish protozoans are not known to infect humans.

Table 14.7. Freshness rating of food fishes (after Anon., 1976; Huss, 1995; Crissey, 1998).

Factor	Acceptable	Inferior	Unacceptable
General appearance	Shine or luster to skin; no breaks in skin; no bloating or protrusion of viscera; no dehydration.	Some loss of sheen.	Luster gone, lumpy.
Eyes	Transparent cornea; black bright pupil; may be slightly sunken.	Dull or cloudy; slightly sunken.	Dull; sunken; cornea opaque (white); red-bordered eyes.
Gills	Bright red to pink; moist; no mucus.	Pink to slight brownish; traces of clear mucus.	Grayish-yellow and covered with milky mucus.
Skin	Bright, iridescent pigmentation, no discoloration; aqueous transparent mucus.	Pigmentation in process of becoming discolored; cloudy mucus.	Dull pigmentation or advanced state of decay; opaque mucus; may split when handled.
Smell	Fresh odor; seaweed smell.	Mild sour or 'fish' odor.	Medium to strong odor, fatty fish may smell rancid.
Feel	Firm and elastic; meat does not stay indented when touched.	Moderately soft; slight indentation left when touched.	Soft, spongy and flabby; exudes juice and easily indented when handled.

Using fresh fish may only be possible when the aquarium is located near a fishing port, where continuity of supply is assured. Fresh food should be thoroughly inspected for unusual coloration, damage, or external parasites, like anchor worms, ciliates, crustaceans, and trematodes. Table 14.7 summarizes suitable criteria for assessing food fish quality. It should be noted that even with this examination, possible parasitic infection might occur.

Spoilage as a result of microbial activity can be stopped, and deterioration as a result of biochemical and physical changes can be retarded, using frozen storage conditions. It is advised for lean fish, like Atlantic cod (*Gadus morhua*) and European plaice (*Pleuronectes platessa*), to freeze at a temperature of -20°C, while for fatty fish, such as herring (*Clupea* spp.) and mackerel (*Scomber* spp.), to freeze at an even lower temperature of -30°C (Keizer, 1995). Crissey (1998) advises a storage temperature of -23°C or lower. Freezing rates for fresh fishes depend on the size and type of species. When freezing is too slow, large ice crystals can form intercellularly and intracellularly, resulting in damage to the cell walls, excessive cell dehydration, and protein agglomeration (denaturation). Freezing rates of 0.15-5.0 cm h^{-1} are adequate and show no difference in quality (Keizer, 1995).

Decrease in nutrient content during freezing can be traced to protein denaturation, lipid oxidation, dehydration, and loss of vitamins (Huss, 1995; Keizer, 1995; Crissey, 1998). To prevent surface dehydration (freezer burn) as a result of low humidity in the freezer, fishes should be packed in plastic (Keizer, 1995) or closed containers. Also glazing, i.e. the application of a thin layer of ice on already frozen products, will provide protection against dehydration and the oxidation of lipids in fatty acids (Keizer, 1995).

Different nutrient losses have been described in stored frozen seafood. Omotosho and Olu (1995) found a decrease in percentage of total lipid, total protein, and moisture content, after frozen storage, for different African freshwater fishes. A decrease in HUFA was shown in Atlantic salmon (*Salmo salar*) stored at -10°C and -20°C (Refsgaard et al., 1998). During a six-month freezing period, oyster lost thiamin (vitamin B1), riboflavin (vitamin B2), niacin, panthothenic acid, and pyridoxine (vitamin B6) (Fennema, 1978). Vitamin E is destroyed during fat oxidation. The extent to which vitamin E is lost depends on the fat content of the fish (Crissey, 1998). Thiaminases are naturally present in fish tissue and may destroy thiamin during storage (Huss,1995; Crissey, 1998).

In general, frozen storage should not exceed 6-12 months and freezer management strategies should ensure that the oldest food is used first.

THAWING AND HYGIENE

Thawing should take place in a covered container or in a closed plastic bag in a refrigerator to prevent bacterial contamination and minimize loss of vitamins. It is suggested that food be removed from the freezer and placed in a refrigerator, to thaw overnight, before preparation and distribution the next day. Crissey (1998) advises that thawing temperature should not exceed 7°C. The rate of thawing is less important, with respect to physical changes to the food, than the rate of freezing.

Thawing in cold or hot standing water is not recommended as this can cause loss of nutrients and an increased microbial activity, resulting in food spoilage (Crissey, 1998). Further, it is not recommended to thaw food using hot or cold running water, as essential water-soluble nutrients, like water-soluble vitamins, can be lost. Loss of nutrients may be less when whole fish, with an intact protective skin, are thawed.

If a large block of fishes is to be thawed, it is advisable to remove the outer, thawed fish as the block defrosts. This helps to ensure thawing of the remaining fishes, while protecting the outer fishes from thawing and sitting unpreserved for a prolonged period (Crissey, 1998).

Proper hygiene should be observed when handling any food items. All cutting surfaces, work areas and preparation surfaces should be cleaned with hot water and a dilute bleach solution to prevent bacterial growth. Other disinfectants can be used, though care should be taken to use products that do not leave residues on preparation surfaces. All knives, strainers, storage containers, and distribution containers should be thoroughly disinfected with hot water and soap after each use.

FOOD PREPARATION

Foods should be distributed as soon as possible after thawing. Frozen foods, once thawed should never be frozen again. If not fed, fish must be discarded 24 hours after removal from the freezer or, if thawed under refrigeration, 24 hours after being thawed (Crissey, 1998). Thawed diets, ready for distribution, should be stored in a refrigerated area and covered with bags or container lids.

Figure 14.1. An assortment of poles, tongs, and other utensils used to target feed elasmobranchs in captivity.

Food items should be of a size that best allows the elasmobranch to feed naturally, whereby the animal can easily grasp and swallow the food in line with its natural feeding behavior. When possible, food fishes should be fed whole so as to include the nutritional content of the internal organs. Feeding only processed fish parts will result in a diminished nutritional value. Removing the head of food fishes results in a substantial decreased calcium content (Crissey, 1998). When cutting food into smaller parts it is advisable to leave the intestine within the food fishes.

When feeding cuttlefish or squid, pens and beaks should be removed prior to feeding. If cut, cephalopods should be cut into strips rather than rings. A ring of squid was entangled around the snout of a starry smooth-hound (*Mustelus asterias*) held at the SeaLife Centre, Scheveningen, Netherlands. The ring of squid could only be removed after catching and restraining the animal.

When feeding bivalves, it is recommended to open the shells and rinse the meat with cool water to remove excess sediment. Bivalve meat can be removed or the bivalve can be fed whole for enrichment. Caution and careful monitoring should be observed when feeding bivalves in this manner. Burgers' Zoo had an Australian cownose ray (*Rhinoptera neglecta*) die from a perforated intestine, inflicted by a shard of razorshell (*Ensis siliqua*). The telsons of food shrimp may cause the same type of injury and should be removed before feeding.

FOOD DISTRIBUTION

Different food distribution techniques have been used to feed elasmobranchs in captivity. Intraspecific and interspecific competition should be reduced as much as possible during feeding. Throwing food in front of animals is the least preferred method of distribution. Individual or target feeding is a more accurate and safer method. Different utensils may be used to target feed elasmobranchs (Figure 14.1). Large tongs, like snake tongs (e.g., Fuhrman Diversified Inc., LaPorte, Texas, USA), should be used for large sharks (Demetrios and Denardo, 1995; Janse, 2003). Bamboo or PVC poles, poles with an attachment clip or airline tubing at the end (Van Dykhuizen and Mollet, 1992), freshwater plant tongs, or aluminum salad tongs can all be used for smaller shark species and most ray species. Feeding specific individuals an appropriate diet and ration is faciliated by target feeding. Target feeding will enable the recording of type and weight of food eaten, vitamins given, changes in appetite, etc. Having a second staff member monitor the animals and record the details of each feeding session will improve the chances of target animals receiving the correct ration and dietary regime.

The use of feeding stations may reduce competition between species and overcrowding within the feeding area. Care must be taken to choose an appropriate feeding station so that unhindered swimming can take place both before and after food acceptance. Feeding sharks beneath the water surface will help protect animal handlers on feeding decks or platforms, preventing conditioned jumping or lunging behavior. In multi-species exhibits, it is recommended to assign conspecifics to the same feeding station. It may be helpful to assign those individuals with the same temperament to the same feeding station. The application of vitamins, within designated food items, and administration of oral medications, will be much easier to achieve when using feeding stations.

Care must be taken to ensure that excess food is not lost into the water column or to competing species. Food lost to the water column and not consumed should be removed immediately, or within 10-15 minutes of the end of the feeding session. Food distribution should not be so slow that the collection loses interest in feeding and should not be so rapid that the collection becomes gorged before consumption of the total ration.

Special feeding devices may be required to effectively feed all the collection. For example, to feed rays near the water surface at Burger's Zoo a feeding ramp (inclined at 45°-60°) was constructed of solid and perforated (for the sides and bottom) PVC sheet. This device, partially submersed when in use, prevented other nuisance fishes (e.g., golden trevally, *Gnathanodon speciosus*, and emperor red snapper, *Lutjanus sebae*) from stealing food from rays (i.e., spotted eagle rays and cownose rays, *Rhinoptera bonasus*) during feeding.

At some aquariums, sharks are fed by divers (Janse, 2003). Larger sharks (e.g., carcharhinids or sand

tiger sharks) can become a problem when they associate divers with food, making diving for other activities more risky. The practice of feeding sharks while diving is not allowed in some countries under the relevant Health and Safety legislation. In general, it is advised to feed elasmobranchs at the surface so that sharks do not associate the divers with food (refer to Chapter 12 of this manual for more detail).

CONDITIONING FOR TARGET FEEDING

It is easier to condition a newly-arrived elasmobranch to feed by pole and/or at a feeding station in quarantine, alone wherever possible, where they can be trained immediately. Where practical, an animal should not be transferred to the exhibit before it is feeding consistently in the manner required.

To condition the shark, throw food in front of the swimming animal in the area where you ultimately want it to station feed. When the animal feeds without hesitation, station training can commence. Start by feeding with a pole, always at the same place. If the animal is too shy, leave the feeding pole inside the tank and throw food in the area adjacent to the feeding station and pole. When feeding swimming sharks or rays, maintain sufficient distances from the side of the tank so that the animals can turn easily. When feeding sedentary sharks or rays, a feeding pole extending to the bottom is recommended. If the display tank is too deep, condition the elasmobranchs to come to the surface, via the side of the tank, and feed at the surface.

INAPPETANCE AND FEEDING ACCLIMATION

Recently acquired or newborn elasmobranchs may exhibit periods of anorexia or inappetance. New acquisitions will use their body reserves to receive nutriment during such periods. The maximum sustainable starvation period for captive sharks varies between species. Gruber and Keyes (1981) mention a loss of about 1.0% BW day^{-1} for young lemon sharks when experimentally fasted. Two examples in the literature show extreme starvation periods of three months for the sand tiger shark (Sabalones, 1995) and five months for a three meter tiger shark, (*Galeocerdo cuvier*) (Clark, 1963). In both cases, the sharks commenced feeding after the period of inappetance. If starvation continues for an extended period, tube-feeding may become necessary (see below).

Newborn elasmobranchs will not start actively feeding until the last of their yolk has been absorbed. Recognizing when an elasmobranch is ready to begin feeding takes careful observation and experience. Once metabolically ready, using a wide variety of foods may help induce the animal to start eating. When available fresh food or even live animals (e.g., fishes, shrimp, decapods, brine and mysid shrimp, shellfish, or earthworms) can help induce feeding.

In the case of newborns and new arrivals, the importance of getting the animal to commence feeding outweighs the risk of parasitic contamination through fresh or live foods. To diminish the risk of parasitism, it is advisable to give the animals a prophylactic treatment once they have started to feed consistently. Elasmobranchs should be moved to a non-live diet as soon as possible. Gradually adding non-live items to a live diet regime, until the diet is completely non-living, is often the easiest method. Another possibility is to feed the rear two-thirds of a food fish, including the tail, causing the food fish to flutter as it sinks through the water column, imitating the swimming motion of a small live fish. Fresh garlic mixed through the food may help trigger feeding in some cases.

At the Aquarium of the Bay, San Francisco, USA, a Pacific angelshark (*Squatina californica*) was fed using a pole with an electrical tape marker on the end. Food fishes were speared through the jaw with the taped end of the pole. The person feeding the shark would stand behind the animal and move the food fish from left to right imitating a live fish, inducing the shark to feed (Howard, pers. com.). This technique was adapted from Fouts and Nelson (1999).

Anorexia may be the result of hormonal changes, exhibit stressors, disease, etc. It is important to understand the cause of anorexia in order to determine how and when to intervene.

TUBE-FEEDING

In some cases, tube-feeding or force-feeding may be necessary to prevent dehydration and extreme body weight loss. This is a potentially stressful procedure and should be delayed as long as possible, without getting to the point of no return (i.e., where the elasmobranch cannot recover from the chronic lack of nutrients). It is advised to start tube-feeding at a body weight decrease of 5%. SeaWorld, Orlando, USA, commences tube-feeding adult elasmobranchs when they have been anorectic for four weeks, six weeks at the absolute extreme.

Food to be tube-fed should be thawed and well blended. Distilled water, electrolytes, or sterile saline should be added to the slurry until it reaches the proper consistency. The mixture should include appetite stimulants and B vitamins.

Tube-feeding or force-feeding is performed using a length of PVC or stainless steel pipe to deliver food into the animal's stomach. At the Artis Aquarium, Amsterdam, The Netherlands, elasmobranch species were tube-fed using stainless steel pipes, 12 mm diameter x 200 mm long or 22 mm diameter x 300 mm long, depending on the size of the animal. The end of the pipes were cut off at an angle of 40° (to the cross section) and the edges rounded. The pipe is carefully inserted through the mouth of an animal and into its esophagus. Care must be taken not to push the pipe too far into the stomach. Food is inserted directly into the pipe and may be gently pushed into the stomach using a small plunging rod. In this manner, larger food particles can be administered, including vitamins or medicines. This technique was successfully applied to horn sharks, Arabian carpetsharks (*Chiloscyllium arabicum*), whitespotted bamboosharks (*Chiloscyllium plagiosum*), brownbanded bamboosharks (*Chiloscyllium punctatum*), smallspotted catsharks (*Scyliorhinus canicula*), and short-tailed nurse sharks

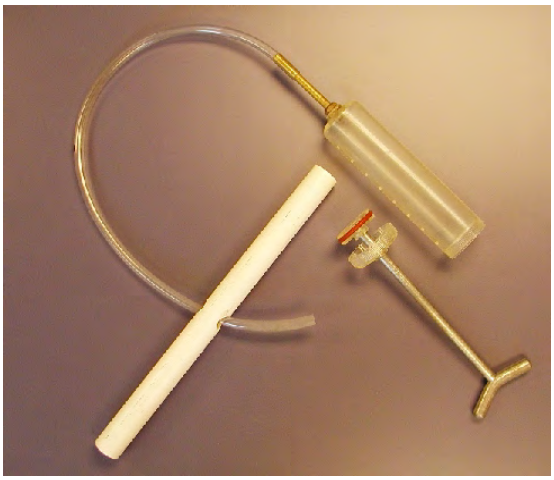


Figure 14.2. Example of a tube-feeding device used to feed blacktip reef sharks (*Carcharhinus melanopterus*) at the Virginia Aquarium and Marine Science Center, Virginia Beach, USA.

(*Ginglymostoma brevicaudatum*) (Dral, pers. com.). Stainless steel may be preferable to PVC as the shark's teeth can scrape plastic shavings off the PVC pipe, which may then be ingested by the animal.

Henningsen (1994) has used a form of hypnotism, tonic immobility (TI), to make force-feeding easier. Sharks and rays were caught and quickly turned on their back, forcing the animals to go into a trance-like state and making handling easier. Henningsen (1994) found that it took 17-46 seconds before the animals became immobile. TI has been induced in leopard sharks (*Triakis semifasciata*), whitetip reef sharks (*Triaenodon obesus*), blacktip reef sharks, Caribbean reef sharks (*Carcharhinus perezi*), swellsharks (*Cephaloscyllium ventriosum*) (Henningsen, 1994), lemon sharks (Gruber and Keyes, 1981), dusky smoothhounds (Whitman et al., 1986), Haller's round rays (*Urolophus halleri*), shovelnose guitarfish (*Rhinobatos productus*), clearnose skates (*Raja eglanteria*), cownose rays, southern stingrays (*Dasyatis americana*) (Henningsen, 1994), and spiny butterfly rays (*Gymnura altavela*) (Henningsen, 1996).

Henningsen (1996) describes a force-feeding technique for spiny butterfly rays whereby a mixture of squid (*Illex* sp.), Atlantic rainbow smelt (*Osmerus mordax mordax*), shrimp (*Penaeus* sp.), and multi-vitamins were given using a tube inserted into the esophagus. Initially, the blended mixture contained ~70% water, but was decreased toward ~25% water. A similar mixture was used at the Virginia Aquarium and Marine Science Center, Virginia Beach, USA, where juvenile blacktip reef sharks were fed with the apparatus shown in Figure 14.2. The recipe included a gelatin-based carnivore diet (Purina Test Diets, Richmond, Indiana, USA) combined with squid and white fish filets. The animals were lightly sedated with Telazol (Tiletamine HCl 100 mg ml^{-1}, Fort Dodge, USA) at a dosage of 10 mg IM for each shark. Induction time was ~10-20 minutes, depending on the state of the shark when first injected.

ACKNOWLEDGEMENTS

We would like to thank Michael Howard for his description of the technique to feed angel sharks, and Anton Dral for the tube-feeding technique.

REFERENCES

Ackman, R. G. 1995. Composition and nutritive value of fish and shellfish lipids. *In:* Fish and Fishery Products, Composition, Nutritive Properties and Stability, p. 117-156. A. Ruiter (ed.). CAB International, Wallingford, Oxfordshire, UK.

Anon. 1976. Laying down common marketing standards for certain fresh or chilled fish. Council Regulation No. 103/76 of 28 January 1976. European Economic Community. Journal of the European Economic Community, Brussels, Belgium L20: 29-34.

Anon. 1981. Nutrient Requirements of Coldwater Fishes. National Research Council. National Academy Press, Washington, DC, USA. 63 p.

Anon. 1983. Nutrient Requirements of Warmwater Fishes and Shellfishes. National Research Council. National Academy Press, Washington, DC, USA. 63 p.

Bowen, S. H. 1987. Dietary protein requirements of fishes-a reassessment. Canadian Journal of Fisheries and Aquatic Sciences 44: 1995-2001.

Boonyaratpalin, M. 1997. Nutrient requirements of marine food fish culture in Southeast Asia. Aquaculture 151: 283-313.

Branstetter, S. 1987a. Age, growth and reproductive biology of the silky shark, *Carcharhinus falciformis*, and the scalloped hammerhead, *Sphyrna lewini*, from the Northwestern Gulf of Mexico. Environmental Biology of Fishes 19(3): 161-173.

Branstetter, S. 1987b. Age and growth validation of newborn sharks held in laboratory aquaria, with comments on the life history of the Atlantic sharpnose shark, *Rhizoprionodon terraenovae*. Copeia 1987(2): 291-300.

Brett, J. R. and J. M. Blackburn. 1978. Metabolic rate and energy expenditure of the spiny dogfish, *Squalus acanthias*. Journal of the Fisheries Research Board Canada 35: 816-821.

Castro, J. I. 1989. The biology of the golden hammerhead, *Sphyrna tudes*, off Trinidad. Environmental Biology of Fishes 24(1): 3-11.

Castro, J. I. 1993. The biology of the finetooth shark, *Carcharhinus isodon*. Environmental Biology of Fishes 36: 219-232.

Castro, J. I. 1996. Biology of the blacktip shark, *Carcharhinus limbatus*, off the Southeastern United States. Bulletin of Marine Science 59(3): 508-522.

Castro, J. I. 2000. The biology of the nurse shark, *Ginglymostoma cirratum* off the Florida east coast and the Bahamas Islands. Environmental Biology of Fishes 58: 1-22.

Chow, K. W. and W. R. Schell. 1980. The minerals. *In:* Aquaculture Development and Coordination Programme, Fish Feed Technology, p. 104-108. United Nations Development Programme/Food and Agriculture Organization of the United Nations, Rome, Italy.

Clark, E. 1963. Maintenance of sharks in captivity with a report on their instrumental conditioning. *In:* Sharks and Survival, p. 115-149. P. W. Gilbert (ed.). D. C. Heath and Co., Boston, Massachusetts, USA.

Crissey, S. D. 1998. Handling fish fed to fish-eating animals. A manual of standard operating procedures. U.S. Department of Agriculture, Agricultural Research Services, National Agricultural Library, USA. 21 p.

Compagno, L. J. V. 1984. FAO Species Catalogue. Sharks of the world. FAO Fisheries Synopsis 125(4): 1- 655.

Compagno, L. J. V., D. A. Ebert, and M. J. Smale. 1989. Guide to the Sharks and Rays of Southern Africa. Struik Publishers, Cape Town, South Africa. 160 p.

Cortés, E. and S. H. Gruber. 1990. Diet, feeding habits and estimates of daily ration of young lemon sharks, *Negaprion brevirostris* (Poey). Copeia 1990(1): 204-208.

Cowey, C. B., A. M. Mackie, and J. G. Bell. 1985. Nutrition and Feeding of Fish. Academic Press, Orlando, Florida, USA. 512 p.

Crow, G. L., J. A. Brock, J. C. Howe, and B. E. Linnon. 1991. Shark bite wounds of the valvular intestine: the cause of an acute mortality syndrome of captive blacktip reef sharks, *Carcharhinus melanopterus*. Zoo Biology 10: 457-463.

Cuzon, G., M. Hew, and D. Cognie. 1982. Time lag effects of feeding on growth of shrimp *Penaeus japonicus* (Bate). Aquaculture 29: 33-44.

Demetrios, M. B. and D. Denardo. 1995. Marine World - Africa USA's Shark Experience. International Zoo Yearbook 34: 87-95.

De Silva, S. S. and T. A. Anderson. 1995. Fish Nutrition in Aquaculture. Chapman & Hall Aquaculture Series 1. Chapman & Hall, London. England. 319 p.

Ensminger, A. H., M. E. Ensminger, J. E. Konlande, and J. R. K. Robson. 1995. The Concise Encyclopedia of Foods and Nutrition. CRC International, London, England. 1178 p.

Fennema, O. 1978. Effect of processing on nutritive value of food: freezing. *In:* Handbook of Nutritive Value of Processed Food, Volume 1: Food for Human Use, p. 31-43. M. Rechcigl Jr. (ed.). CRC Series, Boca Raton, FL, USA.

Fouts, W. R. and D. R. Nelson. 1999. Prey capture by the Pacific angel shark, *Squatina californica*: Visually mediated strikes and ambush-site characteristics. Copeia 1999(2): 304-312.

Gelsleichter, J., J. A. Musick, and S. Nichols. 1999. Food habits of the smooth dogfish, *Mustelus canis*, dusky shark, *Carcharhinus obscurus*, Atlantic sharpnose shark, *Rhizoprionodon terraenovae*, and the sand tiger, *Carcharias taurus*, from the Northwest Atlantic Ocean. Environmental Biology of Fishes 54: 205-217.

Gerking, S. D. 1994. Feeding Ecology of Fish, Academic Press, San Diego, USA. 348 p.

Gray, A. E., T. J. Mulligan, and R. W. Hannah. 1997. Food habits, occurrence, and population structure of the bat ray, *Myliobatis californica*, in Humboldt Bay, California. Environmental Biology of Fishes 49: 227-238.

Gruber, S. H. and R. A. Keyes. 1981. Keeping sharks for research. *In:* Aquarium Systems, p. 373-402. A. D. Hawkins (ed.). Academic Press, New York, USA.

Halver, J. E. 1979. Vitamin requirements of finfish. *In:* Finfish Nutrition and Fish Feed Technology, Volume I, p. 45-58. J. E. Halver, and K. Tiews (eds.). Heenemann Vergsgesel, Berlin, Germany.

Halver, J. E. 1989. The vitamins. *In:* Fish Nutrition, p. 32-109. J. E. Halver (ed.). Academic Press, San Diego, California, USA.

Henningsen, A. D. 1994. Tonic immobility in 12 elasmobranchs: use as an aid in captive husbandry. Zoo Biology 13: 325-332.

Henningsen, A. D. 1996. Captive husbandry and bioenergetics of the spiny butterfly ray, *Gymnura altavela* (Linnaeus). Zoo Biology 15: 135-142.

Hepher, B. 1988. Nutrition of Pond Fishes. Cambridge University Press, Cambridge, England. 388 p.

Heupel, M. R., C. A. Simpfendorfer, and M. B. Bennett. 1999. Skeletal deformities in elasmobranchs from Australian waters. Journal of Fish Biology 54: 1111-1115.

Hilton, J. W., P. V. Hodson, and S. J. Slinger. 1980. The requirements and toxicity of selenium in rainbow trout (*Salmo gairdneri*). Journal of Nutrition 110: 2527-2535.

Huss, H. H. 1995. Quality and quality changes in fresh fish. FAO Fisheries Technical Paper No. 348, Rome, Italy. 195 p.

Janse, M. 2003. Considerations on diet composition and feeding rate of dermersal sharks in 15 European public aquaria. Zoo Biology 22: 203-226.

Jones, B. C. and G. H. Geen, 1977. Food and feeding of spiny dogfish (*Squalus acanthias*) in British Columbia waters. Journal of the Fisheries Research Board Canada 34: 2067-2078.

Karakoltsidis, P. A., A. Zotos, and M. Constantinides. 1995. Composition of the commercially important Mediterranean finfish, crustaceans, and mollusks. Journal of Food Composition and Analysis 8: 258-273.

Keizer, C. 1995. Freezing and chilling of fish. *In:* Fish and Fishery Products. Composition, Nutritive property and Stability, p. 287-313. A. Ruiter (ed.). CAB International, Wallingford, UK.

Ko, R. C. 1999. Fish-borne parasitic zoonoses. *In:* Fish Diseases and Disorders. Volume 1. Protozoan and Metazoan Infections, p. 631-671. P. T. K. Woo (ed.). CABI Publishing, Wallingford, UK.

Lall, S. P. and M. P. Parazo. 1995. Vitamins in fish and shellfish. *In:* Fish and Fishery Products, Composition, Nutritive Properties and Stability, p. 157-186. A. Ruiter (ed.). CAB International, Wallingford, UK.

Lloyd, N. 1995. Treatment of goiter in Atlantic nurse sharks *Ginglymostoma cirratum* at the Blackpool Sea Life Centre. International Zoo Yearbook 34: 95-98.

Lytle, J. S. and T. F. Lytle. 1994. Fatty acid and cholesterol content of sharks and rays. Journal of Food Composition and Analysis 7: 110-118.

Madsen, L. and I. Dalsgaard. 1999. Vertebral column deformities in farmed rainbow trout (*Oncorhynchus mykiss*). Aquaculture 171: 41-48.

Medved, R. J. 1985. Gastric evacuation in the sandbar shark, *Carcharhinus plumbeus*. Journal of Fish Biology 26: 239-253.

Medved, R. J., C. E. Stillwell, and J. G. Casey. 1988. The rate of food consumption of young Sandbar sharks (*Carcharhinus plumbeus*) in Chincoteague Bay, Virginia. Copeia 1988(4): 956-963.

Mohan, P. J. 1996. Using fisheries data to manage the diets of captive elasmobranchs. *In:* AZA Annual Conference Proceedings, September 17-21, Waikiki, Honolulu, Hawaii, p. 265-273. American Zoo and Aquarium Association, Silver Spring, Maryland, USA.

Murru, F. L. 1990. The care and maintenance of elasmobranchs in controlled environments. *In:* Elasmobranchs as Living Resources: Advances in Biology, Ecology, Systematics, and the Status of Fisheries, p. 203-209. H. R. Pratt, S. H. Gruber, and T. Taniuchi (eds.). U.S. Department of Commerce, NOAA Technical Report 90.

Omotosho, J. S. and O. O. Olu. 1995. The effect of food and frozen storage on the nutrient composition of some African fishes. Revista de Biologia Tropical 43(1-3): 289-295.

Pike, C. S., C. A. Manire, and S. H. Gruber. 1993. Nutrition and nutritional diseases in Sharks. *In:* Fish Medicine, p. 763-769. M. K. Stoskopf (ed). W. B. Saunders Company, Harcourt Brace Jovanovich, Inc., Philadelphia, Pennsylvania, USA.

Post, G. W. 1993. Nutrition and nutritional diseases in Salmonids. *In:* Fish Medicine, p. 343-358. M. K. Stoskopf (ed). W. B. Saunders Company, Harcourt Brace Jovanovich, Inc., Philadelphia, Pennsylvania, USA.

Refsgaard, H. H. F., P. B. Brockhoff, and B. Jensen. 1998. Sensory and chemical changes in farmed Atlantic salmon (*Salmo salar*) during frozen storage. Journal of Agricultural Food Chemistry 46: 3473-3479.

Sabalones, J. 1995. Considerations on the husbandry of sharks for display purposes. International Zoo Yearbook 34: 77-87.

Sargent, J., L. McEvoy, A. Estevez, G. Bell, M. Bell, J. Henderson, and D. Toucher. 1999. Lipid nutrition of marine fish during early development: Current status and future directions. Aquaculture 179: 217-229.

Scherz, H. and F. Senser. 1994. Food Composition and Nutrition Tables. Medpharm GmbH Scientific Publishers, Stuttgart, Germany. 1091 p.

Schmid, T. H. and F. L. Murru. 1994. Bioenergetics of the bull shark, *Carcharhinus leucas*, maintained in captivity. Zoo Biology 13: 177-185.

Sidwell, V. D. 1981. Chemical and nutritional composition of finfishes, whales, crustaceans, mollusks and their products. NOAA Tech. Memo. NMFS F/SEC-11, US Department of Commerce, Washington, DC, USA. 432 p.

Sidwell, V. D., P. R. Foncannon, N. S. Moore, and J. C. Bonnet. 1974. Composition of the edible portion of raw (fresh or frozen) crustaceans, finfish, and mollusks, I. Protein, fat, moisture, ash, carbohydrate, energy value, and cholesterol. Marine Fisheries Review 36(3): 21-35.

Sondervan, P. 1997. Iodine in seawater: Measurements and policy in aquarium systems. *In:* Proceedings of the European Union of Aquarium Curators, October 6-12, 1996, Munich, Salzburg, and Innsbruck, p. 15-19. Memoires de l'institut Oceanographique Paul Recard, Marseille, France.

Stevens, J. D. and K. J. McLoughlin. 1991. Distribution, size and sex composition, reproductive biology and diet of sharks from Northern Australia. Australian Journal of Marine and Freshwater Research 42: 151-199.

Stillwell, C. E., and N. E. Kohler. 1982. Food, feeding habits and estimates of daily ration of the shortfin mako (*Isurus oxyrinchus*) in the Northwest Atlantic. Canadian Journal of Fisheries and Aquatic Sciences 39: 407-414.

Stillwell, C. E. and N. E. Kohler. 1993. Food habits of the Sandbar shark *Carcharhinus plumbeus* off the U.S. Northeast coast, with estimates of daily ration. US NMFS Fisheries Bulletin 91: 138-150.

Stoskopf, M. K. 1993a Clinical pathology of sharks, skates, and rays, *In:* Fish Medicine, p. 754-757. M. K. Stoskopf (ed). W. B. Saunders Company, Harcourt Brace Jovanovich, Inc., Philadelphia, Pennsylvania, USA.

Stoskopf, M. K. 1993b Shark pharmacology and toxicology. *In:* Fish Medicine, p. 809-816. M. K. Stoskopf (ed). W. B. Saunders Company, Harcourt Brace Jovanovich, Inc., Philadelphia, Pennsylvania, USA.

Tacon, A. G. J. 1992. Nutritional fish pathology. Morphological signs of nutrient deficiency and toxicity in farmed fish. FAO Fish Technical paper, No. 330, Food and Agriculture Organization of the United Nations, Rome, Italy. 75 p.

Uchida, H. and Abe, Y. 1987. The prevention of goiter in captive sharks. International Zoo Yearbook 26: 59-61.

Van Dykhuizen, G. and H. F. Mollet. 1992. Growth, age estimation and feeding of captive Sevengill sharks, *Notorynchus cepedianus*, at the Monterey Bay Aquarium. Australian Journal of Marine and Freshwater Research 43: 297-318.

Van der Veek, Y. J. M., G. M. Dorrestein, J. A. Mol, and P. Mclean. 2001. The effect of administration of potassium iodine on goiter and serum T4 levels in lesser spotted dogfish (*Scyliorhinus canicula*). Verhandlungsberichte der Internationalen Symposien über die Erkrankungen der Zootiere 2001: 35-40.

Wetherbee, B. M. and S. H. Gruber. 1990. The effects of ration level on food retention time in juvenile lemon sharks, *Negaprion brevirostris*. Environmental Biology of Fishes 29: 59-65.

Wetherbee, B. M., S. H. Gruber, and A. L. Ramsey. 1987. X-radiographic observation of food passage through digestive tracts of lemon sharks. Transactions of the American Fisheries Society 116: 763-767.

Whitman, P. A., J. A. Marshal, and E. C. Keller. 1986. Tonic immobility in the smooth dogfish shark, *Mustelus canis* (Pisces, Carcharhinidae). Copeia (3): 829-832.

Winberg, G. G. 1956. Rate of metabolism and food requirements of fishes. Journal Fisheries Research Board Canada 194: 1960.

PERSONAL COMMUNICATIONS

Dral, A. 2002. Artis Zoo, Amsterdam, the Netherlands, NL-1018 CZ

Howard, M. 2002. Aquarium of the Bay, San Francisco, CA 94133, USA.

The Elasmobranch Husbandry Manual: Captive Care of Sharks, Rays and their Relatives, pages 201-226.
© 2004 Ohio Biological Survey

Chapter 15
Age and Growth of Captive Sharks

PETER J. MOHAN

5802 Thorndale Drive,
Kent, OH 44240, USA.
E-mail: petemohan@aol.com

STEVEN T. CLARK

SeaWorld Inc.
7007 SeaWorld Drive,
Orlando, Florida 32821-2377, USA.
E-mail: steve.clark@seaworld.com

THOMAS H. SCHMID

Texas State Aquarium
2710 North Shoreline,
Corpus Christi, Texas 78251-3002, USA.
E-mail: tschmid@txstateaq.org

Abstract: Understanding the relationship between food intake and growth in elasmobranchs facilitates the establishment of appropriate diets, health management protocols, and collection plans. The Von Bertalanffy growth function (VBGF) is an appropriate method for estimating age and growth parameters. Comparisons between actual captive measurements and mass predictions based on length-weight equations for wild sharks are useful when determining the condition (W_r) of aquarium-held sharks.

This chapter summarizes what is known about the growth rates of commonly-held sharks, both in aquariums and in the wild. Length-weight formulae are provided for a variety of species, and, where known, food rations required to maintain ideal condition are presented.

While some species of sharks seem to thrive at public aquariums without detailed attention to food intake and growth, the long-term health of others, such as sand tiger (*Carcharias taurus*) and sandbar (*Carcharhinus plumbeus*) sharks can benefit from regular weight monitoring and comparison with wild populations (Mohan, 1996). The benefits to these common sharks suggest that attempts at holding delicate species, whose husbandry is not fully established, should only be made after consulting the available literature on age, growth, and diet ration in the wild. We will lay a partial foundation for the captive husbandry of two such desirable species, which to date have not been kept successfully in public aquariums, the white (*Carcharodon carcharias*) and shortfin mako (*Isurus oxyrinchus*) sharks.

Basic research on age and growth in elasmobranchs can benefit from careful accumulation of morphometric data (i.e., measurements) taken from captive sharks held in public aquariums. This is the only environment where precisely timed repeated measures of individual animals can be accomplished.

Among elasmobranchs, the analysis of vertebral centrum rings, or annuli, has been one of the most widely-used and accepted methods of aging individuals (Cailliet et al., 1983) and has been used in a number of shark life history studies (Clarke, 1971; Hoenig, 1979; Thorson and Lacy, 1982; Schwartz, 1983; Cailliet et al., 1986; Branstetter, 1987a; Branstetter, 1987b; Brown and Gruber, 1988; Cailliet et al., 1990; Chen et al., 1990; Branstetter and Musick, 1994; Sminkey and Musick, 1995). As with any age and growth study, it is crucial to accurately determine size (e.g., body length and/or weight) and age. Length and weight measurements are fairly straightforward. However, two inherent assumptions about annulus formation may affect the use of vertebral centra to document age in fishes: (1) centrum rings are thought to be laid down annually, or at least regularly; and (2) annuli can be accurately enumerated and measured. These assumptions are not always valid, leading to difficulty or inaccuracy in age determination, certainly affecting calculations of size-at-age and growth rates.

Rigorous measurement of growth in captive-born sharks is especially beneficial to the scientific community when unforeseen mortalities offer the opportunity to match vertebral annuli with well-documented changes in length.

Successes in the maintenance of captive sharks and rays have afforded researchers the opportunity to gather life history information that may be difficult to obtain from animals in the wild (e.g., Uchida et al., 1990; Van Dykhuizen and Mollet, 1992; Schmid and Murru, 1994; Henningsen, 2000).

While information from age and growth studies on sharks in aquariums may provide valuable life history information, the possible influences of captivity cannot be ignored. Among wild conspecifics, investigators have reported contrasting growth rates at different localities. For example, Branstetter and McEachran (1986) cite the possible influence of water temperature (Wass, 1973), dissolved oxygen (Pauly, 1979; Casey et al., 1985), and predation pressure (Branstetter, 1987b) on growth rate in the silky shark (*Carcharhinus falciformis*). It is reasonable to assume that these environmental variables, predation excepted, will have an important impact on captive growth rates. Food availability has been suggested as a determining factor in the growth of blacktip reef sharks (*Carcharhinus melanopterus*) (Stevens, 1984). Greater feeding rates have been implied as causative for increased growth rates in scalloped hammerhead sharks (*Sphyrna lewini*) (Clarke, 1971), sandbar sharks (Wass, 1973), and the spiny dogfish (*Squalus acanthias*) (Jones and Geen, 1977). A study examining captive Atlantic sharpnose sharks (*Rhizoprionodon terraenovae*) found growth rates similar to those observed in wild animals (Branstetter, 1987c). Thus, the influence of captivity on growth rates may vary between species.

MEASURING SHARKS

Many techniques exist for measuring sharks. Any comparison of the lengths of captive sharks with data collected by field biologists should preferably be made using whatever methods were employed in the published studies. Various authors use total length (TL), fork length (FL), or precaudal length (PCL) when measuring particular species. Conversions from one of these length measures to another are often provided by the authors (as per Table 15.1).

Fork and precaudal lengths are typically taken as straight-line measurements from the tip of the rostrum to the tail fork and anterior end of the precaudal pit, respectively (Compagno, 1984). TL is measured in a variety of ways. When obtaining straight-line measurements, some authors stretch the tail posteriorly to its maximum extent, while others leave it in a natural position. Some public aquariums have historically used an “over-the-curve” technique for PCL, FL, and TL. A measuring tape is placed on the tip of the rostrum, curved over the dorsal surface, and stretched to the precaudal pit, fork, or extended upper lobe of the caudal fin.

Exact length measurements are not critically important for general husbandry purposes. The difference in weight between a dangerously thin or obese shark and a typical wild animal will be considerably more than the few kilograms of error introduced by inexact measurement. TL determined using one of the methods described above will be within five percent of TL determined using another of the methods. However, we recommend all new studies of captive elasmobranchs use the straight-line methods of determining length as this is considered standard methodology by research biologists. Simple calipers constructed out of rigid pipe will greatly improve the measurement process.

Photographic evaluation of length is a useful alternative to direct measurement, minimizing handling. An animal can be photographed while passing in front of a grid of known dimensions (Govender et al., 1991). Caution is necessary when this technique is used on larger animals as parallax can create measurement inaccuracies. More recently, indirect measurement of sharks has been achieved using a video camera, convergent laser lighting, and image analysis techniques (Romero, pers. com.). It is possible to achieve a similar result inexpensively by aligning an object of known length against the side of the animal to be photographed. When using either of these methods the tail of the animal will be in a natural position. Since TL data on wild sharks has frequently been collected with the tail extended, it would be prudent to record PCL or FL from image-derived measurements. Using available conversions (to and from TL) provided in the literature, it will be possible to compare various data sets.

After an animal has been measured and weighed, comparisons with "normal" wild animals of similar length can be made. It will quickly become obvious whether ration changes are required when actual and expected weights are compared. Do not hesitate to increase rations significantly if an animal appears thin; however, rations should be reduced conservatively for obese animals to limit predation on teleosts that may co-inhabit the display.

Length-weight equations can be used to predict what a typical wild shark of a particular length should weigh. Similarly, it is possible to use other allometric relationships to predict TL, if either FL or PCL are known. The study of allometry involves an examination of the relationship in growth between a part of an organism and the entire organism. For example, an allometric study may examine the growth relationship between FL and TL, or more commonly, the relationship between weight and TL.

When length vs. weight relationships are graphed, a curvilinear plot is produced which can be converted to a linear relationship by performing log or natural log (ln) transformations on both raw length and weight data. For ease of use, the resulting regression equation (i.e., ln weight vs. ln length) is usually converted from logarithmic format to an untransformed (i.e., exponential) equation, whereby $W = aTL^b$, (where W = total weight (kg), TL = total length (cm), and a and b are constants). Allometric relationships relating FL, or PCL, to TL are inherently linear and require no transformation. A variety of length-weight equations for wild and captive sharks, and conversions between various measures of length, are provided in Table 15.1.

Ideally, animal weight should be obtained by placing the shark in a stretcher and suspending it from a scale. However, indirect methods of estimating weight may be more practical in some situations. Girth measurements have been used successfully in formulae to estimate the weight of white sharks (Mollet and Cailliet, 1996). It seems likely that measurements of shark body "height", extracted from photographs, could be used as an index of girth for such equations. Predictable ratios between height and width of elliptical shark cross-sections should allow a one-dimensional height or width measurement (e.g., taken from a photo) to be inserted into a formula that estimates volume and therefore weight. Trunk height (TRH) and trunk width (TRW), both taken at the level of pectoral insertion (Compagno, 1984), are unlikely to be useful for this purpose. One of the authors (Mohan) confirmed that girth measurements taken directly behind the insertion of the pectoral fins, again as per Compagno (1984), was a relatively crude index of weight when applied to sand tiger sharks. This is probably because the area near the pectoral girdle is less affected by weight changes than the region around the belly. More suitable circumferences for weight estimation can probably be taken posteriorly to the pectoral insertion. An ideal location to obtain TRH may be species-specific.

COLLECTING DATA AND ASSESSING GROWTH

To ensure age and growth data collected from captive specimens constitute an accurate representation of animal growth, certain guidelines should be followed:

1. Morphometrics should be recorded soon after collection of wild specimens. This procedure will allow for approximate age assessments when current literature predictions of size at age are available. Estimation of age at capture will be an essential component of further captive age and growth studies. If morphometric information is not recorded until some time after collection, the influence of captivity on growth rate may produce an error in assessing age approximations and thereby lead to inaccurate growth modeling.

2. Captive-born animals should be measured as soon as possible after birth, and juveniles should be subjected to an increased frequency of measurement. Dramatic growth rate changes occur in newly born elasmobranchs, so an increased frequency of data collection will provide more accurate descriptions of growth during these early periods.

3. Morphometric data should be collected on a regular basis, especially if internal or published research is previewed. Ideally, measurements should be performed monthly or quarterly. As growth rates may be influenced by seasonal fluctuations (e.g., photoperiod, water temperature, etc.), data collected on a less frequent basis may not be sufficient to capture these changes. Data collection associated with husbandry procedures does not always create uniform data sets appropriate for statistical analyses. A non-parametric statistical technique known as "bootstrapping" can be employed to correct this fault (Sokal and Rohlf, 1995).

ESTIMATING AGE AND GROWTH

Growth in elasmobranchs is typically characterized by the von Bertalanffy growth function (VBGF) as described by Cailliet et al. (1986) in the following form:

$$L_t \text{ or } W_t = L_{inf} \text{ or } W_{inf} \left[1 - e^{-k(age + t_0)} \right]$$

where L_t or W_t is length or weight at time t; L_{inf} or W_{inf} is asymptotic (maximum) length or weight; e is the base of the natural log; k is the growth coefficient; age is in years (for the results presented in this chapter); and t_0 is the theoretical age at which the animal was size 0. Estimates of growth parameters (L_{inf}, W_{inf}, k, and t_0) can be obtained by fitting size at age data to the VBGF model using statistical software packages (e.g., SYSTAT; SPSS, FISHPARM, etc.).

Estimates of age are desirable for institutional records and may be useful in collection planning. In cases where the age of the animal is unknown but repeated measurements over time are available (e.g., tag-recapture studies), VBGF parameters can be estimated using the technique described in Gulland and Holt (1959). This procedure requires an annualized growth rate (cm $year^{-1}$) as the dependent variable, plotted against average TL (i.e., the independent variable) for each animal of a particular species in a collection (or, where relevant, tagged and released). Annualized growth rate is defined as the increase in length or weight between two measurements, divided by the time period between the measurements. Average length or weight is defined as the mean of the initial and most recent measurements. To illustrate, a nurse shark (*Ginglymostoma cirratum*) measuring 195.0 cm on 5 April 1994 and 233.0 cm on 5 April 2001 (seven years since the first measurement), would have an annualized growth rate (GR_a) of:

$$GR_a = \frac{233.0 - 195.0}{7.0} = 5.4 \text{ cm year}^{-1}$$

and an average TL of:

$$\overline{TL} = \frac{195 + 233}{2} = 214 \text{ cm}$$

By calculating these two variables for each specimen in a collection, a scatter plot of GR_a on TL is produced and a linear regression line fitted. The slope of the regression line is an estimate of the VBGF growth coefficient (k), while the regression line x-intercept is an approximation of L_{inf}. The approximate time at which the animal was size 0 (t_0), theoretically at the moment of conception, is calculated by substitution into the VBGF. Literature-reported length or weight at birth is entered into the VBGF as L_t or W_t, with "age in years" entered as 0. Computation of t_0 is thus:

$$t_n = \frac{\text{natural log}\left(1 - \frac{\text{birth size}}{L_{inf} \text{ or } W_{inf}}\right)}{-k}$$

In addition to the Gulland and Holt method, there exists a newer, albeit more complex, method to estimate VBGF parameters using a statistical procedure known as the maximum likelihood technique (Francis, 1988; Simpendorfer, 2000; Simpendorfer et al., 2000; Natanson et al., 2001). The procedures are beyond the scope of this manual, but readers are encouraged to examine this technique if it is desired to present elasmobranch age and growth analyses in a more scientifically rigorous fashion.

Longevity is difficult to estimate for any elasmobranch. Mollet (www1) notes that a good longevity estimate may range from 5*ln2/k to 7*ln2/k years (Fabens, 1965; Cailliet et al., 1992).

These ages correspond to 95% and 99% of the theoretical age at L_{inf}, respectively.

GROWTH RATES AND ALLOMETRY

Allometric relationship equations relating weight to TL, FL, or PCL, and FL to TL, for several wild and captive elasmobranch species are presented in Table 15.1. Length (either TL, PCL, or FL) and weight at known or estimated ages, with accompanying VBGF equations, are presented for a number of species, along with model parameter estimates in Figures 15.1-15.16. For example, Figure 15.1 depicts VBGF model parameter estimates for captive nurse sharks, held at SeaWorld, using the Gulland and Holt (1959) method described above. Growth rates may vary substantially between different ages, as the VBGF describes accelerated growth early in an organism's life with diminishing growth rates near the asymptotic length or weight.

INDIVIDUAL SPECIES ACCOUNTS

The following accounts summarize what is known about the age and growth of selected species in nature and captivity. They report growth at specific rations. More detailed information on feeding habits and ration is presented in Chapter 14.

Sand tiger shark (*Carcharias taurus*)

Age and growth data for captive sand tiger sharks were first reported by Schmid et al. (1990) for a group of Northwest Atlantic sub-adult and adult specimens maintained at SeaWorld, Orlando, Florida, USA. Govender et al. (1991) tracked the growth of a number of South African animals, born by Caesarian section, from 1964 to 1991. The maximum age for wild sand tiger sharks is unconfirmed; however, sharks captured as adults commonly live for 10-15 years. One of the captive born animals studied by Govender et al. (1991) survived for 16.6 years. Henningsen (pers. com.) reports the display of a 20-year-old animal at the National Aquarium in Baltimore, while Paul Loiselle and others at the New York Aquarium (pers. com.) believe one of their animals, which was acquired as an adult, has now (2002) been in captivity for 31-36 years. Goldman (pers. com.) predicted maximum ages of 30 years for males, and 40 years for females, using back-calculated data to extrapolate his VBGF curves. However, no animals sampled for his study were older than 16-17 years (based on counts of annuli) agreeing with typical average maximum ages for captive specimens. Older animals appear uncommon in both aquariums and wild populations.

Captive growth issues are inseparable from considerations of diet ration in sand tigers. Sand

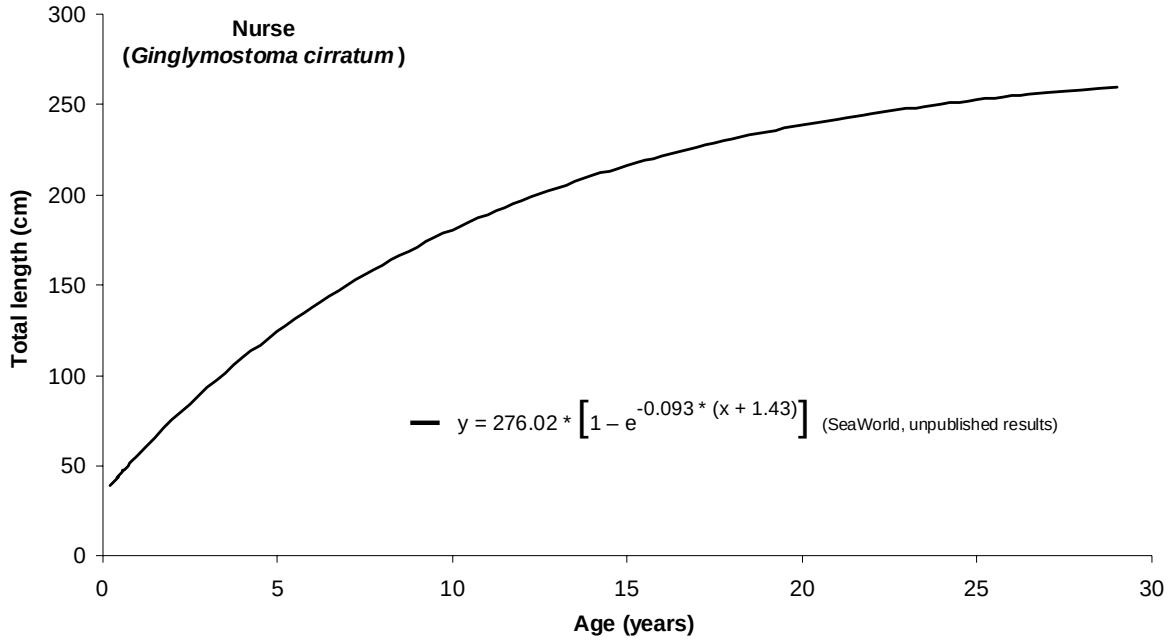


Figure 15.1. Von Bertalanffy growth function modeling growth in total length (cm) versus age (years) for captive nurse sharks (*G. cirratum*) (n=9) from SeaWorld. Parameter estimates were obtained using method described in text developed by Gulland and Holt (1959). Total length / age relationship is appropriate only for those ages and sizes plotted.

tigers can become grossly obese in captive situations. Some facilities feed daily, while others schedule 2-3 sessions per week, feeding to satiation on one or all days. Often, some attempt is made to limit predation on teleosts within the sand tigers' exhibit by providing food to the sharks beyond their basic requirement. Obesity, or other diet-related issues, may contribute to the high incidence of spinal deformity observed in sand tigers (Berzins, pers. com.). For example, increased body weight might necessitate obese animals to ingest additional air to maintain neutral buoyancy and increase stress on the spine where it passes dorsal to the air-filled stomach. This is the region where most spinal subluxations occur (Berzins and Jesselson, 1999; Berzins, pers. com.). Observations on five neonates, obtained and reared by SeaWorld Ohio between 1999 and 2004, further suggest that diet ration alone may not prevent spinal curvature. While none of these animals developed sub-luxations, four of the five exhibited slight to moderate curvature of the spine. The authors agree with Berzins's (pers. com.) suggestion that water depth, and it's relationship to the size of air bubbles within the sand tiger shark's stomach, may influence spinal morphology. The five specimens mentioned above were reared to ~1.6 m TL in a 1.0 m deep pool.

Sand tigers are known to require a food ration as low as 1% BW week^{-1}. Even at this low ration, captive sand tigers will typically be heavier than wild sharks of a similar length (Mohan, 1996; Mohan, 2000). Regularly feeding sand tigers to satiation is likely to produce obesity. For example, seven adult sand tiger sharks maintained at SeaWorld, Aurora, Ohio, USA were fed to satiation twice per week for a seven month period, consuming an average of 2.3% BW week^{-1} (Mohan, 1996). The majority of these sharks gained weight rapidly, one specimen increasing by 28.5 kg during this short period. When feeding ration was reduced to 1.6% BW week^{-1}, continued weight gains (of a generally lower magnitude) were observed in all sharks. Consistent weight loss could not be achieved until average ration was reduced to <1% BW week^{-1}.

Sand tigers are capable of consuming many times their weekly food requirement during a single feeding session. Even when sated at one feeding session per week (in addition to a single vitamin-filled food-fish on another day), staff at SeaWorld Florida observed that individual sharks could attain weekly food consumption rates as high as 4-5% BW week^{-1} (Kerivan, pers. com.). While studying juvenile sharks, one of the authors (Mohan) established that young sand tigers share this ability to "binge" feed and should be provided rations that vary according to TL and age.

Juvenile sand tiger shark

Sand tiger pups are approximately one meter in length at birth. North American specimens typically weigh around 5 kg at birth, but sharks born at SeaWorld, Durban, Natal, South Africa were somewhat heavier, weighing 7.5 kg (Anon., 1999). Unpublished length-weight equations developed by the Natal Sharks Board seem to confirm this difference. A typical 100 cm TL sand tiger from South Africa weighs approximately 6.5 kg (i.e., 0.9 kg heavier than the predicted weight for a sand tiger pup from North America (Cliff, pers. com.; Farquhar, pers. com.; Mohan, 2000).

Neonates have large livers and may not need to feed for some time after birth (Gilmore et al., 1983). At SeaWorld Florida (March, 1981), a neonate (born to a female fertilized in the wild) did not eat for 28 days following birth. A 91 cm TL neonate was received inappetant by SeaWorld Ohio (August, 1999) and did not feed unassisted for 10 days.

At SeaWorld Ohio, two newly-acquired (August, 1999) female neonates were fed to satiation every day for ten weeks. During this period the highest average weekly ration for the neonates was 14.1% BW week^{-1}. After the acclimation period both animals were rationed to 6% BW week^{-1} spread over three feeding days. This starting ration was based on an anecdotal report of slightly larger juveniles consuming ~5% BW week^{-1} (Charbeneau, pers. com.). Each animal was initially offered a variety of food items, including Atlantic silversides (*Menidia menidia*), Atlantic herring (*Clupea harengus*), capelin (*Mallotus villosus*), and Pacific chub mackerel (*Scomber japonicus*). Both sharks preferred Pacific chub mackerel. Food was supplemented with a 1:2 admixture of vitamins (Vi-Sorbin®, Pfizer, USA; and either PolyVitamin, Watson Pharmaceuticals Inc., USA; or Poly-Vi-Sol®, Mead Johnson and Company, USA). The sharks were gradually weaned off all foods, other than mackerel, before ad lib feeding was discontinued. Feeding rations were reduced when condition, expressed as relative weight (W_r), began to increase steadily over several sampling intervals. Relative weight is expressed as a percentage and is obtained by the following:

$$W_r = \left(\frac{\text{Observed weight}}{\text{Expected weight generated by L-W equation}} \right) \text{X } 100$$

Each shark was fed for 26 weeks at 6% BW week^{-1}, then for 36 weeks at 5% BW week^{-1}, and finally for 22 weeks at 4.5% BW week^{-1}. By the 95th week (post-acquisition) the animals were placed on a ration of 4.0% BW week^{-1}. Subsequent data is unavailable, but rations continued to decrease as the animals grew. The intent was to eliminate both obesity and abnormally high growth rates, by controlling dietary intake and matching weights to those observed in wild caught sharks of equivalent length. Observed weights were compared to a table of expected weights that was generated using a length-weight equation based on wild sand tiger morphometric data (Mohan, 2000). The equation: R_W=0.01918*TL-1.666 describes the relationship between weekly ration (R_W) in kilograms of 1.1-1.5 kcal g^{-1} Pacific chub mackerel, and TL in centimeters for animals within the length range of 105-180 cm TL ("over-the-curve"). Average W_r increased from ~100% at 105 cm TL to ~125% at 180 cm TL, suggesting that rations were higher than required for these young sand tigers. The equation therefore did not generate an optimum diet ration, and an adjustment of the feeding ration was warranted to maintain a W_r closer to 100%. Different results could be expected under different feeding and environmental conditions.

Anecdotal reports from Underwater World, Mooloolaba, Queensland, Australia (Fischer, pers. com.) suggest two sand tiger sharks (born November, 1997) reached ~200 cm TL by age 2.5 years; consistent with growth rates observed at SeaWorld Ohio. The effect of temperature on captive growth is unclear. Unlike SeaWorld Ohio, where temperature variations in closed systems were limited to 22-24 ^{o}C, the semi-open system at Underwater World, Mooloolaba exposed sharks to water temperatures mirroring an ambient range of 18-28 ^{o}C. SeaWorld Durban also relied on a natural source of seawater, with a temperature range of 21-28 ^{o}C (Davis, pers. com.), yet Govender et al. (1991) described a slower growth rate for sand tiger sharks maintained at this facility. This observation could be attributable to high, continuous, therapeutic copper levels used within the system, or other environmental or endogenous biological variables. Hazel and Meith (1970) report that 0.20 mg l^{-1} copper inhibits the growth of young Chinook salmon (*Oncorhynchus tshawytscha*). Two 24 month-old sand tigers at SeaWorld Durban were only 150 cm and 156 cm TL, while a pair of specimens held at SeaWorld Ohio were 178 cm and 180 cm TL at the same age, 17% longer on average.

Sub-adult and adult sand tiger shark

Schmid et al. (1990) studied the captive growth of several shark species at SeaWorld Florida. Reports on intermediate-sized sand tigers held at 24 ^{o}C were included. One 160 cm TL female grew to 205 cm TL in 16 months. A 160 cm TL male grew to 198 cm in 20 months. Two sub-adults increased from 202 cm and 209 cm TL to 211 cm and 217 cm in 11 months, respectively (TL back-calculated from FL using Branstetter and Musick's (1994) equation from Table 15.1).

Using growth reports for female sand tigers from SeaWorld Ohio and SeaWorld Florida, it is possible to demonstrate potential growth rates for specimens kept in closed systems with relatively high, static water temperatures (i.e., 22-24 ^{o}C). At SeaWorld Ohio two females reached an average length of 160 cm TL at 1.3 years of age. At SeaWorld Florida, the 160 cm TL female (described above) grew 45 cm in 1.3 years, and the two sub-adult females (206 cm mean TL; described above) grew an average of ~8 cm in 0.9 years. Captive female sand tigers may therefore reach 214 cm TL in as little as 3.5 years. At this rate of growth, sexual maturity could be reached in less than 4.5 years (using Branstetter and Musick's (1994) estimate of minimum length for sexual maturity at 220 cm TL). Goldman (2002) predicts that wild females reach sexual maturity at 9-10 years. Govender et al. (1991) present data that suggest captive sharks at SeaWorld Durban required seven years to reach 220 cm TL. It appears that the sharks at Underwater World Mooloolaba and SeaWorld Ohio will exceed this growth rate and reach sexual maturity in a time frame more consistent with the rough estimate calculated above (i.e., ~4.5 years). Goldman (2002) estimated wild male sand tigers may reach sexual maturity in 6-7 years. As for captive female sand tigers, males reared under artificial conditions may mature more rapidly (i.e., in a period of less than four years). Two VBGF of TL versus age are given in Figure 15.2.

The influence of captivity on accelerated growth rates has been well documented in sandbar sharks (Wass, 1973; Mohan, 1996; Branstetter, 1987c). Wass (1971) reported that captive grey reef sharks (*Carcharhinus amblyrhynchos*) grew ten times faster than conspecifics in the field. Gruber and Stout (1983) found that captive lemon sharks (*Negaprion brevirostris*) grew nine times faster than wild counterparts.

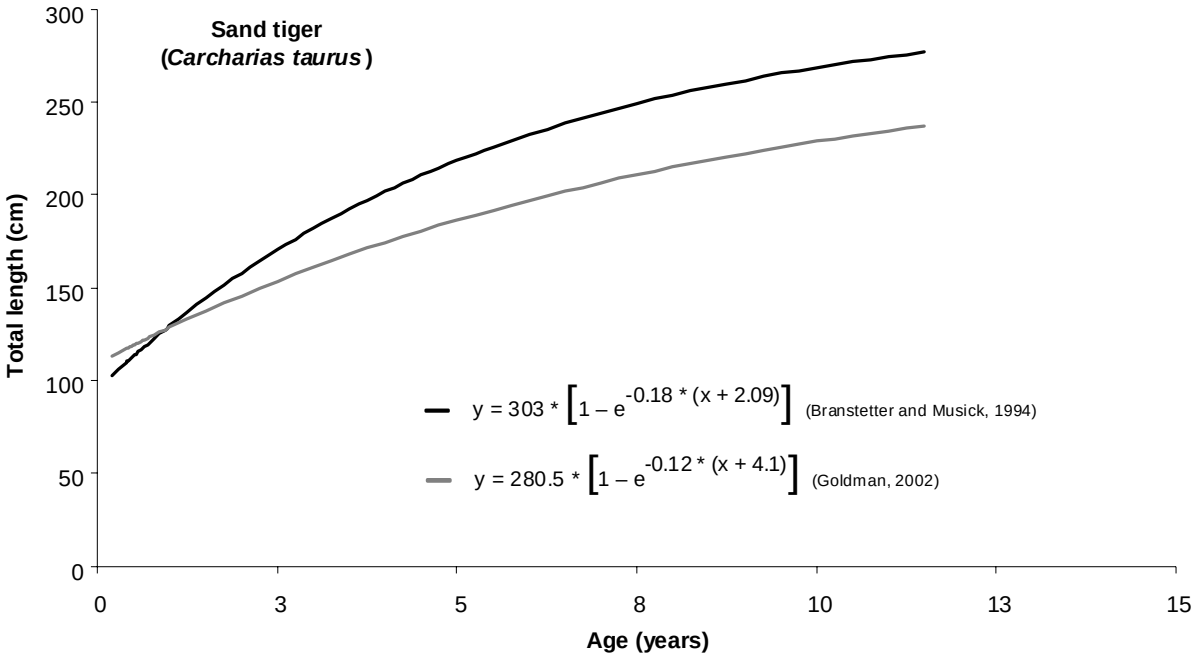


Figure 15.2. Von Bertalanffy growth function modeling growth in total length (cm) versus age (years) for wild sand tiger sharks (*C. taurus*). Total length / age relationship is appropriate only for those ages and sizes plotted. Functions are shown for both Branstetter and Musick (1994) and Goldman's unpublished dissertation (2002).

Diet management of captive sand tiger shark

The equation for R_w given above provides target feeding rations for sand tigers from 100-180 cm TL, and probably overestimates ideal ration at the high end of this size range. During this growth period, juveniles can be expected to require rations that gradually drop from 6% to 4% BW week^{-1} (or slightly less). Sharks above 200 cm TL will likely require no more than 3% BW week^{-1}. The data in Schmid et al. (1990) suggests that animals approaching sexual maturity (i.e., 200-220 cm TL) require around 2% BW week^{-1}, and Mohan (1996) shows fully grown animals (230-280 cm TL) require as little as 1% BW week^{-1}. These figures should be viewed as starting points when establishing rations for an animal of known length. Rations will vary depending on individual metabolic differences, environmental conditions (e.g., temperature, etc.), and caloric variation in food fishes.

Estimated normal weights for North American sand tigers of known length can be calculated using the length-weight equations provided in Table 15.1. The first equation is based primarily on public aquarium capture records from the Delaware Bay (i.e., SeaWorld Florida in 1991 and Ripley's Aquariums, Orlando, Florida, USA in 1999). Three neonate data points were taken from Gilmore et al. (1983), and one data point from a healthy neonate received by SeaWorld Ohio (in 1999). Branstetter and Musick's (1994) equation is based on a larger sample size, but without neonate data. The formula appears differently in the original article, due to a confirmed typographical error. From a husbandry perspective, the two equations are not appreciably different for sharks above 165 cm TL. However, it should be noted that TL was measured differently in each study. Where necessary, conversions between the "straight-line" (SL) method used by Branstetter and Musick (1994) and the "over-the-curve" (OC) method used by Mohan (2000) can be achieved using the formula (n=12; R^2=0.998; range=104-281cm):

$$\mathbf{TL_{SL}(cm) = 0.159 + 0.985 * TL_{OC}(cm)}$$

Both methods assume the tail is stretched to its full extent during measurement. Data for young South African sharks (Cliff, pers. com.) suggest a difference between sharks from South Africa and those from North America (Table 15.1).

While minimum acceptable weights are not yet quantifiable for sand tigers, it is safe to assume a problem exists when it becomes difficult to maintain weights approximately equal to, or slightly greater than, those of wild sharks of similar

Table 15.1. Allometric relationships for selected elasmobranchs. Unless other units are specified, WT = weight in kilograms, TL = total length in centimeters, FL = fork length in centimeters, PCL = precaudal length in centimeters. Measurements marked † were taken "over the contour".

Species	WT vs. TL, FL, PCL	Size Range	FL, PCL vs. TL	Size Range	Reference
Carcharhinus brevipinna (spinner shark)					
combined, Gulf of Mexico	$WT = 7.51x10^{-6}*TL^{2.97}$	103-208	FL = -2.61+0.86*TL	103-208	Branstetter, 1987b
Carcharhinus falciformis (silky shark)					
combined, NW Atlantic	$WT = 1.54x10^{-5}*FL^{2.92}$	73-212	FL = -2.65+0.84*TL	73-212	Kohler et al., 1995
combined, Gulf of Mexico	$WT = 2.01x10^{-6}*TL^{3.23}$	80-255	FL = 0.97+0.83*TL	80-255	Branstetter, 1987a
Carcharhinus leucas (bull shark)					
combined, captive	$WT = 3.30x10^{-5}*TL^{2.76}$	152-253	FL = 10.59+0.78*TL	152-253	SeaWorld, unpublished results
combined, Gulf of Mexico	$WT = 2.71x10^{-6}*TL^{3.20}$	84-268	FL = -6.37+0.86*TL	84-268	Branstetter and Stiles, 1987
Carcharhinus limbatus (blacktip shark)					
combined, Gulf of Mexico	$WT = 1.44x10^{-5}*TL^{2.87}$	62-181	FL = -4.92+0.86*TL	62-181	Branstetter, 1987b
combined, SE US waters	$WT = 2.51x10^{-6}*TL^{3.13}$	65-195			Castro, 1996
Carcharhinus longimanus (oceanic whitetip shark)					
males	$WT = 3.08x10^{-5}*PCL^{2.86}$	50-195			Seki et al., 1998
females	$WT = 5.08x10^{-5}*PCL^{2.76}$	50-195			Seki et al., 1998
Carcharhinus obscurus (dusky shark)					
combined, NW Atlantic	$WT = 3.24x10^{-5}*FL^{2.79}$	79-287	FL = -3.19+0.84*TL	79-287	Kohler et al., 1995
Carcharhinus plumbeus (sandbar shark)					
females, captive	$WT = 1.05x10^{-4}*TL^{2.52}$	175-226	FL = 2.32+0.78*TL	175-226	SeaWorld, unpublished results
combined, NW Atlantic	$WT = 1.09x10^{-5}*FL^{3.01}$	44-201	FL = 2.57+0.82*TL	44-201	Kohler et al., 1995
Carcharias taurus (sand tiger shark)					
combined†, Delaware Bay	$WT = 2.59x10^{-6}*TL^{3.17}$	103-278			Mohan, 2000
combined, Chesapeake Bay	$WT = 1.62x10^{-6}*TL^{3.24}$	113-272	FL = -2.97+0.85*TL	113-272	Branstetter and Musick, 1994
combined, South Africa	$WT = 2.70x10^{-8}*PCL(mm)^{2.94}$		TL(mm) = 44.1+1.33*PCL(mm)		Cliff, unpublished results
combined, Argentina			TL = 18.13+1.24*PCL	89-267	Lucifora, et al., 2002
combined, NW Atlantic			FL = 0.592+0.847*TL	95-277	Goldman, 2002

Table 15.1 (continued). Allometric relationships for selected elasmobranchs. Unless other units are specified, WT = weight in kilograms, TL = total length in centimeters, FL = fork length in centimeters, PCL = precaudal length in centimeters. Measurements marked † were taken "over the contour".

Species	WT vs. TL, FL, PCL	Size Range	FL, PCL vs. TL	Size Range	Reference
Carcharodon carcharias (white)					
combined, US Atlantic	$WT = 7.58x10^{-6}*FL^{3.09}$	112-499	FL = -5.74+0.94*TL	112-499	Kohler et al., 199
combined, US California +	$WT = 4.34x10^{-6}*TL^{3.14}$	127-554			Compagno, 1984
Galeocerdo cuvier (tiger)					
combined, NW Atlantic	$WT = 2.53x10^{-6}*FL^{3.26}$	92-389	FL = -13.35+0.88*TL	145-375	Kohler et al., 199
combined, Virginia & Gulf of Mexico	$WT = 1.41x10^{-6}*TL^{3.24}$	91-381	FL = -11.5+0.86*TL	91-381	Branstetter et al.
Ginglymostoma cirratum (nurse)					
combined, captive	$WT = 3.43x10^{-5}*TL^{2.69}$	170-272	FL = 2.32+0.78*TL	170-272	SeaWorld, unpub
males, Florida Keys	$WT = 3.44x10^{-5}*TL^{2.60}$	50-180			Carrier and Luer,
females, Florida Keys	$WT = 4.09x10^{-5}*TL^{3.04}$	30-180			Carrier and Luer,
Isurus oxyrinchus (shortfin mako)					
combined, NW Atlantic	$WT = 5.24x10^{-6}*FL^{3.14}$	65-338			Kohler et al., 199
Negaprion brevirostris (lemon)					
combined, captive	$WT = 1.71x10^{-7}*TL^{3.67}$	237-274	FL = 22.05+0.76*TL	237-274	SeaWorld, unpub
Notorynchus cepedianus (sevengill)†					
combined, captive	$WT = 8.74x10^{-7}*TL^{3.33}$	65-188			Van Dykhuizen a
combined, worldwide	$WT = 3.44xTL(m)^{3.32}$	all			Ebert, unpublishe
Prionace glauca (blue)					
combined, NW Atlantic	$WT = 3.18x10^{-6}*FL^{3.13}$	52-288	FL = 1.39+0.89*TL	64-337	Kohler et al., 199
Sphyrna lewini (scalloped hammerhead)					
combined, captive	$WT = 2.07x10^{-5}*TL^{2.68}$	56-232	FL = -0.47+0.78*TL	56-232	SeaWorld, unpub
combined, NW Atlantic	$WT = 7.78x10^{-6}*FL^{3.07}$	79-243	FL = -0.31+0.78*TL	82-278	Kohler et al., 199
combined, Gulf of Mexico	$WT = 1.26x10^{-5}*TL^{2.81}$	105-232	FL = 0.97+0.83*TL	105-232	Branstetter, 1987
males, Taiwan	$WT = 1.35x10^{-6}*TL^{3.25}$	75-270			Chen et al., 1990
females, Taiwan	$WT = 2.82x10^{-6}*TL^{3.13}$	60-330			Chen et al., 1990
Squatina californica (Pacific angel shark)					
males, California	$WT = 1.33–0.00831TL(mm)+0.0000152TL(mm)^2$	all			Natanson, unpub
females, California	$WT = 2.095–0.0115TL(mm)+0.0000182TL(mm)^2$	all			Natanson, unpub

length. There is evidence to suggest that long-term captives (possibly geriatric specimens) may require increasingly larger rations to maintain weight. For example, individuals collected as adults for SeaWorld Ohio in 1991 began to increase their average food consumption after five years in captivity, and as of early 2002 some individuals required up to 2.3% BW week^{-1}. Increasing demands across the entire captive population were not observed, suggesting that the energy content of the food was not responsible. Violetta (pers. com.) observed a male sand tiger, in captivity for 14 years, exhibiting a chronic weight loss problem in spite of generous rations; weight declining to 67% of the value expected for a wild shark of similar length.

Bull shark (*Carcharhinus leucas*)

Growth rates for the bull shark (*Carcharhinus leucas*) have been reported at 15-20 cm year^{-1} TL for the period from birth to five years, decreasing to 10 cm year^{-1} TL from 6-10 years, diminishing further to 4-5 cm year^{-1} TL from 11-16 years, and falling to <4-5 cm year^{-1} TL for animals >16 years old (Branstetter and Stiles, 1987). Thorson and Lacy (1982) documented more rapid growth rates during the first two years of 16-18 cm year^{-1} TL, dropping to 11-12 cm year^{-1} TL, and finally declining to 9-10 cm year^{-1} TL for older animals. Growth rate does not appear to be sexually dimorphic and thus investigators from both studies calculated gender-combined VBGF models for age and growth. Growth rates for captive pups have been recorded as high as 23.0 cm year^{-1} FL, declining to rates comparable to wild specimens (i.e., 7.0 cm year^{-1} FL) by year four (Schmid and Murru, 1994). Branstetter and Stiles (1987) indicated an asymptotic, or predicted maximum, TL of 285 cm. This length is less than the reported maximum size of 340 cm TL and typical maximum sizes of 299 cm TL and 324 cm TL, for males and females respectively, as indicated by Compagno (1984). Male bull sharks reach sexual maturity at 14-15 years, measuring 210-220 cm TL, while females mature at >225 cm TL. Dodrill (1977) suggested that bull shark pups are born at sizes ranging from 60-75 cm TL, while Compagno (1984) listed a size at birth of 56-81 cm TL. Our model predicted a size at birth of 47.3 cm TL; significantly less than quoted in the literature. This finding is most likely a result of missing size-at-age data for young animals as measurements were not taken until animals were >2 years old. VBGF for wild and captive bull sharks are given in Figure 15.3 and Figure 15.4. According to Schmid et al. (1990), juvenile bull sharks consumed 3.5% BW week^{-1} resulting in growth rates of 12.6 cm year^{-1} FL and 10.1 kg year^{-1} BW. The same study reported that an adult male consumed 2.8% BW week^{-1}.

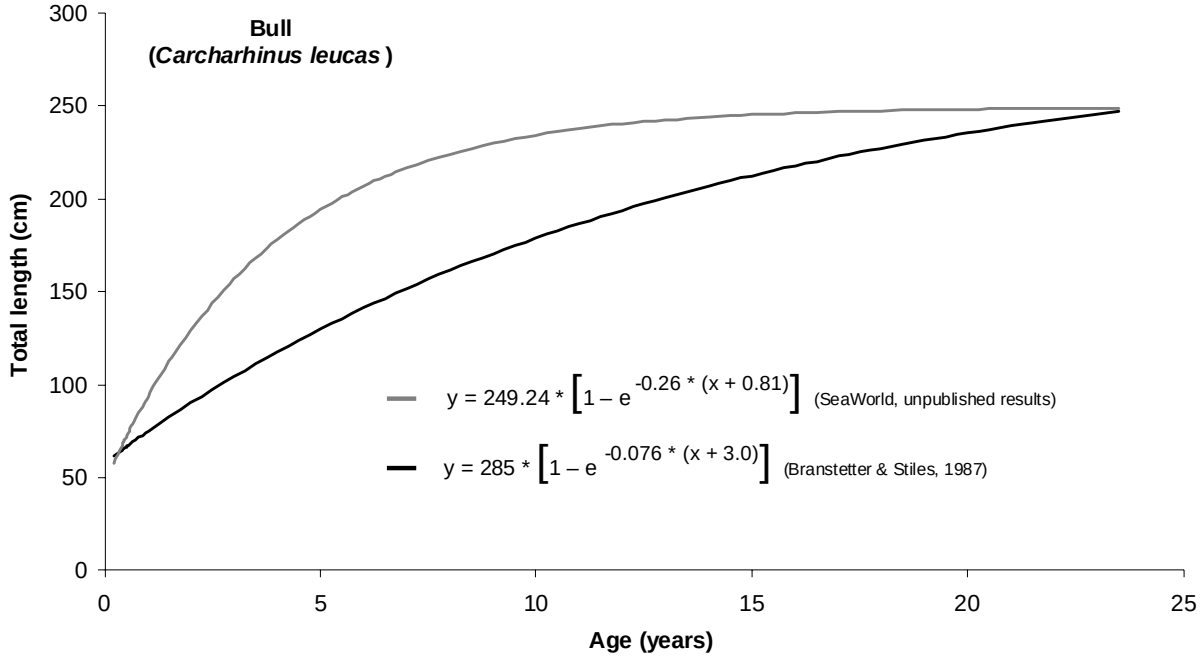


Figure 15.3. Von Bertalanffy growth function relationship between growth in total length (cm) and age (years) for known-age captive bull sharks (*C. leucas*) (n=110 from 5 specimens) from SeaWorld compared to modeled curves obtained from wild animal data. Total length / age relationship is appropriate only for those ages and sizes plotted.

Figure 15.4. Von Bertalanffy growth function modeling growth in weight (kg) and age (years) for known-age captive bull sharks (*C. leucas*) (n=109 from 5 specimens) from SeaWorld. Weight / age relationship is appropriate only for those ages and weights plotted.

Lemon shark (*Negaprion brevirostris*)

Lemon sharks (*Negaprion brevirostris*) are reported as having a slow growth rate, living a minimum of 20 years (Brown and Gruber, 1988), and reaching an asymptotic length of ~300 cm at age ~27 years (Gruber, 1981). In another study Brown (1988) found that lemon sharks reach 95% of their maximum size after 50 years, while Gruber and Keyes (1981) observed that small captive lemon sharks (average 61 cm TL) only grew 5 cm year^{-1} over a three-year period. In the same report, Gruber and Keyes (1981) found that juvenile lemon sharks grew ~26 cm year^{-1} when kept in large pools that allowed them to swim unrestricted. Maximum size is ~340 cm TL, males maturing near 224 cm TL and females at ~239 cm TL, and young are born at 60-65 cm TL (Compagno, 1984). Brown and Gruber (1988) predicted birth size at 39.0 cm PCL, and maturity at 11.6 years for males and 12.7 years for females. A VBGF of PCL versus age is given in Figure 15.5.

Work with captive lemon sharks showed that young animals (~70 cm) eat 3% BW day^{-1}, doubling their weight in 100 days (Gruber, 1981). Animals starved for three days and subsequently fed 3% BW digested all food in one day, whereas feeding rates of 20% BW result in food retention in the stomach for >2 days (Gruber, 1981). Gruber and Keyes (1981) found that young, fasting animals lost 1% BW day^{-1}. Gruber (1984) observed that 11.5% BW day^{-1} was sufficient for weight maintenance, while values of 12% BW week^{-1} and 15% BW week^{-1} resulted in weight gains of 28 grams and 56 grams, respectively, over 14 days. In the same study, sharks allowed to feed ad libitum consumed 18.3% BW day^{-1}, yet did not feed every day.

Sandbar shark (*Carcharhinus plumbeus*)

Like lemon sharks, sandbar sharks have been described as slow growing, long-lived species (>30 years) with somewhat constant, gender-indiscriminate growth rates of 5.5-5.9 cm year^{-1} FL (based on wild sharks initially tagged at 50-109 cm FL) (Casey et al., 1985). In contrast, two captive males (150 cm FL and 165 cm FL) grew 8 cm year^{-1} during a six-month observation period, and four captive females (165-185 cm FL) grew 4.0 cm year^{-1} FL over one year (Schmid et al., 1990). Growth rates in specimens taken from both captive and wild populations are highly variable.

Maximum size for sandbar sharks is generally 239 cm TL, but may reach 300 cm TL (Compagno, 1984). Size at birth is 56-75 cm TL (Compagno, 1984) similar to the 47.0 cm PCL reported by Wass (1973). Tooth replacement analyses

suggest maturation at ~10 years for males and ~13 years for females (Compagno, 1984), while a study by Casey et al. (1985) set maturation at 12 years and 13 years for females and males, respectively. Springer (1960) set a size-at-maturity of 152 cm FL, while Sminkey and Musick (1995) reported maturity at 15-16 years. Tagging studies have suggested some individuals may take 30 years to reach maturity (Casey and Natanson, 1992). In stark contrast, Wass (1973) tracked the growth of sandbar sharks held in an outdoor pool and reported a size-at-maturity of 110-115 cm PCL

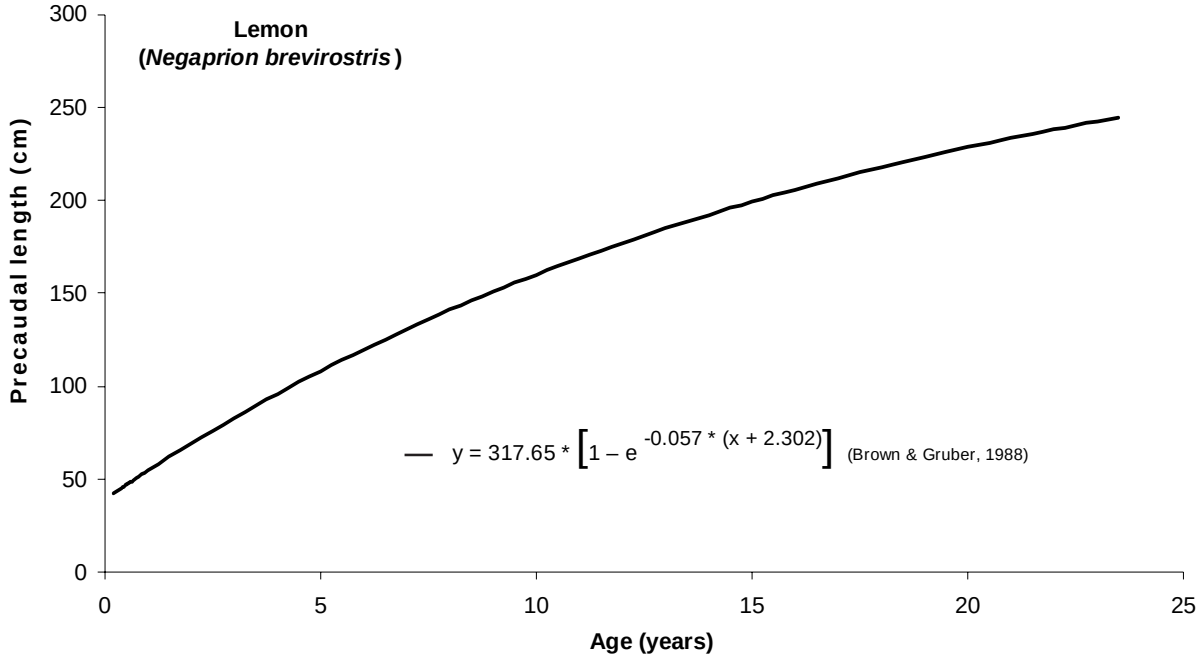


Figure 15.5. Von Bertalanffy growth function modeling growth in precaudal length (cm) versus age (years) for wild lemon sharks (*N. brevirostris*). Precaudal length / age relationship is appropriate only for those ages and sizes plotted.

Figure 15.6. Von Bertalanffy growth function modeling growth in precaudal length (cm) versus age (years) for wild sandbar sharks (*C. plumbeus*). Precaudal length / age relationship is appropriate only for those ages and sizes plotted.

after only three years. The observed younger age and smaller size at maturity was probably attributable to the overall small size of Hawaiian sandbar sharks and the generous feeding regime applied to the captive specimens. Growth rates of young, captive specimens have been reported at ~23 cm year^{-1} PCL from birth to three years (Wass, 1973) and 20 cm year^{-1} PCL from birth to 16 months (Branstetter, 1987c). A captive-born sandbar shark at SeaWorld Ohio reached 103 cm FL at an age of two years (Mohan, 1996), nearly matching the 109 cm FL (transformed from PCL data) observed by Wass (1973) for animals of the same age. This finding suggests that environmental conditions (i.e., a continuous warm water temperature and an abundant food supply) produces rapid growth in both Hawaiian and Northeast Atlantic sandbars. Overall, it appears that wild sandbars mature at 10-16 years; although captive specimens have achieved sexual maturity in as little at three years (Compagno, 1984). VBGFs for wild sandbar sharks are given in Figure 15.6 and Figure 15.7.

Estimates of weekly ration have been established for wild sandbar sharks at 10.0% BW week^{-1} for pups (average 55 cm FL) and 2.9% BW week^{-1} for a mixed group of juveniles and adults (average 144 cm FL) (Stillwell and Kohler, 1993). The authors assumed a caloric value of 1.235 kcal g^{-1} for food given to pups, based on a diet of $^{2}/_{3}$ crustaceans (e.g., blue crab, *Callinectes sapidus*) and $^{1}/_{3}$ fish (e.g., Atlantic menhaden, *Brevoortia tyrannus*), and 1.195 kcal g^{-1} of food given to juveniles and adults, based on a higher percentage of elasmobranch and teleost prey. Three captive-born pups (all 82 cm FL) consumed an average of 9.0% BW week^{-1} during a six-month period, their mass increasing by an average of 71%. Subsequently, two of these pups (97 cm FL and 99 cm FL) consumed an average weekly ration of 5.8% BW week^{-1} (correction to Mohan, 1996). Using growth and food ration data for these and other young sharks held at SeaWorld Ohio (1993-1996), a simple regression equation was calculated relating R_w to FL for young (82-136 cm FL) sandbar sharks:

$$R_w = 22.882 + 0.147 * FL_{(cm)}$$

The young sharks at SeaWorld Ohio were fed primarily on Pacific chub mackerel, thought to have a similar caloric value (i.e., 1.1-1.5 kcal g^{-1}) as the food given to young sand tiger sharks (discussed above). Another group of six captive sandbar sharks (165-185 cm TL) fed at a rate of 3.3% BW week^{-1} grew 4.5 cm year^{-1} FL and gained 4.6 kg year^{-1} BW (Schmid et al., 1990). Male consumption rates (3.8% BW week^{-1}) were higher than female consumption rates (3.0% BW week^{-1}). Unlike sand tigers, which are prone to obesity if fed to satiation twice a week, young sandbar sharks at SeaWorld Ohio typically weighed about the same as wild sharks of similar length (Mohan,

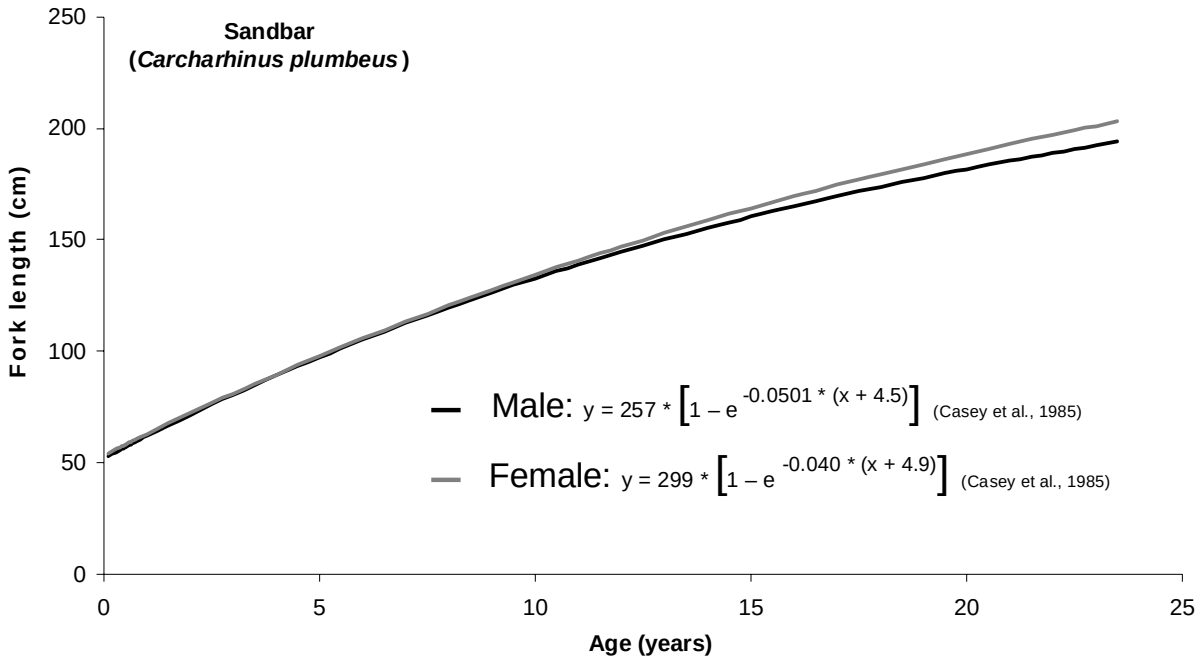


Figure 15.7. Von Bertalanffy growth function modeling growth in fork length (cm) versus age (years) for wild sandbar sharks (*C. plumbeus*). Fork length / age relationship is appropriate only for those ages and sizes plotted.

1996). The Kohler et al. (1995) VBGF equation for wild sharks (Table 15.1) was an equally good fit for young captive sharks.

While there are no studies quantifying a minimum weight for healthy sandbar sharks, Mohan (1996) used raw data from wild populations (Kohler, pers. com.) to estimate a "lowest viable weight". The weight of the lightest sharks taken from each length interval (n>1600) was used to build the equation:

$$WT = 1.434x10^{-6}*FL^{3.358}$$

where WT is weight in kilograms. This formula is intended for use as a husbandry aid, identifying animals that may need exceptional care (e.g., intubation), and has been used successfully for this purpose at a number of institutions.

Nurse shark (*Ginglymostoma cirratum*)

Due to their wide tolerance of temperatures and dissolved oxygen levels, nurse sharks do well in captivity and specimens have been kept successfully for 24-25 years (Compagno, 1984). Most adult nurse sharks are less than 300 cm TL. Males achieve a maximum size of 257 cm TL and mature at ~225 cm TL. Females grow to an asymptotic length in excess of 259 cm TL; maturing at ~230-240 cm TL. The size of newborn nurse sharks is reported to be 27-30 cm TL (Compagno, 1984). In a study of growth rates for wild and captive nurse sharks, investigators found that GR_a for captive animals (19.1 cm year^{-1} TL and 4.0 kg year^{-1} BW) were higher than those for wild sharks (13.1 cm year^{-1} TL and 2.3 kg year^{-1} BW) (Carrier and Luer, 1990). These differences were not significant and disappeared when analyses were conducted on captive and wild specimens similar in size. A study of nurse sharks held at SeaWorld Florida showed a growth rate of 9.0 cm year^{-1} FL (Schmid et al., 1990). Two captive-born nurse pups at the Curacao Sea Aquarium grew at rates of 0.25 cm day^{-1} TL and 29.6 grams day^{-1} BW during 188 days, and 0.24 cm day^{-1} TL and 26.8 grams day^{-1} BW during 173 days (Kuenen, 2000). These figures translate to 90.3 cm year^{-1} TL and 1.1 kg year^{-1} BW, and 86.5 cm year^{-1} TL and 1.0 kg year^{-1} BW. Observed elevated TL growth rates, well beyond reports in other studies, were probably the result of limited data sets for young animals that were only measured during the first six months of age. A VBGF for captive nurse sharks has been provided in Figure 15.1.

According to a study by Schmid et al. (1990) mean feeding rates for captive nurse sharks was 2.2% BW week^{-1} with a resultant growth of 9.0 cm year^{-1} and 4.0 kg year^{-1} BW.

Scalloped hammerhead shark (*Sphyrna lewini*)

Scalloped hammerheads reach a maximum size of 370-420 cm TL, with males growing to 295 cm TL, and females growing to a slightly larger 309 cm TL (Compagno, 1984). Size and age of maturation was reported in Branstetter (1987a) at 180 cm TL and 10 years for males, and 250 cm TL and 15 years for females. These estimates are higher than those reported by Chen et al. (1990) for a population of Taiwanese scalloped hammerheads where males matured at 198 cm TL and 3.8 years, and females matured at 210 cm TL and 4.1 years. Age estimates for this study may not be directly comparable to those for Branstetter (1987a) due to differences in age determination techniques. Growth rates obtained from a VBGF based on captive scalloped hammerheads held at SeaWorld, San Antonio, Texas, USA (Violetta, pers. com.) were estimated to be 44.3 cm year^{-1} TL for the 1st year and 20.3 cm year^{-1} TL for the 2nd year. Data from Branstetter (1987a) indicated wild growth rates from birth (i.e., late spring to early summer) through the first winter of 15 cm year^{-1} TL, becoming 15-20 cm year^{-1} TL until ~2.5 years of age, decreasing to 10-15 cm year^{-1} TL, and continuing to diminish to 5-10 cm year^{-1} TL in older animals. Greater growth rates were observed in animals studied off Taiwan by Chen et al. (1990), who reported fast and variable growth rates for both males and females. Female growth was 63 cm TL for the 1st year, then 23-50 cm year^{-1} TL until year five; and, male growth was 54 cm TL for the 1st year, 22-42 cm year^{-1} for years 2-5, and 11-18 cm year^{-1} TL for years 6-8. A length-at-birth of 54.0 cm TL was calculated for the sharks held at SeaWorld Texas using the VBGF. This figure was greater than reported in previous studies; 43 cm TL (Casey, 1964), ~50 cm TL (Bass et al., 1975), 38-45 cm TL (Castro, 1983), 42-55 cm TL (Compagno, 1984), 49.0 cm TL (Branstetter, 1987a), and 31.3 cm TL for males and 48.9 cm TL for females (Chen et al., 1990); and yet less than the 59.7 cm TL documented by Hoenig (1979). Direct calculation of model-predicted size at ages 0, 1 year, and 2 years showed SeaWorld Texas animals grew at greater rates than those observed by Hoenig (1979) or Branstetter (1987a), and less than those observed in Taiwanese waters (Chen et al., 1990). VBGFs for captive and wild scalloped hammerhead sharks are given in Figures 15.8-15.10.

In a study examining the growth of captive scalloped hammerheads in Hawaii (Clarke, 1971), the influence of feeding rates appeared to be pronounced. Five animals were initially measured (48.5-56.7 cm TL), fed to satiation twice a day, and subsequently re-measured at either 30 days or 60 days. Growth rates for these periods were 73.0-91.8 cm year^{-1}, with an average of 81.4 cm year^{-1} TL. Another group of five animals were measured (54.7-57.2 cm TL), fed once a day (~5% BW day^{-1}), and re-measured at 92 days or 100 days. Resultant growth rates were significantly

Figure 15.8. Von Bertalanffy growth function relationships between growth in total length (cm) and age (years) for known-age captive scalloped hammerheads (*S. lewini*) (n=117 from 15 specimens) from SeaWorld. Total length / age relationship is appropriate only for those ages and sizes plotted.

Figure 15.9. Von Bertalanffy growth function relationships between growth in total length (cm) and age (years) for wild scalloped hammerheads (*S. lewini*). Total length / age relationship is appropriate only for those ages and sizes plotted.

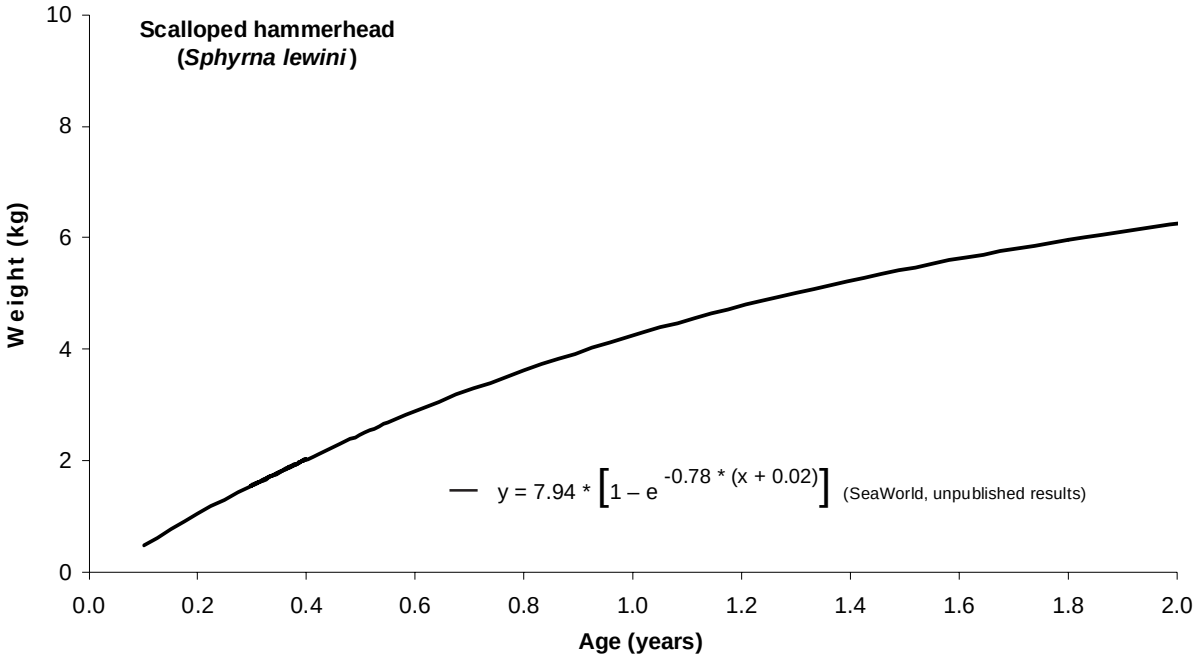


Figure 15.10. Von Bertalanffy growth function modeling growth in weight (kg) and age (years) for known-age captive scalloped hammerheads (*S. lewini*) (n=114 from 15 specimens) from SeaWorld. Weight / age relationship is appropriate only for those ages and weights plotted.

less, 23.4-64.6 cm year^{-1} TL, with an average of 42.4 cm year^{-1} TL, or ~½ of the growth rate seen for animals fed to satiation. Since these animals were captured as neonates, growth rates represent first-year growth and were similar to first-year growth rates calculated for the SeaWorld Texas specimens (44.3 cm year^{-1} TL).

White Shark (*Carcharodon carcharias*)

White sharks are 120-150 cm TL and 22-54 kg BW at birth (Wintner and Cliff, 1999), and increase in length by 30% in their first year of life (Branstetter, 1990). Males mature in 8-10 years at 300-365 cm TL, while females may take 12-14 years to reach maturity at ≧ 445 cm TL (Wintner and Cliff, 1999).

As of this writing, no aquariums have successfully kept the white shark for more than a few weeks. Two factors appear to have contributed to the difficulty in maintaining this species: the generally poor condition of most captives on arrival; and an initial rapid depletion of energy reserves, aggravated by inappetence. Discussion about age and growth of captive white sharks must be limited to what is known about length-weight relationships and feeding activities for the species.

Hewitt (1984) describes the common chain of events, observed for many years, when weakened white sharks were brought to aquariums. He identifies collection by amateurs, ill-prepared transportation efforts, and resulting physiological problems as major barriers to keeping this species. Additionally, he notes the significance and consequences of energy depletion as an important complication. Stress and other factors may influence feeding behavior of captive white sharks. Reidarson and McBain (1994) report two cases of elevated blood-glucose in inappetant captive white sharks at SeaWorld, San Diego, California, USA. Sources of stress and their relationship to feeding readiness appear to be an important focus for the husbandry of new captives.

Several attempts to display white sharks have been partially successful. Gordon (www2) and Ellis and McCosker (1991) report that Manly Marineland, Sydney, Australia acquired a 2.3 m white shark in 1968. Despite rough handling by the angler, it began feeding after ~3 days. Amazing as it may seem to us today, this animal was euthanazed after it began to show an unnerving interest in the diving staff. Three sharks displayed during 1994 by SeaWorld California (two females of 30 kg BW) and Manly Oceanworld, Sydney, Australia (a single specimen of 2.1 m TL) remained inappetant. One specimen survived for approximately two weeks, while the other two were released within 5-10 days before their condition deteriorated (Reidarson and

McBain, 1994; Gordon, www2). Two additional specimens, a 1.5 m animal acquired by the Monterey Bay Aquarium, Monterey, California, USA in 1984, and a 1.7 m shark held at SeaWorld California in 1981 (Ellis and McCosker, 1991), appear to have followed a similar pattern of inappetence and declining physiological condition.

Speculation on diet ration for white shark

The observed weakening of white sharks after a week or less in captivity suggest that capture, tank negotiation, and related stress may deplete energy reserves, and/or that inappetence over this relatively short interval is of critical concern.

White sharks are known to maintain a higher body temperature than the surrounding water. While observing a 4.6 m specimen, Carey et al. (1982) noted muscle temperatures up to 5°C higher than the water. McCosker (1987) found the stomach temperature of a 3.5 m male to be as much as 7.4°C above ambient seawater, while Goldman et al. (1996) recorded stomach temperatures 13.7°C above ambient. These findings appear to be in the same range, or greater than, records for shortfin mako sharks (Carey and Teal, 1969). Early estimates of metabolic rates (Carey et al., 1982) suggested that a 943 kg BW shark might be able to fast for 45 days following a 30 kg meal of whale blubber. Mollet, (pers. com.) and the authors believe that this may be an underestimate of metabolic rate. Mollet (pers. com.) suggests that a maintenance metabolism of ~1% BW week^{-1} would be required for a 30 kg meal to last a 943 kg BW animal 45 days; concluding that this would be low for an endotherm, especially for a growing white shark. It is more likely that juvenile white sharks require a ration similar to those required by young shortfin mako (Mollet, pers. com.), reported by Stillwell and Kohler (1982) to be as much as ~20-30% BW week^{-1}. This finding implies young white sharks eat large and/or frequent meals and may explain why animals previously exhausted by capture will deplete their energy reserves in a week or less. A stomach-content analysis of young whites in the New York Bight (Casey and Pratt, 1985) indicated that these sharks focus on relatively abundant, small fishes such as Atlantic menhaden and searobins (*Prionotus* spp.), suggesting that frequent small meals may be typical.

While appropriate rations can only be estimated for white sharks, length-weight relationships are well documented for some populations (Table 15.1). It seems likely that, like sand tigers, maintaining white sharks at, or slightly above, weights expected for wild conspecifics would be desirable.

Shortfin mako shark (*Isurus oxyrinchus*)

Size-at-birth for the shortfin mako is thought to be ~70 cm (Gilmore, 1993; Mollet et al., 2000). Pratt and Casey (1983) estimate a 55% first-year length increase for wild sharks (i.e., growth from 76 cm FL to 118 cm FL). Captive shortfin mako can be expected to exceed this rate of increase. Shortfin mako may reach 230 cm FL in 4.5 years, and their lifespan has been estimated to be at least 11.5 years (Pratt and Casey, 1983); although Mollet (pers. com.) reports that recent reinterpretations of vertebral data may double these ages. A VBGF for wild shortfin mako sharks is given in Figure 15.11.

Unlike the white shark, which is usually coastal in distribution, the shortfin mako is a largely offshore littoral and epipelagic species (Compagno, 1984), and is an extremely active swimmer. Atlantic populations feed primarily on bluefish (*Pomatomus saltatrix*) when inshore. Offshore feeding preferences include miscellaneous teleosts and squid (Stillwell and Kohler, 1982).

Attempts to maintain shortfin mako (≧ 100 cm TL) at SeaWorld California in the early 1970's were unsuccessful. Shaw (pers. com.) reports additional attempts were made when SeaWorld California's Shark Encounter opened in 1978. Two 120-150 cm TL animals survived 1-2 days but swam stiffly and had difficulty negotiating the tank walls. At least two other facilities, the Okinawa Expo Aquarium, Okinawa, Japan and the New Jersey State Aquarium, Camden, New Jersey, USA have attempted to hold shortfin mako (Uchida et al., 1990; Steslow, pers. com.). The shark at the Okinawa Expo Aquarium survived for only one day. However, the 107 cm TL male shortfin mako held at the New Jersey State Aquarium lived for five days and was relatively successful at negotiating the tank.

Shortfin mako have an extremely high metabolism and maintain a body temperature 7-10°C above ambient (Carey and Teal, 1969). Average stomach capacity is 10% BW (reaching as high as 23.3% BW) and large meals can be digested quickly (Stillwell and Kohler, 1982). The authors estimate that an average mako (i.e., 63 kg BW) would eat

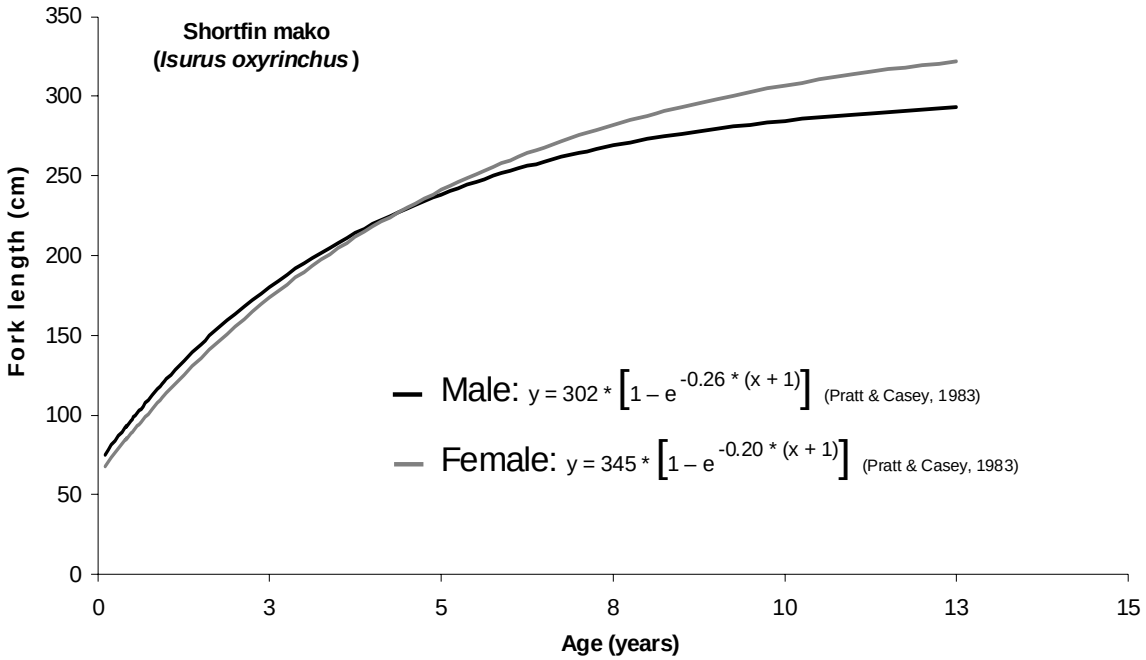


Figure 15.11. Von Bertalanffy growth function modeling growth in fork length (cm) versus age (years) for wild shortfin mako sharks (*I. oxyrinchus*). Fork length / age relationship is appropriate only for those ages and sizes plotted.

20% BW week^{-1}, and perhaps 30% BW week^{-1}, if estimated energy expenditures during active metabolism are included in ration calculations.

Stress and food availability may be of even more concern for captive shortfin mako than for small white sharks. Steslow (pers. com.) reported that the shortfin mako held at the New Jersey State Aquarium was inappetant and showed signs of increasing energy depletion over time. Shaw (pers. com.) noted a similar “crash and burn” scenario. Like other offshore species (e.g., blue shark, *Prionace glauca*), shortfin mako may not adapt easily to environments with vertical barriers (e.g., aquarium walls). However, the obstacle and wall-negotiating abilities of the animal held at the New Jersey State Aquarium were encouraging. Sharks of the family Lamnidae have been likened to tuna (*Thunnus* spp.), both for their ability to control internal body temperature, and their global distribution (Carey et al., 1985). It seems reasonable to assume that many of the capture and husbandry techniques applied to tuna can be modified to develop protocols for mako, white, and other shark species of similar physiology and behavior.

Sevengill shark (*Notorynchus cepedianus*)

Size-at-birth for the sevengill is estimated to be 35-45 cm TL (Ebert, 1989; Mollet, pers. com.). Male sevengill reach sexual maturity at ~153 cm TL in 4.3-5.0 years, while females mature at 218-244 cm TL in 11-21 years (Ebert, 1989; Van Dykhuizen and Mollet, 1992).

Over the past 40 years, a number of North American Pacific Coast aquariums have displayed sevengill sharks (Rupp, 1984) and regard them as an analog to the sand tiger, probably because of their passive demeanor and size.

Sevengill sharks display a punctuated feeding pattern, both in the wild (Ebert, pers. com. cited in Van Dykhuizen and Mollet, 1992) and in aquariums (Rupp, 1984). Depending on the amount of food offered, 3-5 days of inappetence is not uncommon. Animals held at the Monterey Bay Aquarium were typically fed once or twice a week (Van Dykhuizen and Mollet, 1992). Wild sevengill typically feed on elasmobranchs, particularly California bat rays (*Myliobatis californica*) and brown smooth-hound (*Mustelus henlei*), and bony fishes (Ebert, 1989). Sevengill sharks have been known to harass Pacific angelsharks (*Squatina californica*) in captive conditions (Howard, pers. com.).

As is common for other sharks held at public aquariums, length measurements for this species have typically been taken using the “over-the-curve” method, following the contour of the animal, rather than a straight line technique. A conversion factor

of 0.961 can be used to adjust contour measurements to the more widely used straight-line measurements (Van Dykhuizen and Mollet, 1992).

Length-weight equations for both captive and wild sevengill are given in Table 15.1. Comparison with data from wild sharks is useful if condition (W_r) is to be determined. Ebert (pers. com. via Mollet, pers. com.) provides a useful length-weight equation based on worldwide field data for n=524 wild sevengill sharks (Table 15.1). Mollet (pers. com.) notes that both fresh sharks and museum specimens were used for the study, possibly influencing applicability of the length-weight equation to wild sevengill. Assuming that the specimens studied by Ebert were uniform, a comparison of weight projections for wild vs. captive sevengill sharks indicates that the captive animals examined by Van Dykhuizen and Mollet (1992) were ~17-20% BW heavier than wild sharks of similar length.

Van Dykhuizen and Mollet (1992) observed food consumption rates in sevengill sharks similar to those observed for sand tigers (see above). Sevengill pups consumed up to 14% BW $week^{-1}$, the same as the maximum observed ration reported for a pair of slightly larger neonate sand tigers. Two sevengill pups consumed 7% BW $week^{-1}$ in the first year following capture; comparable to 6% BW $week^{-1}$ for juvenile sand tigers fed a diet designed to maintain W_r at, or slightly above, 100%. At three years, female sevengill consumption rate had dropped to 2.8% BW $week^{-1}$. Adult sevengill sharks consumed 1.4% BW $week^{-1}$, similar to the 1-2% BW $week^{-1}$ considered healthy for mature sand tigers (Mohan 1996, Schmid et al., 1990).

Pacific angelshark (*Squatina californica*)

Pacific angelsharks are 25 cm TL at birth and maximum size is 150 cm TL. Both sexes mature at 90-100 cm TL. Captive specimens experience an 80% increase in TL during year one, and may reach ~55-60 cm TL in two years (Natanson and Cailliet, 1990; Cailliet et al., 1992; Schaadt and Landesman, 1997). It has been observed that captive specimens grow more rapidly than tagged wild sharks (Natanson and Cailliet, 1990).

Angelsharks have historically been uncommon in public aquariums. Schaadt and Landesman (1997) document the successful care of a group of neonate sharks obtained from a commercial fisherman after an adult female pupped upon capture. Schaadt (pers. com.) noted that angelsharks thrive when supplied with a fine, deep sand substrate whereby the sharks are able to completely bury themselves. Food was supplied by regular pole feeding, but the continuous availability of supplementary live food (e.g., Californian anchovy, *Engraulis mordax* and South American pilchard, *Sardinops sagax*) was thought to be important to successfully keep this species (Schaadt, pers. com.). Moving non-live food items over the top of an angelshark, to elicit their natural ambush-predator response, was considered a key element in the husbandry of this species at the Aquarium of the Bay (Howard, pers. com.).

Unfortunately, captive weights or ration data have not been kept for this species, so no comparison with wild conspecifics is possible. Natanson (pers. com.) provides length-weight equations for wild male and female Pacific angelsharks over a broad range of sizes (Table 15.1). Weights-at-capture lie near values predicted by these equations (Howard, pers. com.).

Additional shark species

Limitations of space necessitate the omission of detailed discussions for other species used for public exhibition and/or experimentation. However, length-weight relationships for many sharks not addressed in the text are presented in Table 15.1 and will be valuable to those seeking to adjust rations appropriately for those species. VBGFs are presented graphically for the spinner (*Carcharhinus brevipinna*) (Figure 15.12) silky (*Carcharhinus falciformis*) (Figure 15.13) blacktip (*Carcharhinus limbatus*) (Figure 15.14) oceanic whitetip (*Carcharhinus longimanus*) (Figure 15.15), and tiger (*Galeocerdo cuvier*) sharks (Figure 15.16). These figures provide useful estimates of age-at-size for wild specimens. As for other species noted above, captive sharks may exceed the sizes predicted for wild sharks of similar age.

CONCLUSIONS

Growth data obtained from wild shark populations is useful when evaluating the health and condition of captive specimens. While captive diet compositions and feeding frequencies will continue to vary between institutions, regular monitoring of shark lengths and weights allows for adjustments to weekly food rations helping facilities to maintain captive sharks at weights similar to wild conspecifics. While regular

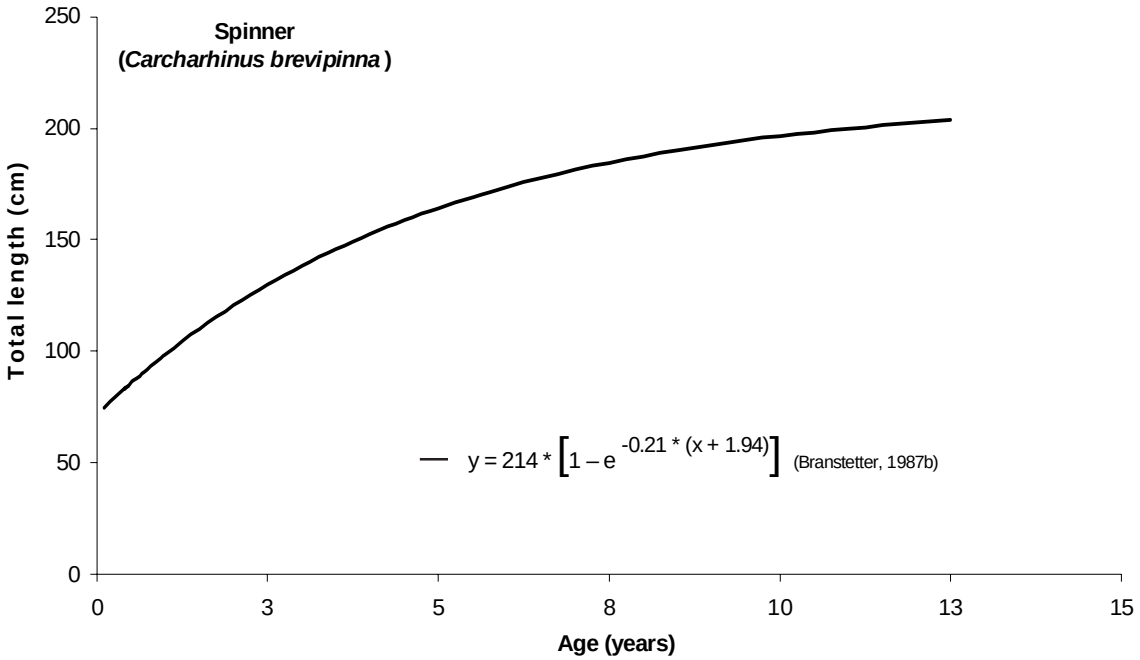


Figure 15.12. Von Bertalanffy growth function modeling growth in total length (cm) versus age (years) for wild spinner sharks (*C. brevipinna*). Total length / age relationship is appropriate only for those ages and sizes plotted.

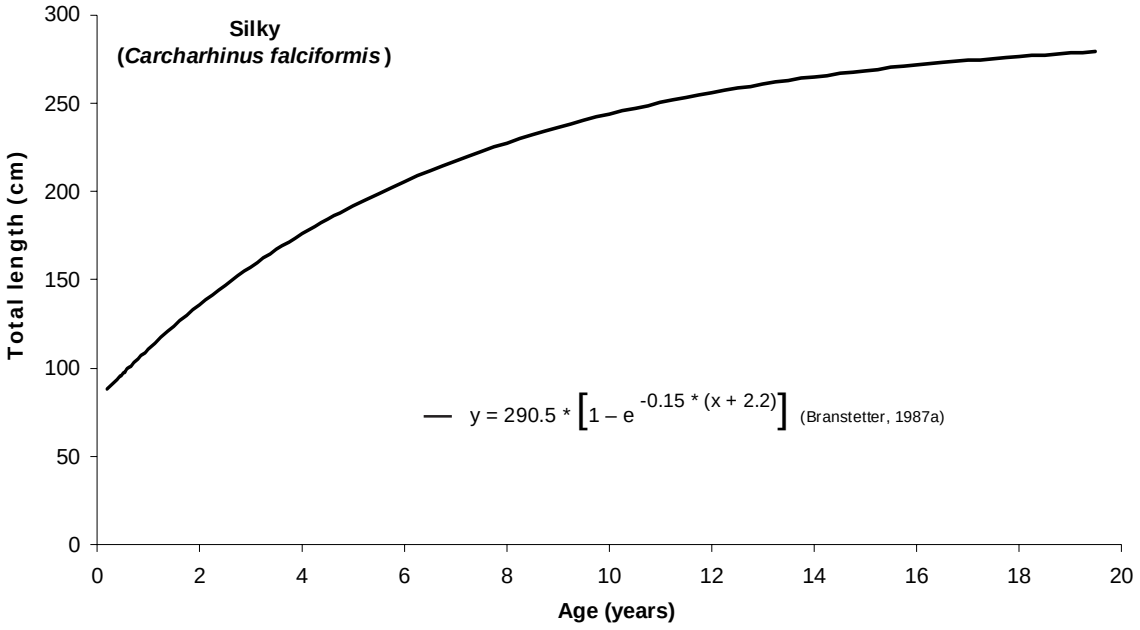


Figure 15.13. Von Bertalanffy growth function modeling growth in total length (cm) versus age (years) for wild silky sharks (*C. falciformis*). Total length / age relationship is appropriate only for those ages and sizes plotted.

measurement of length and weight can be challenging for some species, and difficult in some exhibits, the benefits of improved health outweigh any inconvenience. Normally-proportioned sharks should be a husbandry goal for all aquarists; slim specimens are healthier and better ambassadors for their species than obese animals.

This chapter addresses age and growth in many species commonly held in captivity, and a few that are rarely kept. It is by no means intended as an exhaustive review of the subject. We intentionally avoided discussing growth in chimeras, skates, and rays, but this is expected to be a topic addressed in a second elasmobranch husbandry symposium.

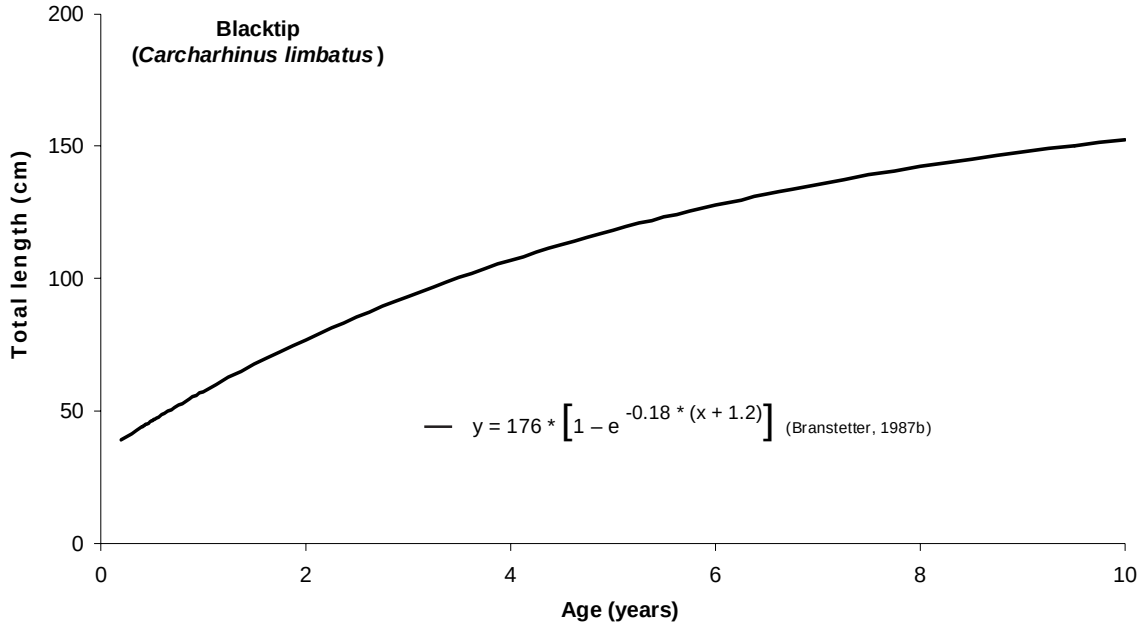


Figure 15.14. Von Bertalanffy growth function modeling growth in total length (cm) versus age (years) for wild blacktip sharks (*C. limbatus*). Total length / age relationship is appropriate only for those ages and sizes plotted.

Figure 15.15. Von Bertalanffy growth function modeling growth in precaudal length (cm) versus age (years) for wild oceanic whitetip sharks (*C. longimanus*). Precaudal length / age relationship is appropriate only for those ages and sizes plotted.

ACKNOWLEDGEMENTS

The aquarists at SeaWorld Ohio (known as Six Flags Worlds of Adventure from 2001 until cessation of operations in 2004) have been very dedicated to both animal care and record keeping during studies of sand tiger and sandbar diet rations. Supervisor Valerie Reischuck, and senior aquarists Don Zeisloft, Denice Teeples, and Mark Telzrow were especially helpful. Appreciation should be extended to the aquarium staff at SeaWorld parks in San Antonio, Texas and Orlando, Florida for specimen collection, data collection, and husbandry of study animals

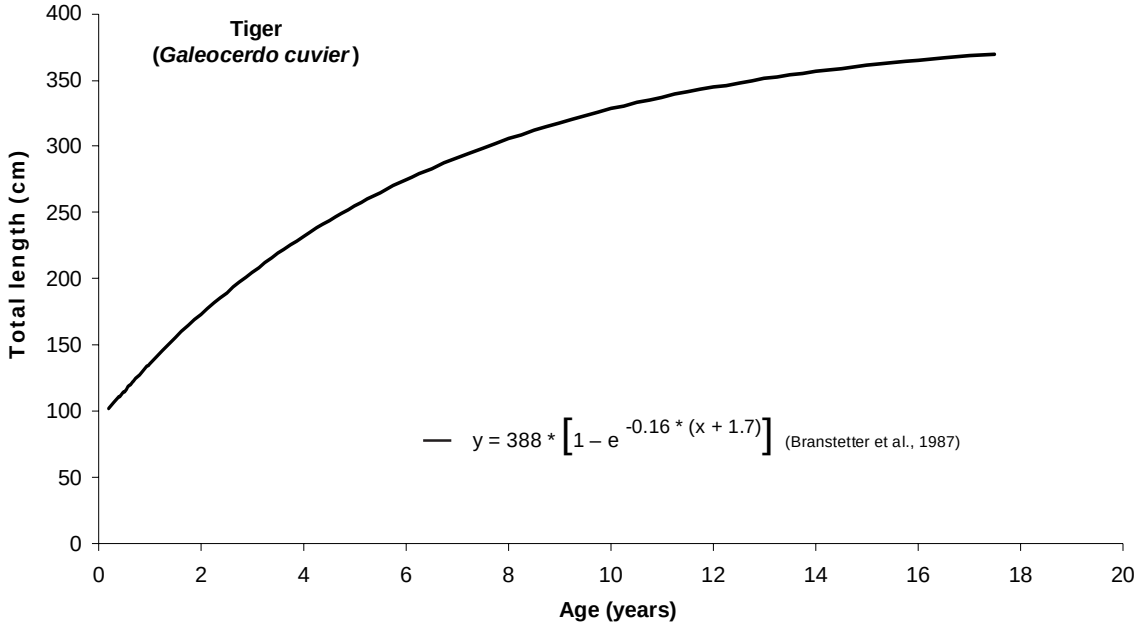


Figure 15.16. Von Bertalanffy growth function modeling growth total length (cm) versus age (years) for wild tiger sharks (*G. cuvier*). Total length / age relationship is appropriate only for those ages and sizes plotted.

throughout this project. Gary Violetta and Joe Keyon provided data on scalloped hammerhead growth at SeaWorld San Antonio.

Henry Mollet maintains an impressive website (http://homepage.mac.com/mollet/) containing a wealth of data on elasmobranch life histories, age, growth, and in some cases diet rations. He commented on early drafts of this manuscript and provided much of the information on sevengill sharks. Henry is a great resource for those in the public aquarium field interested in shark morphometrics.

Ken Goldman of VIMS provided key useful information about North American sand tiger migrations, parturition, and age. Geremy Cliff of the Natal Sharks Board (through Michael Farquhar of the Two Oceans Aquarium) provided unpublished length-weight data for South African sand tiger sharks.

This is SeaWorld technical contribution No. 2001-05-F.

REFERENCES

Anon. 1999. Aquarium. Bulletin of the South African Association for Marine Biological Research 25: 10.

Bass, A. J., J. D. D'Aubrey, and N. Kistnamasy. 1975. Sharks of the East Coast of Southern Africa. III. The Families Carcharhinidae (Excluding Carcharhinus and Mustelus) and Sphyrnidae. Oceanographic Research Institute (Durban) Investigational Report 33. 168 p.

Berzins, I. K. and K. Jesselson. 1999. Spinal problems in captive sand tiger sharks (*Carcharias taurus*). *In:* AZA Annual Conference Proceedings, September 22-28, Minneapolis, Minnesota, p 23. American Zoo and Aquarium Association, Silver Spring, Maryland, USA.

Branstetter, S. 1987a. Age, growth and reproductive biology of the silky shark, *Carcharhinus falciformis*, and the scalloped hammerhead, *Sphyrna lewini*, from the northwestern Gulf of Mexico. Environmental Biology of Fishes 19(3): 161-173.

Branstetter, S. 1987b. Age and growth estimates for blacktip, *Carcharhinus limbatus*, and spinner, *C. brevipinna*, sharks from the northwestern Gulf of Mexico. Copeia 1987(4): 964-974.

Branstetter, S. 1987c. Age and growth validation of newborn sharks held in laboratory aquaria, with comments on the life history of the Atlantic sharpnose shark, *Rhizoprionodon terraenovae*. Copeia 1987(2): 291-300.

Branstetter, S. 1990. Early life-history implications of selected carcharhinoid and lamnoid sharks of the Northwest Atlantic. *In:* Elasmobranchs as Living Resources: Advances in the Biology, Ecology, Systematics, and the Status of the Fisheries, p. 17-28. H. L. Pratt, Jr., S. H. Gruber, and T. Taniuchi (eds.). NOAA Technical Report. NMFS 90.

Branstetter, S. and J. D. McEachran. 1986. Age and growth of four carcharhinid sharks common to the Gulf of Mexico: a summary paper. *In:* Indo-Pacific Fish Biology: Proceedings of the Second International Conference on Indo-Pacific Fishes, p. 361-371. T. Uyeno, R. Arai, T. Taniuchi, and K. Matsuura (eds.). Ichthyological Society of Japan, Tokyo.

Branstetter, S. and J. Musick. 1994. Age and growth estimates for the sand tiger in the northwestern Atlantic Ocean. Transactions of the American Fisheries Society 123: 242-254.

Branstetter, S., J. Musick, and J. Colvocoresses. 1987. A comparison of the age and growth of the tiger shark,

Galeocerdo cuvier, from off Virginia and from the northwestern Gulf of Mexico. Fishery Bulletin 85(2): 269-279.

Branstetter, S. and R. Stiles. 1987. Age and growth estimates of the bull shark, *Carcharhinus leucas*, from the northern Gulf of Mexico. Environmental Biology of Fishes 20(3): 169-181.

Brown, C. A. 1988. Age validation of tetracycline-labeled vertebral centra in a tropical marine predator, the lemon shark, *Negaprion brevirostris* (Poey). Unpublished M.S. Thesis, University of Miami, Florida, USA.

Brown, C. A. and S. H. Gruber. 1988. Age assessment of the lemon shark, *Negaprion brevirostris* using tetracycline validated vertebral centra. Copeia 1988(3): 747-753.

Cailliet, G. M., M. S. Love, and A. W. Ebeling. 1986. Fishes: A Field and Laboratory Manual on their Structure, Identification, and Natural History. Wadsworth Publishing Co., Belmont, California, USA. 194 pp.

Cailliet, G. M., L. K. Martin, D. Kusher, P. Wolf, and B. A. Welde. 1983. Techniques for enhancing vertebral bands in age estimation of California elasmobranchs. NOAA Technical Report NMFS 8: 167-174.

Cailliet, G. M., K. G. Yudin, S. Tanaka, and T. Taniuchi. 1990. Growth characteristics of two populations of *Mustelus manazo* from Japan based upon cross-readings of vertebral bands. *In:* Elasmobranchs as Living Resources: Advances in the Biology, Ecology, Systematics, and the Status of the Fisheries, p. 167-176. H. L. Pratt, Jr., S. H. Gruber, and T. Taniuchi (eds.). NOAA Technical Report. NMFS 90.

Cailliet, G. M., H. F. Mollet, G. G. Pittenger, D. Bedford, and L. J. Natanson. 1992. Growth and demography of the Pacific angel shark (*Squatina californica*), based upon tag returns off California. Australian Journal of Marine and Freshwater Research 43: 1313-1330.

Carey, F. G., J. G. Casey, H. L. Pratt, D. Urquhart, and J. E. McCosker. 1985. Temperature, heat production and heat exchange in lamnoid sharks. Memoirs (Southern California Academy of Science) 9: 92-108.

Carey, F. G., J. W. Kanwisher, O. Brazier, G. Gabrielson, J. G. Casey, and H. L. Pratt, Jr. 1982. Temperature and activities of a white shark, *Carcharodon carcharias*. Copeia 1982(2): 254-260.

Carey, F. G. and J. M. Teal. 1969. Mako and porbeagle: Warm bodied sharks. Comparative Biochemistry and Physiology 28: 199-204.

Carrier, J. C. and C. A. Luer. 1990. Growth rates in the nurse shark, *Ginglymostoma cirratum*. Copeia 1990(3): 686-692.

Casey, J. G. 1964. Angler's guide to sharks of the northeastern United States, Maine to Chesapeake Bay. U.S. Fish and Wildlife Service, Bureau of Sport Fisheries and Wildlife, Circular 179, Washington, D. C., USA 32 p.

Casey, J.G. and L.J. Natanson. 1992. Revised estimates of age and growth of the sandbar shark *(Carcharhinus plumbeus)* from the western North Atlantic. Canadian Journal of Fisheries and Aquatic Sciences 49(7): 1474-1477.

Casey, J. G. and H. L. Pratt, Jr. 1985. Distribution of the white shark, *Carcharodon carcharias*, in the western North Atlantic. Memoirs (Southern California Academy of Science) 9: 2-14.

Casey, J. G., H. L. Pratt, Jr., and C. E. Stillwell. 1985. Age and growth of the sandbar shark (*Carcharhinus plumbeus*) from the western north Atlantic. Canadian Journal of Fisheries and Aquatic Science 42: 963-975.

Castro, J. I. 1983. The Sharks of North American Waters, Texas A & M University Press, College Station, USA. 180 p.

Castro, J. I. 1996. Biology of the blacktip shark, *Carcharhinus limbatus*, off the southeastern United States. Bulletin of Marine Science 59(3): 508-522.

Chen, C. T., T. C. Leu, and S. J. Joung. 1990. Notes on reproduction in the scalloped hammerhead, *Sphyrna lewini*, in Northeastern Taiwan waters. Fishery Bulletin 86(2): 389-393.

Clarke, T. A. 1971. The ecology of the scalloped hammerhead shark, *Sphyrna lewini*, in Hawaii. Pacific Science 25: 133-144.

Compagno, L. J. V. 1984. FAO species catalogue. Sharks of the World. An Annotated and Illustrated Catalogue of Shark Species Known to Date. FAO Fisheries Synopsis, Number 125, Volume 4, Parts 1 and 2. Food and Agriculture Organization of the United Nations, Rome, Italy. 655 p.

Dodrill, J. W. 1977. A hook and line survey of the sharks found within five hundred meters of shore along Melbourne Beach, Brevard County, Florida. M.S. Thesis. Florida Institute of Technology, Melbourne, Florida, USA. 304 p.

Ebert, D. A. 1989. The taxonomy, biogeography, and biology of cow and frilled sharks (Chondrichthyes: Hexanchiformes). Ph.D. Thesis. Rhodes University, Grahamstown, South Africa.

Ellis, R. and J. E. McCosker. 1991. Great White Shark, Stanford University Press, Stanford, California. 270 p.

Fabens, A. J. 1965. Properties and fitting of the von Bertalanffy growth curve. Growth 29: 265-289.

Francis, R. I. C. C. 1988. Maximum likelihood estimation of growth and growth variability from tagging data. New Zealand Journal of Marine and Freshwater Research 22: 42-51.

Gilmore, R. G. 1993. Reproductive biology of lamnoid sharks. Environmental Biology of Fishes 38: 95-114.

Gilmore, R. G., J. Dodrill, and P. Linley. 1983. Reproduction and embryonic development of the sand tiger shark, *Odontaspis taurus* (Rafinesque). Fishery Bulletin 81(2): 201-225.

Goldman, K. J. 2002. Aspects of age, growth, demographics and thermal biology of two lamniform shark species. Ph.D. thesis. College of William and Mary, School of Marine Science, Virginia Institute of Marine Science, Gloucester Point, USA. 220 p.

Goldman, K. J., S. D. Anderson, J .E. McCosker, and A. P. Klimley. 1996. Temperature, swimming depth, and movements of a white shark at the South Faralon Islands, California. *In:* Great White Sharks: The Biology of *Carcharodon carcharias,* p. 111-120. A. P. Klimley and D. G. Ainley (eds.). Academic Press, New York, USA.

Govender, A, N. Kistnasamy, and R. P. Van Der Elst. 1991. Growth of spotted ragged-tooth sharks *Carcharias taurus* (Rafinesque) in captivity. South African Journal of Marine Science 11: 15-19.

Gruber, S. H. 1981. Lemon sharks: supply-side economists of the sea. Oceanus 24(14): 56-64.

Gruber, S. H. 1984. Bioenergetics of the captive and free-ranging lemon shark. *In:* AAZPA Annual Conference Proceedings, Sept 9-13, Miami, Florida, p. 340-373. American Association of Zoological Parks and Aquariums (American Zoo and Aquarium Association), Silver Spring, Maryland, USA.

Gruber, S. H. and R. Keyes. 1981. Keeping sharks for research. *In:* Aquarium Systems, p. 452. A. D. Hawkins (ed.). Academic Press, London, England.

Gruber, S. H. and R. G. Stout. 1983. Biological methods for the study of age and growth in a tropical marine elasmobranch, the lemon shark *Negaprion brevirostris* (Poey). NOAA Tech. Report, U.S. Dept. Commerce, NMFS 8: 193-205.

Gulland, J. A. and S. J. Holt. 1959. Estimation of growth parameters for data at unequal time intervals. Journal du Conseil. Conseil International pour l'Exploration de la Mer 25: 47-49.

Hazel, C. R. and S. J. Meith. 1970. Bioassay of king salmon eggs and sac fry in copper solutions. California Fish and Game 56(2): 121-124.

Henningsen, A. D. 2000. Notes on reproduction in the southern stingray, *Dasyatis americana* (Chondrichthyes: Dasyatidae), in a captive environment. Copeia 2000(3): 826-828.

Hewitt, J. C. 1984. The great white in captivity: A history and prognosis. *In:* AAZPA Annual Conference Proceedings, September 9-13, Miami, Florida, p. 317-324. American Association of Zoological Parks and Aquariums (American Zoo and Aquarium Association), Silver Spring, Maryland, USA.

Hoenig, J. M. 1979. The vertebral centra of sharks and their use in age determination. M.S. thesis. University of Rhode Island, Kingston, USA.

Jones, B. C. and G. H. Geen. 1977. Age and growth of the spiny dogfish (*Squalus acanthias*) in the Strait of Georgia, British Columbia. Environment Canada, Fisheries and Marine Service, Technical Report Number 699. 16 p.

Kohler, N. E., J. G. Casey, and P. A. Turner. 1995. Length-weight relationships for 13 species of sharks from the western North Atlantic. Fishery Bulletin 93: 412-418.

Kuenen, M. 2000. A log of captive births by an Atlantic nurse shark, “Sarah”. Drum and Croaker 31: 22-24. (www3)

Lucifora, L. O., R.C. Menni, and A. H. Escalante. 2002. Reproductive ecology and abundance of the sand tiger shark, *Carcharias taurus*, from the southwestern Atlantic. ICES Journal of Marine Science 59: 553-561.

McCosker, J. E. 1987. The white shark, *Carcharodon carcharias*, has a warm stomach. Copeia 1987: 195-197.

Mohan, P. J. 1996. Using fisheries data to manage the diets of captive elasmobranchs. *In:* AZA Annual Conference Proceedings, September 17-21, Waikiki, Hawaii, p. 265-273. American Zoo and Aquarium Association, Silver Spring, Maryland, USA.

Mohan, P. J. 2000. Some length-weight relationships for sharks and their use in husbandry programs. Drum and Croaker 31: 11-16. (www3)

Mollet, H. F. and G. M. Cailliet. 1996. Using allometry to predict body mass from linear measurements of the white shark. *In:* Great White Sharks: The Biology of *Carcharodon carcharias*, p. 81-89. A. P. Klimley and D. G. Ainley (eds.). Academic Press, New York, USA.

Mollet, H. F., G. Cliff, H. L. Pratt, Jr., and J. D. Stevens. 2000. Reproductive biology or the female shortfin mako, *Isurus oxyrinchus* Rafinesque, 1810, with comments on the embryonic development of lamnoids. Fishery Bulletin 98: 299-318.

Natanson, L. J. and G. M. Cailliet. 1990. Vertebral growth zone deposition in Pacific angel sharks. Copeia 1990(4): 1133-1145.

Natanson, L., S. Mello, and S. Campana. 2001. Validated age and growth of the porbeagle shark (*Lamna nasus*) in the western North Atlantic. Fishery Bulletin 100: 266-278.

Pauly, D. 1979. Gill size and temperature as governing factors in fish growth: A generalization of von Bertalanffy’s growth formula. Berichte des Institut für Meereskunde an der Universität Kiel 63: 156.

Pratt, H. L. and J. G. Casey. 1983. Age and growth of the shortfin mako, *Isurus oxyrinchus*, using four methods. Canadian Journal of Fisheries and Aquatic Sciences 40(11): 1944-1957.

Reidarson, T. H., and J. McBain. 1994. Hyperglycemia in two great white sharks. International Association for Aquatic Animal Medicine (IAAAM) Newsletter 25(4): (unnumbered page).

Rupp, J. H. 1984. The capture and husbandry of cowsharks, family Hexanchidae. *In:* AAZPA Annual Conference Proceedings, September 9-13, Miami, Florida, p. 325-332. American Association of Zoological Parks and Aquariums (American Zoo and Aquarium Association), Silver Spring, Maryland, USA.

Schaadt, M. S. and J. Landesman. 1997. Husbandry of Pacific angel sharks at Cabrillo Marine Aquarium. Drum and Croaker 28: 3-5. (www3)

Schmid, T. H. and F. L. Murru. 1994. Bioenergetics of the bull shark, *Carcharhinus leucas*, maintained in captivity. Zoo Biology 13: 177-185.

Schmid, T. H., F. L. Murru, and F. McDonald. 1990. Feeding habits and growth rates of bull (*Carcharhinus leucas* (Valenciennes)), sandbar (*Carcharhinus plumbeus* (Nardo)), sand tiger (*Eugomphodus taurus* (Rafinesque)) and nurse (*Ginglymostoma cirratum* (Bonnaterre)) sharks maintained in captivity. Journal of Aquariculture and Aquatic Sciences 5(4): 100-105.

Schwartz, F. J. 1983. Shark ageing methods and age estimation of scalloped hammerhead, *Sphyrna lewini*, and dusky, *Carcharhinus obscurus*, sharks based on vertebral ring counts. NOAA Technical Report NMFS 8: 167-174.

Seki, T., T. Taniuchi, H. Nakano, and M. Shimizu. 1998. Age, growth and reproduction of the oceanic whitetip shark from the Pacific Ocean. Fisheries Science 64(1): 14-20.

Simpendorfer, C. 2000. Growth rates of juvenile dusky sharks, *Carcharhinus obscurus* (Lesueur, 1818), from southwestern Australia estimated from tag-recapture data. Fishery Bulletin 98: 811-822.

Simpendorfer, C., J. Chidlow, R. McAuley, and P. Unsworth. 2000. Age and growth of the whiskery shark, *Furgaleus macki*, from southwestern Australia. Environmental Biology of Fishes 58: 335-343.

Sminkey, T. R. and J. A. Musick. 1995. Age and growth of the sandbar shark, *Carcharhinus plumbeus*, before and after population decline. Copeia 1995(4): 871-883.

Sokal, R. R. and F. J. Rohlf. 1995. Biometry, 3rd edition. W.H. Freeman and Company, New York, USA. 887 p.

Springer S. 1960. Natural history of the sandbar shark, *Eulamia milberti*. Fishery Bulletin 61: 1-38.

Stevens, J. D. 1984. Life-history and ecology of sharks at Aldabra Atoll, Indian Ocean. Proceedings of the Royal Society of London 222: 79-106.

Stillwell, C. E. and N. E. Kohler. 1982. Food, feeding habits, and estimates of daily ration of the shortfin mako *(Isurus oxyrinchus)* in the northwest Atlantic. Canadian Journal of Fisheries and Aquatic Sciences 39: 407-414.

Stillwell, C. E. and N. E. Kohler. 1993. Food habits of the sandbar shark *Carcharhinus plumbeus* off the northeast coast, with estimates of daily ration. Fishery Bulletin 91: 138-150.

Thorson, T. B. and E. J. Lacy, Jr. 1982. Age, growth rate and longevity of *Carcharhinus leucas* estimated from tagging and vertebral rings. Copeia 1982(1): 110-116.

Uchida, S., M. Toda, and Y. Kamei. 1990. Reproduction of elasmobranchs in captivity. *In:* Elasmobranchs as Living Resources: Advances in the Biology, Ecology, Systematics, and the Status of the Fisheries, p. 211-237. H. L. Pratt, Jr., S. H. Gruber, and T. Taniuchi (eds.). NOAA Technical Report. NMFS 90.

.Van Dykhuizen, G. and H. F. Mollet. 1992. Growth, age estimation and feeding of captive sevengill sharks, *Notorynchus cepedianus*, at the Monterey Bay Aquarium. Australian Journal of Marine and Freshwater Research 43: 297-318.

Wass, R.C. 1971. A comparative study of the life history, distribution and ecology of the sandbar shark and the grey reef shark in Hawaii. Ph.D. thesis. University of Hawaii, Honolulu, USA.

Wass, R. C. 1973. Size, growth and reproduction of the sandbar shark, *Carcharhinus milberti*, in Hawaii. Pacific Science, 27(4), 305-318.

Wintner, S. P. and G. Cliff. 1999. Age and growth determination of the white shark, *Carcharodon carcharias*, from the east coast of South Africa. Fishery Bulletin 97(1): 153-169.

PERSONAL COMMUNICATIONS

Berzins, I. 2001. The Florida Aquarium, Tampa, FL 33602-5614, USA.
Charbeneau, G. 2001. New Jersey Aquarium, Camden, NJ 08103-1060, USA.
Cliff, G. 2001. Natal Sharks Board, Private Bag 2, Umhlanga 4320, South Africa.
Davis, R. 2001. SeaWorld Orlando, Orlando, FL 32821, USA.
Ebert, D. 2002. Moss Landing Marine Laboratories, Moss Landing, CA 95039, USA.
Farquhar, M. 2001. Two Oceans Aquarium, Cape Town, South Africa.
Fischer, A. 2001. Underwater World, Sunshine Coast, Mooloolaba, QLD 4557, Australia.
Henningsen, A. 2002. National Aquarium in Baltimore, Baltimore, MD 21202, USA.
Howard, M. 2002. Aquarium of the Bay, San Francisco, CA 94133, USA.
Kerivan, J. 1995. SeaWorld Orlando, Orlando, FL 32821, USA.
Kohler, N. 1996. Northeast Fisheries Science Center, Narragansett Laboratory, Narragansett, RI 02882, USA.
Loiselle, P. 2001. New York Aquarium, Brooklyn, NY 11224-2899, USA.
Mollet, H. 2002. Moss Landing Marine Laboratories, Moss Landing, CA 95039, USA.
Natanson, L. 2002. Northeast Fisheries Science Center, Narragansett Laboratory, Narragansett, RI 02882, USA.
Romero, J. 2001. National Marine Aquarium, Plymouth, PL4 0LF, UK.
Schaadt, M. 2001. Cabrillo Marine Aquarium, San Pedro, CA 90731, USA.
Shaw, M. 2001. SeaWorld San Diego, San Diego, CA 92109-7993, USA.
Steslow, F. 2001. New Jersey Aquarium, Camden, NJ 08103-1060, USA.
Violetta, G. 2001. SeaWorld Orlando, Orlando, FL 32821, USA.

INTERNET RESOURCES

www1 http://homepage.mac.com/mollet/VBGF/VBGF.html

www2 http://homepage.mac.com/mollet/Cc/Ian_Gordon.html

www3 http://www.colszoo.org/internal/drumcroaker.htm

The Elasmobranch Husbandry Manual: Captive Care of Sharks, Rays and their Relatives, pages 227-236.
© 2004 Ohio Biological Survey

Chapter 16
Reproduction, Embryonic Development, and Reproductive Physiology of Elasmobranchs

ALAN D. HENNINGSEN

National Aquarium in Baltimore,
Pier 3, 501 E. Pratt Street.
Baltimore, MD 21202, USA.
E-Mail: ahenningsen@aqua.org

MALCOLM SMALE

Port Elizabeth Museum,
P. O. Box 13147.
Humewood 6013, South Africa.
E-Mail: pemmjs@zoo.upe.ac.za

ROD GARNER

58 Carter Road,
Nambour, QLD 4560, Australia.
E-Mail: rodgarner@powerup.com.au

NINO KINNUNEN

Sydney Aquarium,
Aquarium Pier,
Darling Harbour, NSW 2000, Australia.
E-Mail: nkinnunen@hotmail.com

Abstract: Chondrichthyan reproduction is characterized by internal fertilization, diverse reproductive modes, complex reproductive cycles, late sexual maturity, iteroparity (several litters per lifetime), and small brood size. Embryonic development in elasmobranchs ranges from two months to at least two years, and generally proceeds uninterrupted, with the exception of those species in which embryonic diapause has been confirmed. Relatively little information on reproduction in captive elasmobranchs has been published. Information on reproduction from wild conspecifics is therefore useful in assessing reproductive potential in captive elasmobranchs. Reproduction in captive animals may provide insights into hormonal fluctuations, behavior, and maternal-brood relationships. Differences from wild conspecifics may result from constraints associated with the captive environment. Detailed, accurate information relating to reproductive biology and physiology should be collected from captive specimens, and disseminated via peer-reviewed publications.

REPRODUCTION AND DEVELOPMENT

Reproduction in chondrichthyans is variable in terms of the functional morphology of the reproductive tract, biology and behavior, embryonic development, and modes of embryonic nutrition. In general, the reproductive biology of elasmobranchs is characterized by: delayed sexual maturity, diverse modes of embryonic nutrition, different reproductive cycles, and low

fecundity. Several excellent summaries provide extensive detail on reproduction in elasmobranchs and holocephalans (e.g., Wourms 1977; Wourms 1981; Dodd, 1983; Wourms et al., 1988). The intent of this chapter is to summarize the general properties of reproductive biology in chondrichthyans, focusing on elasmobranchs, and apply them to captivity. Compared to wild conspecifics, relatively little has been published on the reproductive biology of captive elasmobranchs in aquariums.

REPRODUCTIVE ANATOMY

Reproductive anatomy is the same for each sex across elasmobranch taxa, although there are some specializations in each sex and asymmetries, particularly with respect to the female reproductive tract. The principal components of the reproductive tract in male elasmobranchs include the: testes; epidymis; Leydig's gland; vas deferens; seminal vesicle; siphon sac, clasper gland, or alkaline gland; and the clasper. The principal components of the reproductive tract in female elasmobranchs include the: ovaries; ostia (ostium); oviduct; shell gland; uterus; and cervix.

There are numerous photographs and drawings available in the literature depicting the reproductive tracts in general, and for several species (e.g., Castro, 1983; Maruska et al., 1996), as well as those for the commonly depicted spiny dogfish (*Squalus acanthias*). The gonads, testes in males and ovaries in females, are located in a dorsal retroperitoneal position, supported by mesenteries, mesorchia, and mesovaria, respectively. Gonad structure varies across taxon groups within the subclass (Pratt, 1988). Primarily, morphological differences occur in the gonad type (Pratt, 1988), claspers in males (Compagno, 1988), and nidamental, oviducal, or shell gland in females (Hamlett et al., 1998). Both testes are active in all species studied to date, and the zonate pattern of the mature elasmobranch testis lends itself well to physiological and histological studies (Dodd, 1983; Callard, 1988; Callard and Klosterman, 1988; Parsons and Grier, 1992). The siphon sac in male sharks is replaced by the clasper gland and alkaline gland in batoids.

Both left and right ovaries and oviducts are functional in some groups (e.g., skates). The right ovary and both oviducts are functional in other groups (e.g., lamniform and carcharhiniform sharks), whereas only the left ovary and oviduct are functional in others (e.g., many myliobatiform rays). The main specializations in the female reproductive tract occur in the shell gland and the uteri. The shell gland is reduced in viviparous forms. Uterine specializations include infoldings, uterine villi or trophonemata (in myliobatiform rays), and compartmentalization (in placental viviparous sharks). Additional specializations have occurred in the reproductive tracts for the storage and packaging of sperm in the seminal vesicles, prior to copulation, in some males (Pratt and Tanaka, 1994), and prior to ovulation and fertilization, in the shell gland, in some females (Pratt, 1993), in those species that have been investigated.

Spermatozoa may be stored in the female reproductive tract from the short-term to periods exceeding two years, in some species (Dodd, 1983; Castro et al., 1988; Pratt, 1993). In other species, such as the Atlantic stingray (*Dasyatis sabina*), there was no evidence for sperm storage by females in a distinct study area (Maruska et al., 1996; Tricas et al., 2000). Mollet et al. (2002) suggested sperm storage for a year in the pelagic stingray (*Dasyatis* [=*Pteroplatytrygon*] *violacea*) based on captive specimens.

Oviducal sperm storage, beyond a few days to weeks, is unlikely in oophagous sharks, due to the volume of ova that passes through the oviducal gland, and long-term sperm storage has not been observed in lamniform species studied to date (Pratt, 1993). The examples provided above illustrate the variability that occurs with regard to sperm storage. For many species, however, it is not known whether or not sperm storage occurs. In addition, it is not known whether captivity may alter (shorten or lengthen) the period of sperm storage in those species in which it has been documented. Sperm storage might have to be taken into account, therefore, when estimating the length of gestation.

The source of specimens is an important consideration as reproductive parameters for a given species may vary depending upon the geographical position within its range (e.g., Parsons, 1993; Lucifora et al., 1999). Parsons (1993) documented differences in mean size at birth and size at maturity in the bonnethead shark (*Sphyrna tiburo*) in two geographically separated populations. The sandbar shark (*Carcharhinus plumbeus*) is another example of a species in which the litter size, mean size at birth, and size at maturity may vary according to the geographical area within its distribution (Springer, 1960; Wass, 1973; Taniuchi, 1971; Joung and Chen, 1995).

MATURITY STATUS

Maturity status in males can be determined from the size and degree of calcification of the claspers, and the ease of opening of the clasper rhipidion, as well as the degree of rotation. Clasper rotation is not a definitive character of maturity in all species, however, as claspers rotate in all size classes of the porbeagle shark (*Lamna nasus*), for example (Jensen et al., 2002). In living specimens, maturity is more difficult to assess with certainty (Pratt and Tanaka, 1994), but external characters related to the claspers usually allow relatively easy assessment. The progression from immaturity to maturity in males can be determined from the rapid increase in clasper length relative to total length (disc width in rays). The presence of viable sperm is a positive indicator of maturity (Pratt, 1979). Upon dissection in males, the progression to maturity can be determined from the vas deferens, as it becomes coiled in adults. In females, maturity is difficult to assess based upon external characters and dissection allows assessment of the status of ovarian recrudescence, oviduct, nidamental gland, and uteri. Furthermore, pregnancy and the degree of development of embryos or fetuses may be determined. The transition to maturity in females is assessed by examining the width of the shell gland, the transition from threadlike undifferentiated uteri to ribbon-like well-differentiated uteri, and ovarian development. In live captive females, diagnostic in vivo imaging (refer Chapter 22 of this manual) is helpful in determining ovarian activity, diameter of the shell gland, oviducts and uteri, and size of embryos or fetuses. These imaging techniques afford a more subjective assessment than when working with dissected specimens.

REPRODUCTIVE CYCLES

Reproductive cycles have been classified by several authors (Wourms, 1977; Dodd and Sumpter, 1984; Koob and Callard, 1999; Hamlett and Koob, 1999). The cycles as defined by Koob and Callard (1999) are:

1. continuous for those species that reproduce throughout the year,
2. seasonal for those species that are reproductively active for only a part of the year, and
3. punctuated for those species that are pregnant for about a year and the next pregnancy is at least a year later.

The stages of the reproductive cycle exhibit certain characteristics. While there is some variation for males, the greatest number of stage-specific characters is displayed by females. In males, the main general stages correspond to mating, the stages of spermatogenesis, and testicular development (Maruska et al., 1996). The stages of the reproductive cycle in females can vary for each type of reproductive cycle. As an example of a seasonal cycle, the placental viviparous bonnethead shark has nine stages: mating, pre-ovulation, ovulation, post-ovulation, early pregnancy, implantation, late pregnancy, parturition, and post-partum. (Manire et al., 1995).

It is important to note that the reproductive cycle in captive animals may differ from that observed in wild conspecifics. Several species have been observed to mate immediately following parturition in captive animals, whereas a longer gap is observed in the wild, in the order of days to weeks in some cases. The Javanese cownose ray (*Rhinoptera javanica*) and the cownose ray (*Rhinoptera bonasus*) are two examples of this phenomenon (Smith and Merriner, 1986; Uchida et al., 1990; Henningsen, personal observation). In addition, parturition and mating may occur at a different time of year than in wild conspecifics. Other aspects of reproductive biology such as maternal-brood relationships may differ between wild and captive conspecifics as has been reported in the southern stingray (*Dasyatis americana*) (Henningsen, 2000). The opportunities provided by aquariums, however, can offer conditions for studies that may otherwise be extremely difficult or expensive. Gestation, for example, can often be estimated as the period between copulation and parturition in captive specimens. Care must be taken to ensure that estimates are placed in the context of a captive setting (i.e., results may be different in wild conspecifics).

DEVELOPMENT

The period from fertilization to hatching in oviparous species, or parturition in viviparous species, is referred to as incubation and gestation, respectively, in this chapter. Similar to other poikilotherms, temperature may have a profound effect on development time, decreasing with an increase in temperature. Some of the best available information on the effects of temperature upon incubation has been obtained for hemiscyllids, as they are commonly maintained and readily reproduce in captivity. For example,

Garner (2003) noted a 12% decrease in incubation from 115 to 101 days with an increase in temperature from 24 to 27°C in the brownbanded bamboo shark (*Chiloscyllium punctatum*). Michael (2001) observed a 27% decrease for the same temperature increase.

REPRODUCTIVE MODES

While reproductive modes have been classified in several ways (see: Breder and Rosen, 1966; Wourms, 1977; Wourms, 1981; Wourms et al., 1988; Compagno, 1990; Hamlett et al., 1992; Hamlett and Koob, 1999), two basic forms of parity, oviparity and viviparity, occur in chondrichthyans. There are variations, however, as some oviparous species deposit eggs at an early stage of development (e.g., skates and some scyliorhinids), while others deposit eggs at an advanced stage of development (e.g., some scyliorhinids) (Wourms et al., 1988). These forms correspond to Compagno's (1990) extended and retained forms of oviparity, respectively. For this chapter, the modes of reproduction will be discussed as described in Hamlett and Koob (1999):

1. oviparity;
2. aplacental yolk sac viviparity;
3. aplacental viviparity with uterine villi or trophonemata;
4. aplacental viviparity with oophagy and (with or without) intrauterine cannibalism; and
5. placental viviparity.

Although reproductive modes of chondrichthyans are not strongly correlated to their phylogeny (Compagno, 1990), there are some trends. As in other vertebrates, oviparity is thought to be the primitive condition and viviparity more derived (Callard et al., 1995; Luer and Gilbert, 1991; Dulvy and Reynolds, 1997). All extant holocephalans and rajoids are oviparous, and although oviparity also occurs in certain shark taxa, approximately two-thirds of the sharks and all other batoids are viviparous (Wourms, 1977; Wourms, 1981; Compagno, 1990). In some families, reproductive mode is consistent, but variations have been documented at both the family and generic level. The genus *Mustelus*, for example, contains species that exhibit aplacental yolk-sac viviparity, while others use placental viviparity. Oophagy is predominant in lamniform sharks; however, the orectolobiform tawny nurse shark (*Nebrius ferrugineus*) and the carcharhiniform false cat shark (*Pseudotriakis microdon*) are both reported to be oophagous (Yano, 1992; Teshima et al., 1995). It is in females, particularly in the uterus, where several specializations have occurred to accommodate developing embryos and fetuses (Hamlett and Hysell, 1998). Furthermore, the frequently observed larger size of females compared to conspecific males has often been attributed to increasing the space available to developing embryos.

Embryonic development in cartilaginous fishes has been reported to range from two months in the pelagic stingray, to at least 3½ years in the frilled shark (*Chlamydoselachus anguineus*) (Ranzi, 1932; Tanaka et al., 1990), although two years has also been suggested for the latter (Gudger, 1940). Generally, development proceeds uninterrupted; exceptions are those species with embryonic diapause such as: the Australian sharpnose shark (*Rhizoprionodon taylori*) (Simpfendorfer, 1992), the bluntnose stingray (*Dasyatis say*) (Snelson et al., 1989), the Brazilian guitarfish (*Rhinobatos horkeli*) (Lessa et al., 1986 in Simpfendorfer, 1992), the shovelnose guitarfish (*Rhinobatos productus*) (Villavicencio-Garayzar, 1993a; Villavicencio-Garayzar et al., 2001), the common guitarfish, (*Rhinobatos rhinobatos*) (Abdel-Aziz et al., 1993), the giant electric ray (*Narcine entemedor*) (Villavicencio-Garayzar et al., 2001), the Brazilian electric ray (*Narcine brasiliensis*) (Villavicencio-Garayzar, 1993b), and the whiptail stingray (*Dasyatis brevis=dipterura*) (Villavicencio-Garayzar et al., 2001). The reader is referred to Wourms (1977) and the references therein for summaries of development. Details of embryonic development have been given for oviparous (Luer and Gilbert, 1985), aplacental yolk-sac viviparous (Natanson and Cailliet, 1986;), aplacental viviparous with uterine villi or trophonemata (Lewis, 1982; Thorson et al., 1983; Amesbury, 1997), aplacental viviparous with oophagy with or without intrauterine cannibalism (Gilmore et al., 1983, Gilmore 1993; Francis and Stevens, 2000), and placental viviparous (e.g., Hamlett, 1993; Wourms, 1993) species. Excellent photographs and drawings that depict the stages of embryonic/fetal development are available in the literature (i.e., Gilmore et al., 1983; Castro, 2000).

REPRODUCTIVE ABNORMALITIES IN CAPTIVITY

Reproductive abnormalities occur in elasmobranchs as well as in other animals. It is difficult to ascertain the occurrence of certain reproductive

abnormalities in wild conspecifics, but in some captive elasmobranch species broods can include both term live fetuses as well as incompletely developed stillborn fetuses. This phenomenon has been observed in the sand tiger shark (*Carcharias taurus*) (Gordon, pers. com.), the southern stingray (Henningsen, personal observation), and the leopard shark (*Triakis semifasciata*) (Ankley, pers. com.). Deformed or "stunted" or "runt of the litter" embryos do occur in nature (Smale and Goosen, 1999). Females have retained encapsulated ova, and there are observations of mortalities associated with "egg-bound" female spotted wobbegongs (*Orectolobus maculatus*) (Gordon, pers. com.). Whether it is unique to captive sharks is unknown, but it is not uncommon for female sand tiger sharks to release infertile ova (Henningsen, personal observation; Gordon, pers. com.) or female nurse sharks (*Ginglymostoma cirratum*) to shed "wind" eggs (fully-formed egg capsules devoid of yolk or embryos).

Another observed abnormality is retention of term fetuses in utero beyond the expected time of parturition in both wild and captive specimens. This "over-gestation" has been noted in some batoids such as the cownose ray (Henningsen, 1999) and the yellow stingray (*Urobatis jamaicensis*). In the former, term fetuses have remained live in utero up to two months past the normal suggested gestation of 11 months (Smith and Merriner, 1986; Henningsen, 1999), and in the latter, up to four months (Stamper, pers. com.) past the normal 3-5 month gestation (Spieler, pers. com.; Hamlett, pers. com.). The two examples cited here correspond to a range of 20-100% over-gestation time. Retention in utero has also been observed in pelagic stingrays (Mollet et al., 2002). In contrast, gravid female elasmobranchs may readily abort when faced with stress, both environmental and physiological (Smith, 1980; Snelson et al., 1988).

In oviparous species, oviposition usually occurs in pairs, several days apart (Luer and Gilbert, 1985, Koob and Callard, 1999; Castro et al., 1988). In placental viviparous species, parturition normally occurs within minutes to hours (Parsons, 1991; Parsons, 1993). However, normal full-term fetuses have been born days to weeks apart in some captive placental viviparous specimens, including the bull shark (*Carcharhinus leucas*) (Uchida et al., 1997) and blacktip reef shark (*Carcharhinus melanopterus*) (Riggles, pers. com.). It is unclear whether this protracted parturition is due to environmentally-driven endocrine factors. Another plausible explanation is that successive parturitions, as well as stillbirths and abortions, originated in a separate uterus. Protracted parturition is normal in some species. In some lecithotrophic, aplacental species, such as the nurse shark, parturition is normally spread out over several days. Ovulation is a prolonged process spread over 2-3 weeks in this species, and embryos may be found at different stages of development in the uterus (Castro, 2000). This "conveyer belt" method occurs in the retained oviparous species, referred to in Wourms et al. (1988) and Compagno (1990).

Conditions in aquariums are suitable for describing other abnormalities. Hermaphroditism has been observed in elasmobranchs, but not as yet in captivity. A case of gynogenesis has been reported in an aquarium (Voss et al., 2001).

REPRODUCTIVE PHYSIOLOGY

The physiological control of reproduction should be considered when attempting to promote or inhibit reproduction in captive animals. Reproductive physiology has been reviewed by several authors (for example see Dodd, 1983; Callard et al., 1988; Hamlett, 1999; Hamlett and Koob, 1999). Demski (1990a; 1990b) provides a focused discussion for reproduction in captive elasmobranchs.

As in other key components of life history, environmental parameters have profound effects upon reproduction. Environmental cues, primarily temperature and photoperiod, are relayed via the central nervous system to target organs such as the gonads, thyroid, and interrenal gland. The effects, both positive and negative, are mediated through the neuroendocrine system (Demski, 1990a; Demski, 1990b; Redding and Patiño, 1993; Henningsen, 1999). Gonadotropin releasing hormone (GnRH) is important in vertebrates in regulating gonadotropin release, and hence reproductive physiology, through the hypothalamus-pituitary-gonadal axis (Demski, 1990a; Pierantoni et al., 1993; Forlano et al., 2000). Unique to chondrichthyans, however, GnRH reaches the gonadotropes, in the ventral lobe of the pituitary in elasmobranchs and in the buccal lobe of the pituitary in holocephalans, via the systemic circulation (Pierantoni et al., 1993: Sherwood and Lovejoy, 1993; Wright and Demski, 1993). It is important to note the effect that environmental parameters have on reproductive physiology in captive elasmobranchs, because

factors such as temperature and photoperiod can readily be altered in aquarium systems.

Reproductive endocrinology is a major component of reproductive physiology and has been described in numerous articles (e.g., Koob et al., 1986; Rasmussen et al., 1992; Manire et al., 1995; Manire et al., 1999a; Snelson et al., 1997). The principal hormones associated with reproduction in elasmobranchs are steroid and peptide hormones similar to other vertebrates. Although about 19 different reproductively-related steroid hormones have been identified in elasmobranchs, detailed investigations conducted throughout the reproductive cycle have, until recently, focused on four of these: 17-β estradiol, progesterone, testosterone, and 5α-dihydrotestosterone (Manire et al., 1999a). Recent work has shown that other steroids, principally other androgens and progestins as well as glucocorticoids, may play important roles at key points during reproduction (Garnier et al., 1999; Manire et al., 1999a; Manire et al., 1999b). It is beyond the scope of this manual to present a review of elasmobranch reproductive endocrinology, but a summary of the hormones associated with reproduction is presented below. Serum steroid titers have been published for oviparous (e.g., Sumpter and Dodd, 1979; Koob et al., 1986; Heupel et al., 1999; Rasmussen et al., 1999), aplacental yolk sac (Lupo di Prisco et al., 1967; Tsang and Callard, 1987; Fasano et al., 1992), aplacental with trophonemata (Snelson et al., 1997; Tricas et al., 2000), oophagous with embryophagy (Rasmussen and Murru, 1992) and placental viviparous species (i.e. Rasmussen and Gruber, 1993; Manire et al., 1995; Manire et al., 1999a; Manire and Rasmussen, 1997). Putative as well as definitive roles for steroids during reproduction in elasmobranchs have been identified; these include regulation of the reproductive tract and modulating behavior (Callard and Koob, 1993; Callard et al., 1993; Sisneros and Tricas, 2000). In addition to steroid hormones, peptide hormones, such as relaxin and the oxytocin-like peptides, have been determined to play key roles during reproduction (Koob et al., 1984; Callard and Koob, 1993; Sorbera and Callard, 1995).

The levels of specific steroids not only play key roles in reproduction, but clearly can be associated with stages of the reproductive cycle in some cases. In addition, the levels of the steroids rise in accordance with maturational status (Rasmussen and Gruber, 1993). Most studies of the endocrine cycle in elasmobranchs have focused on females, and few studies have examined the steroid levels over the entire reproductive cycle in male elasmobranchs (Manire and Rasmussen, 1997). In general, the levels of androgens in males peak prior to the period of maximum sperm production and mating. The patterns for estradiol and progesterone vary in those species that have been investigated. The levels of steroids in females over the entire reproductive cycle show some variations, but some trends are consistent. The levels of estradiol, for example, increase prior to and during vitellogenesis, when yolk products are stored in the developing oocytes (follicular phase). Progesterone peaks in the peri-ovulatory and post-ovulatory periods, with some differences observed in the timing of this peak. The duration of the post-ovulatory peak in progesterone, when it occurs, is correlated to the functional life of the corpora lutea (post-ovulatory cycle), the source of the progesterone. Despite these similarities, the steroid levels and the timing of peaks vary considerably in those species examined.

To date, the sole published values of reproductively-related hormones in elasmobranchs in a public aquarium were by Rasmussen and Murru (1992). The titers obtained from carcharhinids were comparable to those in non-stressed, wild sharks. In two captive populations of sand tiger sharks, one of the authors (Henningsen) observed reproductively-related hormone differences between the groups, particularly in males. In addition, monthly sampling of one of these captive populations revealed significant individual variation with respect to the levels of steroids as well as the timing of steroid peaks. Similar studies would be valuable for determining reproductive status in captive elasmobranchs.

SUGGESTIONS FOR THE FUTURE

Acquisition of many species of elasmobranchs, for display in aquariums, is becoming increasingly restricted (refer to Chapter 3 of this manual). Captive specimens must be viewed as a resource, both for captive breeding programs (refer to Chapter 17 of this manual) and for obtaining data relevant to the biology and conservation of wild populations. Valuable, detailed information relating to reproduction can be obtained with relatively little effort from existing captive specimens. Such information should be obtained from all available specimens. Focus should be placed on documenting this information and publishing it in peer-reviewed outlets. Examples of such studies include investigations into the reproductive biology of nurse sharks (Castro,

2000) and reproductive parameters for Southern stingrays (Henningsen, 2000).

Imaging techniques can be used to collect details on reproductive tract development in live specimens (refer to Chapter 22 of this manual). Measurements taken (oocyte diameter, etc.), however, should be validated. The directive is to collect more quantitative data on reproductive biology and physiology from captive elasmobranchs. Serum hormone titers, coupled with morphological and behavioral correlates should be monitored.

By collecting and publishing information on reproduction of elasmobranchs in aquariums, the gap between what is known and published for wild conspecifics and what is known for captive specimens will be closed. In addition, more differences or similarities between wild and captive conspecifics can be documented.

ACKNOWLEDGEMENTS

We acknowledge the support provided by our respective institutions: the National Aquarium in Baltimore, Port Elizabeth Museum, and the Sydney Aquarium. Appreciation is extended to Dr. José Castro and H. L. “Wes” Pratt for critical reviews of an early draft of the manuscript, as well as for support to the senior author. Special thanks go to Mark Smith and Doug Warmolts for making this volume possible, as well as to all of the editors, Mark Smith, Doug Warmolts, Robert Hueter, and Dennis Thoney.

REFERENCES

Abdel-Aziz, S. H., A. N. Khalil, and S. A. Abdel-Maguid. 1993. Reproductive cycle of the common guitarfish, *Rhinobatos rhinobatos* (Linnaeus, 1758), in Alexandria waters, the Mediterranean Sea. Australian Journal of Marine and Freshwater Research 44: 507-517.

Amesbury, E. 1997. Embryo development and nutrition in the Atlantic stingray, *Dasyatis sabina* (Elasmobranchii: Dasyatidae). M. S. Thesis, University of Central Florida. Orlando, Florida, USA. 92 p.

Breder, C. M., Jr. and D. E. Rosen. 1966. Modes of Reproduction in Fishes. Natural History Press, Garden City, New York, USA. 941 p.

Callard, G. V. 1988. Reproductive physiology, Part B. *In:* Physiology of Elasmobranch Fishes p. 293-317. T. J. Shuttleworth (ed.). Springer-Verlag, New York, USA.

Callard, I. P. and L. Klosterman. 1988. Reproductive physiology, Part A. *In:* Physiology of Elasmobranch Fishes p. 277-292. T. J. Shuttleworth (ed.). Springer-Verlag, New York, USA.

Callard, I. P., O. Putz, M. Paolucci, and T. J. Koob. 1995. Elasmobranch reproductive life-histories: Endocrine correlates and evolution. *In:* Proceedings of the Fifth International Symposium on the Reproductive Physiology of Fish, p. 204-208. F. Goetz and P. Thomas (eds.). University of Texas, Austin, Texas, USA.

Callard, I. P., L. A. Fileti, and T. J. Koob. 1993. Ovarian steroid synthesis and the hormonal control of the elasmobranch reproductive tract. Environmental Biology of Fishes 38: 175-185.

Callard, I. P. and T. J. Koob. 1993. Endocrine regulation of the elasmobranch reproductive tract. Journal of Experimental Zoology 266: 368-377.

Castro, J. I. 1983. The Sharks of North American Waters. Texas A & M University Press, College Station, Texas, USA. 180 p.

Castro, J. I., P. M. Bubucis, and. N. A. Overstrom. 1988. The reproductive biology of the chain dogfish, *Scyliorhinus retifer*. Copeia 1988(3): 740-746.

Castro, J. I. 2000. The biology of the nurse shark, *Ginglymostoma cirratum*, off the Florida east coast and the Bahama Islands. Environmental Biology of Fishes 58: 1-22.

Compagno, L. J. V. 1988. Sharks of the Order Carcharhiniformes. Princeton University Press, Princeton, New Jersey, USA. 486 p.

Compagno, L. J. V. 1990. Alternative life-history styles of cartilaginous fishes in time and space. Environmental Biology of Fishes 38: 33-75.

Demski, L. S. 1990a. Neuroendocrine mechanism controlling sexual development and behavior of sharks and rays. Journal of Aquariculture and Aquatic Sciences 5: 53-67.

Demski, L. S. 1990b. Elasmobranch reproductive biology: implications for captive breeding. Journal of Aquariculture and Aquatic Sciences 5: 84-95.

Dodd, J. M. 1983. Reproduction in cartilaginous fishes. *In:* Fish Physiology, Vol. IX, Part A, p. 31-95. W. S. Hoar, D. J. Randall, and E. M. Donaldson (eds.). Academic Press, New York, USA.

Dodd, J. M. and J. P. Sumpter. 1984. Fishes. *In:* Marshall’s Physiology of Reproduction, p. 1-126. G. E. Lamming (ed.). Churchill Livingstone, Edinburgh, Scotland.

Dulvy, N. K. and J. D. Reynolds. 1997. Evolutionary transitions among egg-laying, live bearing and maternal inputs in sharks and rays. Proceedings of the Royal Society of London B, 264: 1309-1315.

Fasano, S., M. D’Antonio, R. Pierantoni, and G. Chieffi. 1992. Plasma and follicular tissue steroid levels in the elasmobranch fish, *Torpedo marmorata*. General and Comparative Endocrinology 85: 327-333.

Forlano, P. M., K. P. Maruska, S. A. Sower, J. A. King, and T. C. Tricas. 2000. Differential distribution of gonadotropin-releasing hormone immunoreactive neurons in the stingray brain: functional and evolutionary considerations. General and Comparative Endocrinology 118: 226-248.

Francis, M. P and J. D. Stevens. 2000. Reproduction, embryonic development, and growth of the porbeagle shark, *Lamna nasus*, in the southwest Pacific Ocean. U. S. Fishery Bulletin 98: 41-63.

Garner, R. 2003. Annual fecundity, gestation period and egg survivorship in the brown-banded bamboo shark, *Chiloscyllium punctatum*, in captivity. Thylacinnus 27(3): 4-9

Garnier, D. H., P. Sourdaine, and B. Jégou. 1999. Seasonal variations in sex steroids and male sexual characteristics in *Scyliorhinus canicula*. General and Comparative Endocrinology 116: 281-290.

Gilmore, R. G., J. W. Dodrill, and P. A. Linley. 1983. Reproduction and embryonic development of the sand tiger shark, *Odontaspis taurus* (Rafinesque). U. S. Fishery Bulletin 81(2): 201-225.

Gilmore, R. G. 1993. Reproductive biology of lamnoid sharks. Environmental Biology of Fishes 38: 95-114.

Gudger, E. W. 1940. The breeding habits, reproductive organs and external embryonic development of *Chlamydoselachus*, based on notes and drawings by Bashford Dean. The Bashford Dean Memorial Volume, Archaic Fishes, Article VII: 525-633.

Hamlett, W. C. 1993. Ontogeny of the umbilical cord and placenta in the Atlantic sharpnose shark, *Rhizoprionodon terraenovae*. Environmental Biology of Fishes 38: 253-267.

Hamlett, W. C. 1999. Male reproductive system. *In:* Sharks, Skates and Rays: The Biology of Elasmobranch Fishes, p. 444-470. W. C. Hamlett (ed.). Johns Hopkins University Press, Baltimore, Maryland, USA.

Hamlett, W. C., A. G. Ferri, and M. A. Miglino. 1992. Modes of reproduction in the elasmobranchs of Brazil. *In:* Reproductive Biology of South American Vertebrates, p. 3-18. W. C. Hamlett (ed.). Springer-Verlag, New York, USA.

Hamlett, W. C. and M. K. Hysell. 1998. Uterine specializations in elasmobranchs. Journal of Experimental Zoology 282: 438-459.

Hamlett, W. C., D. P. Knight, T. J. Koob, M. Jezior, T. Luong, T. Rozycki, N. Brunette, and M. K. Hysell. 1998. Survey of oviducal gland structure and function in elasmobranchs. Journal of Experimental Zoology 282: 399-420.

Hamlett, W. C. and T. J. Koob. 1999. Female reproductive system. *In:* Sharks, Skates, and Rays: The Biology of Elasmobranch Fishes, p. 398-443. W. C. Hamlett (ed.). Johns Hopkins University Press, Baltimore, Maryland, USA.

Henningsen, A. D. 1999 Levels of recirculating reproductively-related steroid hormones in female elasmobranchs. Implications for reproduction in a captive environment. Aquarium Sciences and Conservation 2: 97-116.

Henningsen, A. D. 2000. Notes on reproduction in the southern stingray, *Dasyatis americana* (Chondrichthyes: Dasyatidae) in a captive environment. Copeia 2000(3): 826-828.

Heupel, M. R., J. M. Whittier, and M. B. Bennett. 1999. Plasma steroid hormone profiles and reproductive biology of the epaulette shark, *Hemiscyllium ocellatum*. Journal of Experimental Zoology 284: 586-594.

Jensen, C. F, L. J. Natanson, H. L. Pratt, Jr., N. E. Kohler, and S. E. Campana. 2002. The reproductive biology of the porbeagle shark *(Lamna nasus)* in the Western North Atlantic Ocean. Fishery Bulletin 100: 727-738.

Joung, S. and C. Chen. 1995. Reproduction in the sandbar shark, *Carcharhinus plumbeus*, in the waters of Northeastern Taiwan. Copeia 1995(3): 659-665.

Koob, T. J., J. J. Laffan, and I. P. Callard. 1984. Effects of relaxin and insulin on reproductive tract size and early fetal loss in *Squalus acanthias*. Biology of Reproduction 31: 231-238.

Koob, T. J., P. Tsang, and I. P. Callard. 1986. Plasma estradiol, testosterone, and progesterone levels during the ovulatory cycle of the little skate (*Raja erinacea*). Biology of Reproduction 35: 267-275.

Koob, T. J. and I. P. Callard. 1999. Reproductive endocrinology of female elasmobranchs: Lessons from the little skate (*Raja erinacea*) and the spiny dogfish (*Squalus acanthias*). Journal of Experimental Zoology, 284: 557-574.

Lewis, T. C. 1982. The reproductive anatomy, seasonal cycles, and development of the Atlantic stingray, *Dasyatis sabina* (Lesueur), from the northeastern Gulf of Mexico. Ph. D. dissertation. Florida State University, Tallahassee, Florida, USA. 206 p.

Lucifora, L. O., J. L. Valero, and V. B. Garcia. 1999. Length at maturity of the greeneye spurdog shark, *Squalus mitsukurii* (Elasmobranchii: Squalidae), from the SW Atlantic, with comparisons with other regions. Marine and Freshwater Research 50: 629-632.

Luer, C. A. and P. W. Gilbert. 1985. Mating behavior, egg deposition, incubation period and hatching in the clearnose skate, *Raja eglanteria*. Environmental Biology of Fishes 13: 161-171.

Luer, C. A. and P. W. Gilbert. 1991. Elasmobranch fish: oviparous, viviparous, and ovoviviparous. Oceanus 34(3): 47-53.

Lupo di Prisco, C., C. Vellano, and G. Chieffi. 1967. Steroid hormones in the plasma of the elasmobranch *Torpedo marmorata* at various stages of the sexual cycle. General and Comparative Endocrinology 8: 325-331.

Manire, C. A., L. E. L. Rasmussen, D. L. Hess, and R. E. Hueter. 1995. Serum steroid hormones and the reproductive cycle of the female bonnethead shark, *Sphyrna tiburo*. General and Comparative Endocrinology 97: 366-376.

Manire, C. A. and L. E. L. Rasmussen. 1997. Serum concentrations of steroid hormones in the mature male bonnethead shark, *Sphyrna tiburo*. General and Comparative Endocrinology 107: 414-420.

Manire, C. A., L. E. L. Rasmussen, and T. S. Gross. 1999a. Serum steroid hormones including 11-ketotestosterone, 11-ketoandrostenedione, and dihydroprogesterone in juvenile and adult bonnethead sharks, *Sphyrna tiburo*. Journal of Experimental Zoology 284: 595-603.

Manire, C. A., L. E. L. Rasmussen, and T. Tricas. 1999b. Elasmobranch corticosterone concentrations: related to stress or sex or what? 79th Annual meeting of the American Society of the Ichthyologists and Herpetologists, 15th Annual Meeting American Elasmobranch Society. Pennsylvania State University, State College, PA, USA. June 24-30, Abstract, 156 p.

Maruska, K. P., E. G. Cowie, and T. C. Tricas. 1996. Periodic gonadal activity and protracted mating in elasmobranch fishes. Journal of Experimental Zoology 276: 219- 232.

Michael, S. W. 2001. Aquarium Sharks and Rays. Tropical Fish Hobbyist Publications, Neptune City, New Jersey, USA. 254 p.

Mollet, H. F., J. M. Ezcurra, and J. B. O'Sullivan. 2002. Captive biology of the pelagic stingray, *Dasyatis violacea* (Bonaparte, 1832). Marine and Freshwater Research 53: 531-541.

Natanson, L. J. and G. Cailliet. 1986. Reproduction and development of the Pacific angel shark, *Squatina californica*, off Santa Barbara, California. Copeia 1986: 987-994.

Parsons, G. R. 1991. Notes on the behavior of the bonnethead shark, *Sphyrna tiburo* (Linnaeus) during birth. Journal of Aquariculture and Aquatic Sciences 6(1): 6-8.

Parsons, G. R. and H. J. Grier. 1992. Seasonal changes in shark testicular structure and spermatogenesis. Journal of Experimental Zoology 261: 173-184.

Parsons, G. R. 1993. Geographic variation in reproduction between two populations of the bonnethead shark, *Sphyrna tiburo*. Environmental Biology of Fishes 38: 25-35.

Pierantoni, R., M. D'Antonio, and S. Fasano. 1993. Morpho-functional aspects of the hypothalamus-pituitary-gonadal axis of elasmobranch fishes. Environmental Biology of Fishes 38: 187-196.

Pratt, H. L. 1979. Reproduction in the blue shark, *Prionace glauca*. Fishery Bulletin 77(2): 445-470.

Pratt, H. L. 1988. Elasmobranch gonad structure: A description and survey. Copeia 1988: 719-729.

Pratt, H. L., Jr. 1993. The storage of spermatozoa in the oviducal glands of western North Atlantic sharks. Environmental Biology of Fishes 38: 139-149.

Pratt, H. L. and S. Tanaka. 1994. Sperm storage in male elasmobranchs: A description and survey. Journal of Morphology 219: 297-308.

Ranzi, S. 1932. Le basifisio-morfologiche dello svillupo embrionale dei selaci. Parte I, Pubblicacio Stazion Zoologio Napoli 13: 209-290.

Rasmussen, L. E. L., D. L. Hess, and S. H. Gruber. 1992. Serum steroid hormones during reproduction in elasmobranchs. *In:* Reproductive Biology of South American Vertebrates, p. 19-42. W. C. Hamlett (ed.). Springer-Verlag, New York, USA.

Rasmussen, L. E. L. and F. L. Murru. 1992. Long-term studies of serum concentrations of reproductively related steroid hormones in individual captive carcharhinids. Australian Journal of Marine and Freshwater Research 43: 273-281.

Rasmussen, L. E. L. and S. H. Gruber. 1993. Serum concentrations of reproductively-related circulating steroid hormones in the free-ranging lemon shark, *Negaprion brevirostris*. Environmental Biology of Fishes 38: 167-174.

Rasmussen, L. E. L., D. L. Hess, and C. A. Luer. 1999. Alterations in serum steroid concentrations in the clearnose skate, *Raja eglanteria*: correlations with season and reproductive status. Journal of Experimental Zoology 284: 575-585.

Redding, J. M. and R. Patiño. 1993. Reproductive physiology. *In:* The Physiology of Fishes, p. 503-534. D. H. Evans (ed.). CRC Press, Boca Raton, Florida, USA.

Sherwood, N. M. and D. A. Lovejoy. 1993. Gonadotropin-releasing hormone in cartilaginous fishes: Structure, location, and transport. Environmental Biology of Fishes 38: 197-208.

Simpfendorfer, C. A. 1992. Reproductive strategy of the Australian sharpnose shark, *Rhizoprionodon taylori* (Elasmobranchii: Carcharhinidae), from Cleveland Bay, northern Queensland. Australian Journal of Marine and Freshwater Research 43: 67-75.

Sisneros, J. A. and T. C. Tricas. 2000. Androgen-induced changes in the response dynamics of ampullary electrosensory primary afferent neurons. Journal of Neuroscience 20(22): 8586-8595.

Smale, M. J. and A. J. J. Goosen. 1999. Reproduction and feeding of spotted gully shark, *Triakis megalopterus*, off the Eastern Cape, South Africa. U. S. Fishery Bulletin 97: 987-998.

Smith, J. W. 1980. The life history of the cownose ray, *Rhinoptera bonasus* (Mitchill, 1815), in lower Chesapeake Bay, with notes on the management of the species. M. A. Thesis, Virginia Institute of Marine Sciences, College of William and Mary, Williamsburg, Virginia, USA. 151 p.

Smith, J. W. and J. V. Merriner. 1986. Observations on the reproductive biology of the cownose ray, *Rhinoptera bonasus*, in Chesapeake Bay. U. S. Fishery Bulletin 84(4): 871-877.

Snelson, F. F., Jr., S. E. Williams-Hooper, and T. H. Schmid. 1988. Reproduction and ecology of the Atlantic stingray, *Dasyatis sabina*, in Florida coastal lagoons. Copeia 1988(3): 729-739.

Snelson, F. F., Jr., S. E. Williams-Hooper, and T. H. Schmid. 1989. Biology of the bluntnose stingray, *Dasyatis say*, in Florida coastal lagoons. Bulletin of Marine Science 45: 15-25.

Snelson, F. F. Jr., L. E. L. Rasmussen, M. R. Johnson, and D. L. Hess. 1997. Serum concentrations of steroid hormones during reproduction in the Atlantic stingray, *Dasyatis sabina*. General and Comparative Endocrinology 108: 67-79.

Sorbera, L. A. and. I. P. Callard. 1995. Myometrium of the spiny dogfish *Squalus acanthias*: peptide and steroid regulation. American Journal of Physiology 269(38): 389-397.

Springer, S. 1960. Natural history of the sandbar shark, *Eulamia milberti*. U. S. Fish and Wildlife Service Fishery Bulletin 61: 1-38.

Sumpter, J. P. and J. M. Dodd. 1979. The annual reproductive cycle of the female lesser spotted dogfish, *Scyliorhinus canicula* L., and its endocrine control. Journal of Fish Biology 15: 687-695.

Tanaka, S., Y. Shiobara, S. Hioki, H. Abe, G. Nishi, K. Yano, and K. Suzuki. 1990. The reproductive biology of the frilled shark, *Chlamydoselachus anguineus*, from Suruga Bay, Japan. Japanese Journal of Ichthyology 37(3): 273-291.

Taniuchi, T. 1971. Reproduction of the sandbar shark, *Carcharhinus milberti*, in the East China Sea. Japanese Journal of Ichthyology 18: 94-98.

Teshima, K., Y. Kamei, M. Toda, and S. Uchida. 1995. Reproductive mode of the tawny nurse shark taken from the Yaeyama Islands, Okinawa, Japan with comments on individuals lacking the second dorsal fin. Bulletin of Sekai National Fisheries Research Institute 73: 1-12.

Thorson, T. B., J. K. Langhammer, and M. I. Oetinger. 1983. Reproduction and development of the South American freshwater stingrays, *Potamotrygon circularis* and *P. motoro*. Environmental Biology of Fishes 9(1):3-24.

Tricas, T. C., K. P. Maruska, and L. E. L. Rasmussen. 2000. Annual cycles of steroid hormone production, gonad development, and reproductive behavior in the Atlantic stingray. General and Comparative Endocrinology 118: 209-225.

Tsang, P. C. W. and I. P. Callard. 1987. Morphological and endocrine correlates of the reproductive cycle of the aplacental viviparous dogfish, *Squalus acanthias*. General and Comparative Endocrinology 66: 182-189.

Uchida, S., M. Toda, N. Tanaka, and Y. Kamei. 1990. Reproduction of elasmobranchs in captivity. *In:* Elasmobranchs as Living Resources: Advances in the Biology, Ecology, Systematics, and the Status of the Fisheries, p. 211-237. H. L. Pratt, Jr., S. H. Gruber, and T. Taniuchi (eds.). U. S. Department of Commerce, NOAA Technical Report NMFS 90.

Uchida, S., M. Toda, and Y. Kamei. 1997. Reproduction of elasmobranchs in captivity (II). *In:* Proceedings of the Fourth International Aquarium Congress, June 23-27, 1996, Tokyo. p. 99-107. Tokyo, Japan by the Congress Central Office of IAC '96, Tokyo Sea Life Park. 402 pp.

Villavicencio-Garayzar, C. J. 1993a. Biologia reproductive de *Rhinobatos productus* (Pisces: Rhinobatidae), en Bahia Almejas, Baja California Sur, México. Revista de Biologia Tropical 41(3): 777-782.

Villavicencio-Garayzar, C. J. 1993b. Observaciones sobre la biologia reproductive de *Narcine brasiliensis* (Olfers) (Pisces: Narcinidae), en Bahia Almejas, B. C. S. México. Revista de Investigaciones Cientificas 4(1): 95-99.

Villavicencio-Garayzar, C. J., M. E. Mariano, and C. H. Downtonn. 2001. Reproductive biology of three ray species in the North Pacific of Mexico. 6th Indo-Pacific Fish Conference, May 20-25, 2001, Durban, South Africa. p. 62. Abstract. Oceanographic Research Institute, Durban, South Africa.

Voss, J., L. Berti, and C. Michel. 2001. *Chiloscyllium plagiosum* (Anon., 1830) born in captivity: hypothesis for gynogenesis. Bulletin of the Institute of Oceanography, Monaco 20(1): 351-353.

Wass, R. C. 1973. Size, growth and reproduction of the sandbar shark, *Carcharhinus milberti*, in Hawaii. Pacific Science 27(4): 305-318.

Wourms, J. P. 1977. Reproduction and development in chondrichthyan fishes. American Zoologist 17: 379-410.

Wourms, J. P. 1981. Viviparity: Maternal-fetal relationships in fishes. American Zoologist 21: 473-515.

Wourms, J. P., B. D. Grove, and J. Lombardi. 1988. The maternal-embryonic relationship in viviparous fishes. *In:* Fish Physiology, Vol. X1, Part B, p. 1-134. W. S. Hoar and D. J. Randall (eds.). Academic Press, New York, USA.

Wourms, J. P. 1993. Maximization of evolutionary trends for placental viviparity in the spadenose shark, *Scoliodon laticaudus*. Environmental Biology of Fishes 38: 269-294.
Wright, D. E. and L. S. Demski. 1993. Gonadotropin-releasing hormone (GnRH) pathways and reproductive control in elasmobranchs. Environmental Biology of Fishes 38: 209-218.
Yano, K. 1992. Comments on the reproductive mode of the false cat shark *Pseudotriakis microdon*. Copeia, 1992(2): 460-468.

PERSONAL COMMUNICATIONS

Ankley, M. 1999. Aquarium of the Pacific, Long Beach, CA 90802, USA.
Gordon, I. 2000. Off The Edge Research, Sydney, NSW, 2100, Australia.
Hamlett, W. C. 1999. Indiana University School of Medicine Notre Dame, Indiana 46556, USA.
Riggles, G. 2000. Indianapolis Zoo, Indianapolis, IN 46222, USA.
Spieler, R. 1999. Nova Southeastern University Oceanographic Center, FL 33004, USA.
Stamper, A. 1999. Disney's Epcot The Living Seas, Orlando, FL 32830-1000, USA.

The Elasmobranch Husbandry Manual: Captive Care of Sharks, Rays and their Relatives, pages 237-248.
© 2004 Ohio Biological Survey

Chapter 17
Captive Breeding and Sexual Conflict in Elasmobranchs

ALAN D. HENNINGSEN

National Aquarium in Baltimore,
Pier 3, 501 E. Pratt Street,
Baltimore, MD 21202, USA.
E-Mail: ahenningsen@aqua.org

MALCOLM J. SMALE

Port Elizabeth Museum,
P.O. Box 13147,
Humewood, 6013, South Africa.
E-Mail: pemmjs@zoo.upe.ac.za

IAN GORDON

Off The Edge Research,
P.O. Box W356.
Warringah Mall, 2100, Sydney, Australia.
E-mail: ote@ozemail.com.au

ROD GARNER

58 Carter Road,
Nambour, 4560, Queensland, Australia.
E-Mail: rodgarner@powerup.com.au

RAUL MARIN-OSORNO

Acuario de Veracruz,
A.C. Blvd. Manuel Av Camacho s/n,
Playón de Hornos, Veracruz, México, 91700.
E-Mail: marinosorno@hotmail.com

NINO KINNUNEN

Sydney Aquarium,
Aquarium Pier, Darling Harbour,
NSW, 2000, Australia.
E-Mail: nkinnunen@hotmail.com

Abstract: Successful reproduction has been recorded in many different species of cartilaginous fish held in captivity, representing the various reproductive modes recorded in chondrichthyans. Documentation of behaviors of captive chondrichthyans has provided a foundation to our knowledge of reproductive behavior, as these interactions are rarely witnessed in the wild and difficult to infer from freshly caught wild specimens. Reproductive behavior often results in conspecific and interspecific conflicts. Conspecific sexual conflict may be consexual as well as intersexual. Although specific reproductive behaviors have

been reported for many species, mating systems remain poorly understood. Captive breeding may reduce pressure on wild populations, particularly for those species where severe declines have been documented. Such efforts may be opportunistic, directed, or undertaken in collaboration with other institutions. Detailed behavioral records relevant to reproduction should be collected and maintained for all captive elasmobranchs and shared through peer-review publication.

Reproductive behaviors in chondrichthyans are often complex and, until recently, few qualitative studies of reproductive behaviors in elasmobranchs have been published (Pratt and Carrier, 2001). Several reviews of reproductive behavior have been presented in the last decade (Bres, 1993; Demski, 1990a; Demski, 1990b; Pratt and Carrier, 2001). The majority of reproductive behaviors reported in the literature have been observed in captive elasmobranchs, as it is difficult to closely monitor wild conspecifics. One hundred species of chondrichthyans are known to have exhibited reproductive behaviors or reproduced in captivity: in aquariums, semi-natural confinements, and laboratories. These species include one holocephalan and 99 elasmobranchs; oviparous and viviparous species comprise 40% and 60%, respectively (Table 17.1).

As noted by Parker (1979), Davies (1992, in Birkhead and Parker 1997), and Reynolds (1996), all mating systems may be the result of intrasexual and intersexual conflict. Mating systems in elasmobranchs have resulted in adaptations in both sexes, such as sexual dimorphism in skin thickness (Pratt, 1979; Kajiura et al., 2000) and sexually dimorphic dentition (McCourt and Kerstitch, 1980; Kajiura and Tricas, 1996). The intent of this chapter is to provide a brief summary of the following: chondrichthyans bred in captivity (including a closer examination of five sample species), the range of observed sexual conflicts, methods of controlling reproduction, and suggestions for the future.

SEXUAL CONFLICT

Intra- and intersexual behaviors evolved in environments very different from those in aquariums. Captive animals are confined to the limited space provided by the aquarium system, and the full spectra of behaviors are almost always modified or attenuated. Consequently, captive sharks, skates, or rays may be subject to persistent chasing and biting by members of the same or opposite sex, from which they may have limited ability to escape. In addition, wounds inflicted during pre-copulatory or copulatory behaviors in captive elasmobranchs may act as entry sites for pathogens such as bacteria and fungi (refer to Chapter 26 of this manual), particularly if they are aggravated by teleost cohabitants.

Several behaviors relating to reproduction have been documented in semi-natural and captive settings. Intersexual interactions may range from one or more males following a female, to nosing the female, to grasping and copulation (Johnson and Nelson, 1978; Uchida et al., 1990; Gordon, 1993). Nosing, as observed in sand tiger sharks, *Carcharias taurus* (Gordon, 1993) and blacktip reef sharks, *Carcharhinus melanopterus* (Gordon, 1993; Riggles, pers. com.) consists of the male positioning its snout just under the cloaca of the female. In other animal taxa, some behaviors, and specifically reproductive behaviors, are often induced via biochemical compounds. Pheromones have been identified in several invertebrate and vertebrate groups, including teleosts (Sorensen et al., 1995; Sorensen et al., 2000). Although no pheromones have been identified in elasmobranchs to date, behavioral observations during reproduction (i.e., Springer, 1967; Johnson and Nelson, 1978; Castro et al., 1988; Gordon, 1993) suggest that pheromones may be released by the female and may induce part of the male behavioral repertoire. Ongoing but unpublished investigations on reproductively active clearnose skates, *Raja eglanteria*, strongly suggest that male skates respond to secretions released by reproductively active females (Rasmussen, pers. com.). In some skates and rays, many social and reproductive behaviors are mediated via electroreception using the ampullary system (New, 1994; Tricas et al., 1995; Sisneros et al., 1998; Sisneros and Tricas, 2002). It is likely therefore that reproductive behavior is mediated via visual, biochemical, and electroreceptive cues in elasmobranchs; the importance of each cue may differ across species or groups.

Additional interactions include, but are not limited to, pectoral fin biting in sharks and rays and male gouging of the dorsal surface of the female in myliobatiform rays. The occurrence and type of male-induced bites on female pectoral fins in dasyatids can be used to determine reproductive behavior as well as seasonality (Kajiura et al., 2000). Although Kajiura et al. (2000) observed

Table 17.1. Chondrichthyan reproduction in captivity showing species that have completed the reproductive cycle in a captive environment, as well as those that have exhibited mating behavior in captivity. The list includes species from aquariums, laboratories, and semi-natural environments. It does not refer to species that were known to be gravid when retained in captivity. Reproductive modes, as per Hamlett and Koob (1999), include the following: O = Oviparous; VA1 = Viviparous - aplacental - yolksac; VA2 = Viviparous - aplacental - with uterine villi or trophonemata; VA3 = Viviparous - aplacental - with oophagy and (with or without) intrauterine cannibalism; and VP = Viviparous - placental. Unless otherwise specified, source references were the International Zoo Yearbook, 1971-1997, Vols. 11-35, Zoological Society of London.

Species name	Common name	Mode	Reference
Aetobatus narinari	spotted eagle ray	VA2	Uchida, 1982; Uchida et al., 1990; Uchida et al., 1997
Apristurus brunneus	brown cat shark	O	
Atelomycterus macleayi	Australian marbled cat shark	O	
Atelomycterus marmoratus	coral cat shark	O	
Brachaelurus waddi	blind shark	O	
Carcharhinus acronotus	blacknose shark	VP	Kaiser, pers. com.
Carcharhinus leucas	bull shark	VP	Uchida et al., 1997
Carcharhinus melanopterus	blacktip reef shark	VP	Riggles, pers. com.
Carcharhinus perezi	Caribbean reef shark	VP	Kaiser, pers. com.
Carcharhinus plumbeus	sandbar shark	VP	Engelbrecht, pers. com.
Carcharias taurus	sand tiger shark	VA3	Gordon, 1993; Garner, 1997
Cephaloscyllium umbratile	Japanese swell shark	O	Hagiwara, 1990
Cephaloscyllium ventriosum	swell shark	O	
Chiloscyllium arabicum	Arabian carpet shark	O	
Chiloscyllium griseum	gray bamboo shark	O	Dral, 1980
Chiloscyllium indicum	slender bambooshark	O	
Chiloscyllium plagiosum	whitespotted bamboo shark	O	
Chiloscyllium punctatum	brownbanded bamboo shark	O	Schmid and Murru, 1991; Garner, 1998
Dasyatis akajei	red stingray	VA2	
Dasyatis americana	southern stingray	VA2	Henningsen, 2000
Dasyatis brevicaudata	short-tail stingray	VA2	
Dasyatis chrysonata	blue stingray	VA2	
Dasyatis fluviorum	estuary stingray	VA2	
Dasyatis izuensis	Izu stingray	VA2	
Dasyatis matsubarai	pitted stingray	VA2	
Dasyatis pastinaca	common stingray	VA2	
Dasyatis sabina	Atlantic stingray	VA2	
Etmopterus lucifer	blackbelly lantern shark	VA1	Uchida et al., 1990
Ginglymostoma cirratum	nurse shark	VA1	Klimley, 1980; Kuenen, 2000; Marin-Osorno, personal observation
Gymnura altavela	spiny butterfly ray	VA2	
Gymnura japonica	Japanese butterfly ray	VA2	
Gymnura micrura	smooth butterfly ray	VA2	
Haploblepharus edwardsii	puffadder shy shark	O	

Table 17.1 (continued). Chondrichthyan reproduction in captivity showing species that have completed the reproductive cycle in a captive environment, as well as those that have exhibited mating behavior in captivity. The list includes species from aquariums, laboratories, and semi-natural environments. It does not refer to species that were known to be gravid when retained in captivity. Reproductive modes, as per Hamlett and Koob (1999), include the following: O = Oviparous; VA1 = Viviparous - aplacental - yolksac; VA2 = Viviparous - aplacental - with uterine villi or trophonemata; VA3 = Viviparous - aplacental - with oophagy and (with or without) intrauterine cannibalism; and VP = Viviparous - placental. Unless otherwise specified, source references were the International Zoo Yearbook, 1971-1997, Vols. 11-35, Zoological Society of London.

Species name	Common name	Mode	Reference
Haploblepharus pictus	dark shy shark	O	
Hemiscyllium hallstromi	Papauan epaulette shark	O	
Hemiscyllium ocellatum	epaulette shark	O	West and Carter, 1990; Schmid and Murru, 1991
Heterodontus francisci	horn shark	O	Dempster and Herald, 1961
Heterodontus galeatus	crested bullhead shark	O	Last and Stevens, 1994
Heterodontus japonicus	Japanese bullhead shark	O	Uchida et al., 1989; Hagiwara, 1990
Heterodontus mexicanus	Mexican horn shark	O	
Heterodontus portusjacksoni	Port Jackson shark	O	
Heteroscyllium colcloughi	blue-gray carpet shark	O	Horton, pers. com.
Hydrolagus colliei	spotted ratfish	O	Sathyanesan, 1966; Van Dykhuizen et al., 1997
Leucoraja erinacea	little skate	O	
Leucoraja ocellata	winter skate	O	
Mustelus californicus	gray smooth-hound	VP	
Mustelus canis	dusky smooth-hound	VP	
Mustelus manazo	star-spotted smooth-hound	VA1	
Mustelus norrisi	Florida smooth-hound	VP	
Myliobatis californicus	bat eagle ray	VA2	
Negaprion brevirostris	lemon shark	VP	
Okamejei kenojei	spiny rasp skate	O	
Orectolobus japonicus	Japanese wobbegong	VA1	Whitley, 1940 in Demski, 1990b; Hagiwara, 1990; Uchida et al., 1997)
Orectolobus maculatus	spotted wobbegong	VA1	Whitley, 1940 in Demski, 1990b
Orectolobus ornatus	ornate wobbegong	VA1	
Parmaturus xaniurus	filetail cat shark	O	
Poroderma africanum	striped cat shark	O	
Poroderma pantherinum	leopard cat shark	O	
Potamotrygon histrix	porcupine river stingray	VA2	
Potamotrygon magdalenae	Magdalena river stingray	VA2	
Potamotrygon motoro	ocellate river stingray	VA2	Thorson et al., 1983
Potamotrygon ocellata	red-blotched river stingray	VA2	
Potamotrygon orbignyi	smooth back river stingray	VA2	
Potamotrygon schroederi	rosette river stingray	VA2	
Pristis pectinata	smalltooth sawfish	VA1	Liu, pers. com.
Pteroplatytrygon violacea	pelagic stingray	VA2	Mollet et al., 2002; Morales, pers. com.

Table 17.1 (continued). Chondrichthyan reproduction in captivity showing species that have completed the reproductive cycle in a captive environment, as well as those that have exhibited mating behavior in captivity. The list includes species from aquariums, laboratories, and semi-natural environments. It does not refer to species that were known to be gravid when retained in captivity. Reproductive modes, as per Hamlett and Koob (1999), include the following: O = Oviparous; VA1 = Viviparous - aplacental - yolksac; VA2 = Viviparous - aplacental - with uterine villi or trophonemata; VA3 = Viviparous - aplacental - with oophagy and (with or without) intrauterine cannibalism; and VP = Viviparous - placental. Unless otherwise specified, source references were the International Zoo Yearbook, 1971-1997, Vols. 11-35, Zoological Society of London.

Species name	Common name	Mode	Reference
Raja binoculata	big skate	O	
Raja clavata	thornback ray	O	
Raja eglanteria	clearnose skate	O	Luer and Gilbert, 1985
Raja microocellata	small-eyed skate	O	
Raja montagui	spotted skate	O	
Raja rhina	longnose skate	O	
Raja texana	roundel skate	O	
Raja undulata	undulate ray	O	
Rhina ancyclostoma	bowmouth guitarfish	VA1	Uchida et al., 1990
Rhinobatos hynnicephalus	ringstraked guitarfish	VA1	
Rhinobatos lentiginosus	Atlantic guitarfish	VA1	
Rhinobatos productus	shovelnose guitarfish	VA1	
Rhinoptera bonasus	cownose ray	VA2	Davis, pers. com.; Henningsen, personal observation
Rhinoptera javanica	Javanese cownose ray	VA2	Uchida, 1982; Uchida et al., 1990; Uchida et al., 1997
Rhynchobatus djiddensis	giant guitarfish	VA1	Bok, pers. com.
Scyliorhinus canicula	smallspotted cat shark	O	Bolau, 1881; Schensky, 1941, in Pratt and Carrier, 2001)
Scyliorhinus retifer	chain dogfish	O	Castro et al., 1988
Scyliorhinus stellaris	nursehound	O	
Scyliorhinus tokubee	Izu cat shark	O	
Scyliorhinus torazame	cloudy cat shark	O	Uchida, 1982, Hagiwara, 1990
Sphyrna tiburo	bonnethead shark	VP	
Squalus acanthias	spiny dogfish	VA1	
Squatina japonica	Japanese angel shark	VA1	
Stegostoma fasciatum	zebra shark	O	Uchida et al., 1990; Uchida et al., 1997
Taeniura lymma	bluespotted ribbontail stingray	VA2	Riggles, pers. com.
Taeniura meyeni	blotched fantail ray	VA2	Garner and Mackness, 1998b
Torpedo marmorata	marbled electric ray	VA2	
Triaenodon obesus	whitetip reef shark	VP	Uchida, 1982; Garner and Mackness, 1998a
Triakis scyllium	banded houndshark	VA1	
Triakis semifasciata	leopard shark	VA1	Ankley, pers. com.
Trygonnorhina sp. A (undescribed)	eastern fiddler ray	VA1	
Urobatis halleri	round stingray	VA2	
Urobatis jamaicensis	yellow stingray	VA2	
Urolophus aurantiacus	sepia stingray	VA2	Hagiwara, 1990; Uchida et al., 1990

these behaviors in wild Atlantic stingrays, *Dasyatis sabina*, similar observations can readily be made in captive elasmobranchs. As many as five or more males may chase a captive female cownose ray, *Rhinoptera bonasus*, during mating behaviors, something also observed in the field (Pratt, pers. com.; Henningsen, personal observation). This behavior has also been observed in the Javanese cownose ray, *Rhinoptera javanica* (Uchida et al., 1990). Sexual conflict in captive rhinopterids may be so profound as to cause severe lacerations on the trailing edges of the pectoral fins of females and even mortality (Uchida et al., 1990; Henningsen, personal observation).

EXAMPLE SPECIES

Captive breeding and sexual conflict and has been observed in many species of elasmobranchs. We present brief summaries for five species: sand tiger sharks, sandbar sharks (*Carcharhinus plumbeus*), whitespotted bamboo sharks (*Chiloscyllium plagiosum*), nurse sharks (*Ginglymostoma cirratum*), and southern stingrays (*Dasyatis americana*) to point out the importance of recording and clearly defining reproductive behaviors and reproductive events in captive elasmobranchs. These examples serve as models only and a complete coverage of all species is beyond the scope of this chapter.

Sand tiger shark

The sand tiger is widely distributed in warm temperate waters (Compagno, 1984; Castro et al. 1999), and undergoes coastal seasonal migrations that are coupled with the reproductive cycle (Gilmore et al., 1983; Cliff, 1989; Gilmore, 1993; Pollard et al. 1996) and governed by water temperature (Compagno, 1984; Parker and Bucher, 2000).

In Australia, males are predominant in southern Queensland during July to October, while a high proportion (77.4%) of the catch from beach meshing off central New South Wales (NSW) at the same time of year is composed of females (Reid and Krogh, 1992). The sex ratio of the sand tiger population shifts from a majority of females in spring (September-November) to a majority of males in autumn/winter (March-August) at the northern sites, indicating that the movements of the sexes may differ (Parker & Bucher, 2000). Migrations of sand tigers in South African waters appear to follow a similar seasonal pattern to those described by Reid and Krogh (1992), Pepperell (1992), and Pollard et al. (1996) for conspecifics in Australian waters.

Although the reproductive cycle of the sand tiger has been reported to be annual (Gilmore et al., 1983, Gilmore, 1993), a biennial cycle (punctuated cycle: refer to Chapter 16 of this manual) appears to be the case at least in females (Cliff, 1989; Branstetter and Musick, 1994; Castro et al., 1999; Goldman, 2002). Reproductive behaviors for sand tigers in aquariums have occurred in South Africa, Australia, and the USA. To date, successful captive reproduction from copulation to parturition has occurred only in Australia and South Africa. Pre-copulatory as well as copulatory behavior in sand tigers was described by Gordon (1993) from captive specimens at Manly Oceanworld, Sydney, NSW, Australia. The most recent sequence of reproduction in the existing captive population of three mature males and four mature females occurred from September to November 2000 and lasted approximately 53 days (Kinnunen, personal observation). Gordon (1993) reported pre-copulatory and copulatory behavior occurring 14 months apart, of just over a month in duration, and suggested that captive sharks may mate annually. Information from Seaworld Durban and the National Aquarium in Baltimore corroborate this suggestion as annual pre-copulatory behavior has been observed (Bok, pers. com.; Henningsen, personal observation). Annual copulation was witnessed by one of the authors (Garner) at Underwater World, Mooloolaba, Queensland, Australia. It is possible that the reproductive cycles are annual and biennial for males and females, respectively, but further work is required to confirm this suggestion.

One of the authors (Garner) and Fischer (pers. com.) have documented reproduction in sand tiger sharks at Underwater World, Mooloolaba, from 1993 to 2001. Three successful parturitions by one female, “Big Mamma,” in 1992 (wild-copulation), 1997 (captive copulation), and 1999 (captive copulation) were observed. Further, two pre-term stillborn pups (~70-80 cm TL), born in 2000, were attributed to a female shark born of “Big Mamma” in 1992. The age of the latter female corroborates the estimate of the age at maturity given by Branstetter and Musick (1994).

It is worthwhile noting that although most of the sexual conflicts in sand tiger sharks, at several institutions, conform to Gordon’s (1993) basic descriptions, duration and seasonality vary (Bok,

pers. com.; Choromanski, pers. com.; Zoller, pers. com.). Temperature, in addition to social structure of the captive population, has been suggested by one of the authors (Garner) to be a critical factor for successful captive reproduction in sand tigers. It was noted, however, on one occasion, when pre-copulatory behaviors extended for several months, that salinity appeared to play a role in cessation of the behaviors (Zoller, pers. com.). Despite these suggestions, critical cues have not been positively identified, as captive sand tigers maintained at different institutions under similar temperature, photoperiod, and social structures may or may not be reproductively active. It has been suggested that the disruption of a stable, reproducing captive colony can severely delay, if not extinguish, reproductive success in sand tigers, which may of course be illustrative of several other species of elasmobranchs. It should be noted that annual intrasexual conflicts have been observed in male sharks in the absence of females. The conflicts between males may be severe, and previously undescribed behaviors have been observed between male sharks (Henningsen, personal observation). These observations highlight the need for ongoing detailed behavioral studies in this species. [Author's note (September, 2004): Revised estimates of age and growth in sand tiger sharks in the Northwest Atlantic indicate that it grows more slowly than previously thought and that annual bands, verified by validation, are laid down in the vertebral centra (Goldman, 2002). Consequently, ages at maturity are considered to be 6-7 years and 9-10 years for males and females, respectively, in this population, rather than four years for males and five years for females (Branstetter and Musick, 1994; Goldman, 2002). This result may be indicative of the species or yet another example of differences between populations. A further observation in this species in the southwestern Atlantic by Lucifora et al. (2002) was that males appeared lighter in color coincident with the mating period, an observation made in captive males (Gordon, 1993; Henningsen, in prep.).]

Nurse shark

A mating group of nurse sharks has been the subject of an ongoing investigation in the Dry Tortugas National Park, Dry Tortugas (Carrier et al., 1994; Pratt and Carrier, 2001). This investigation has provided detailed observations on social structure and mating behavior, and provides documented cases of polygyny and polyandry (Pratt and Carrier, 2001). Nurse sharks have been commonly maintained in aquariums for extended periods of time (Clark, 1963; Castro, 2000), yet their reproductive biology has only recently been detailed by Castro (2000). Mating behavior and copulation in captivity has been previously described for this species (Klimley, 1980).

During 1997 one of the authors (Marin-Osorno) observed reproductive behaviors, including copulation, in a captive population of nurse sharks (consisting of five males and four females) at the Aquario de Veracruz. Only two of the nine nurse sharks in the 1,250 m^3 multi-species exhibit were mature, a 267 cm TL male and a 250 cm TL female. Behavioral observations included the presence of a "blocking male", as described by Carrier et al. (1994). Other behaviors were more in accord with field observations described by Carrier et al. (1994), rather than Klimley's (1980) observations of captive nurse sharks.

In captive nurse sharks there have been instances of conflict, involving adult males, directed towards immature conspecifics, and also involving immature animals, directed towards mature consexual conspecifics. Interspecific conflicts by mature and immature nurse sharks have been directed toward tiger (*Galeocerdo cuvier*), sandbar, and sand tiger sharks (Marin-Osorno, personal observation; Henningsen; personal observation; Martel-Bourbon, pers. com.). Such conspecific and interspecific interactions have been observed in several facilities. The reason for these presumably non-reproductively mediated behaviors is not known.

Sandbar shark

The sandbar shark is a widely distributed species that is commonly maintained in public aquariums. Reproduction in this species has been described for captive specimens (Uchida et al., 1990). Although the authors did not observe mating, mating scars were noted and subsequent parturition described. Other instances of reproduction in sandbar sharks have occurred at several institutions (Areitio, pers. com.; Engelbrecht, pers. com.). For the purpose of illustration, a summary of three successive pregnancies, in the same adult female sandbar shark, at the Madrid Zoo Aquarium, is given below. The sharks, four males and one female, were obtained in May of 1985, each ~170 cm TL. The sharks were maintained in a multi-species display using a combination of natural and artificial lighting, with temperature ranging annually from 21-26°C. The first mating was observed in May

of 1997, with subsequent parturition in May of 1998. The second and third mating occurred in May of 1999 and May 2001, with parturition occurring in May 2000 and May 2002, respectively (Areitio, pers. com.). These observations agree with the biennial cycle of wild female conspecifics. Observations indicate a shorter, more direct, pre-copulatory period (as short as 1-3 days, preceding copulation) than that observed in sand tiger sharks.

Whitespotted bamboo shark

The whitespotted bamboo shark is a commonly maintained hemiscyllid that is often available in the hobbyist trade. Its biology is poorly known despite its abundance within public aquariums. Like several other similar hemiscyllids it reproduces readily in captivity, given the proper conditions. Males mature at 50-65 cm TL and females mature at ~80 cm TL (Michael, 1993; Compagno, 2001; Michael, 2001). Captive whitespotted bamboo sharks are often maintained at a constant temperature and photoperiod. The lack of a temperature change may allow continuous breeding rather than a restricted annual cycle. Similar observations have been reported for the epaulette shark, *Hemiscyllium ocellatum* (Heupel et al., 1999).

Although few observations on reproduction in whitespotted bamboo sharks have been published (e.g., Michael, 2001), its mating behavior is similar to that described in other hemiscyllids, notably the gray bamboo shark, *Chiloscyllium griseum* (Dral, 1980 in Pratt and Carrier, 2001), and the epaulette shark (West and Carter, 1990). In addition to the male initiating mating behavior, West and Carter (1990) observed instances of the female initiating mating in the epaulette shark, although this has not yet been observed in whitespotted bamboo sharks. In wild epaulette sharks, mating was focused from July to November on Heron Island Reef, Heron Island, Australia (Heupel et al., 1999). The end of the mating season was coincident with an increase in water temperature, but it was not determined whether water temperature was a critical cue (Heupel et al., 1999). Similar to other hemiscyllids, female epaulettes may store sperm, allowing sperm to fertilize ova for a period of at least several months. In addition, females will occasionally produce “wind eggs,” or empty egg cases without yolk or embryo, as reported in horn and nurse sharks (Castro, 2000; Michael, 2001).

Female whitespotted bamboo sharks produce pairs of eggs every 7-10 days, over the course of the egg-laying season. It is advisable to separate egg cases from adults, particularly adult males, as they may prey upon the egg cases (Michael, 2001). Incubation time and embryonic development vary with temperature, but eggs hatch after about 125-128 days at 25°C (Tullis et al., 1997; Michael, 2001). Although not verified, a possible case of gynogenesis was reported in the whitespotted bamboo shark (Voss et al., 2001).

Southern stingray

The southern stingray is common in coastal subtropical and tropical waters of the western Atlantic, including the Gulf of Mexico and the Caribbean (Bigelow and Schroeder, 1953). Maturity has been reported to occur at 51 cm DW (disc width) and 75-80 cm DW, for males and females, respectively (Bigelow and Schroeder, 1953; Schmid et al., 1988). It is a hardy species that has been successfully maintained long-term in captivity. Many details on the life history of this species are lacking in the literature. It is noteworthy that average size at parturition, and litter size, reported for one captive population, differs from that reported for wild conspecifics (Henningsen, 2000). A positive relation between maternal DW and litter size, and an inverse relation between litter size and mean DW of neonates, has been observed. Age at sexual maturity has been recorded as 3-4 years and 5-6 years, for males and females, respectively. Size at maturity was found to be similar to that reported for wild conspecifics (Henningsen, 2002). Multiple males have been observed to mate with a single female, as is the case for the Javanese cownose ray (Uchida et al., 1990). Mating occurred immediately, to within hours, after parturition and was always venter to venter. Intersexual interactions have been observed between mating and subsequent copulation, and male-inflicted bites on females are similar to those described by Kajiura et al. (2000) in Atlantic stingrays. [Author’s note (September, 2004): The mating behavior of southern stingrays in the wild was reported by Chapman et al. (2003) to be similar to that described in the manta ray, *Manta birostris* (Yano et al. 1999) and consisted of a sequence five distinct steps. As described here, multiple males mating with a single female, and copulation occurring within minutes to hours following parturition, was observed. Observations on captive animals may therefore reflect behavior in the wild.]

PROMOTION AND INHIBITION OF REPRODUCTION

Reproduction in captive elasmobranchs can be promoted or inhibited by several means. Demski (1990b) and Henningsen (1999) describe physiological as well as environmental methods of promoting reproduction. Important biological cues such as temperature and photoperiod can be manipulated, as can social structure (e.g., mature males:mature females), which may be essential to successful captive reproduction. An application of the use of environmental factors to control reproduction is given in Luer and Gilbert (1985) and Luer (1989) for the clearnose skate, with temperature being the critical factor. The temperature during captive breeding in the clearnose skate mimics conditions during the reproductive cycle in wild conspecifics (Luer, 1989).

Both reproduction and sexual conflicts among captive elasmobranchs can be controlled through a judicious approach to husbandry. The easiest method of eliminating reproduction is by maintaining a single sex within a collection. Reproduction occurs throughout the year in both southern stingrays and cownose rays at the National Aquarium in Baltimore, Baltimore, Maryland, USA, where both sexes are maintained continuously within the same aquarium system (Henningsen, 2000). At SeaWorld Orlando, Florida, USA, male elasmobranchs are kept separated from females until reproductive activity is desired (Davis, pers. com.).

Other important physiological processes that can have negative or positive impacts on reproduction include stress, thyroid status, and metabolism (Henningsen, 1999). Although not yet investigated in elasmobranchs, future studies may show that gonadotropin releasing hormone (GnRH) agonists and antagonists are useful for controlling reproduction as they are in some other vertebrates (e.g., Atkinson et al., 1998, Felberbaum et al., 2000).

MANAGEMENT OF A CAPTIVE BREEDING PROGRAM

The implementation of a captive breeding program requires proper management. Once the target species is selected, all necessary details need to be worked out, including initial and on-going requirements for the species and its offspring. Suggested requirements vary from those that must be met before the program can begin, to those that are more of a program management type. Even in its simplest form, several steps are involved in a well-designed captive breeding program and these have been summarized in Table 17.2.

RECOMMENDATIONS

There are many species of chondrichthyans maintained in aquariums that are not included in the 100 species listed in Table 17.1. Of the species not bred in captivity, several populations of wild

Table 17.2 . Steps involved in a well-designed captive breeding program for elasmobranchs, showing those steps that should be considered before and during the program, and those steps that should be considered on a continuous basis.

	Tasks	Before	During	Continued
1.	Select species.	●		
2.	Gather information from other institutions and the literature.	●	●	●
3.	Determine environmental requirements.	●	●	
4.	Determine spatial requirements.	●		
5.	Determine social structure.	●	●	
6.	Determine methods (i.e., natural, hormonally induced, etc.).	●	●	●
7.	Develop alternative methods.	●	●	●
8.	Plan for surplus, broodstock, and progeny.	●		●
9.	Ensure adequate holding space for all life stages.	●	●	●
10.	Develop plan to inhibit reproduction if desired.	●		●
11.	Maintain complete and accurate records.	●	●	
12.	Disseminate information: publish in peer-reviewed outlets.	●	●	

conspecifics have declined severely, locally as well as globally. Vulnerable and depleted species, especially those that are frequently in demand for display in aquariums (e.g., pristids, sand tiger sharks, sandbar sharks, etc.), should be the target of research and captive breeding programs.

The smalltooth sawfish (*Pristis pectinata*), for example, is listed as critically endangered in the western North Atlantic and has been extirpated from much of its range (Simpfendorfer, 2000). Due to a paucity of biological information on the smalltooth sawfish, Simpfendorfer's (2000) demographic analysis used information from the large-tooth sawfish (*Pristis perotteti*) to estimate population recovery rates for both species. It is only recently, during the revision of this chapter, that promising news of reproductive behavior has been recorded for captive smalltooth sawfish. Pre-copulatory behavior was observed in a captive population of smalltooth sawfish (one male and four females) at the Atlantis Paradise Resort and Casino, New Providence Island, Bahamas. The male sawfish showed great interest in some of the females, notably in the late summer to fall, although attempted or successful copulation was not observed (Kelley, pers. com.). During March of 2003, one of the female sawfish gave birth to, or aborted, young. Unfortunately, the remains of only two pups/fetuses were found, the others probably preyed on by sharks (Liu-Ferguson, pers. com.). This event was quite significant because it was the first known case of captive reproduction in smalltooth sawfish or any other pristid.

A cooperative effort between institutions may aid greatly in breeding species such as the smalltooth sawfish, sand tiger sharks, sandbar sharks, etc. The principal objective of such programs would be to reduce the number of animals taken from the wild, not necessarily to restock wild populations. Although the latter is certainly possible, it is beyond the scope of this chapter to consider all of the benefits and risks associated with introducing captive-born animals into wild populations.

With few exceptions, mating systems of elasmobranchs are not well known and specimens in aquariums represent a valuable source of information for many species. However, the effects of captivity must be taken into consideration when interpreting results and drawing conclusions about wild conspecifics. We urge the collection and publication of detailed observations relating to reproduction and reproductive behaviors, particularly for those species or behaviors not described in the literature.

ACKNOWLEDGEMENTS

The authors thank their respective institutions. In particular, the senior author expresses his thanks for support to the Biological Programs Department of the National Aquarium in Baltimore. Appreciation is extended to Wes Pratt for graciously providing extremely helpful comments on an earlier draft. Special thanks go to Mark Smith and Doug Warmolts for making this volume possible, as well as to all of the editors, Mark Smith, Doug Warmolts, Robert Hueter, and Dennis Thoney

REFERENCES

Atkinson, S., T. J. Ragen, W. G. Gilmartin, B. L. Becker, and T. C. Johanos. 1998. Use of a GnRH agonist to suppress testosterone in wild male Hawaiian monk seals (*Monachus schauinslandi*). General and Comparative Endocrinology 112: 178-182.

Bigelow, H. B. and W. C. Schroeder. 1953. Fishes of the western North Atlantic. No. 1, Part 2. Sawfishes, Guitarfishes, Skates, Rays, and Chimaeroids. Sears Foundation for Marine Research, Yale University, New Haven, Connecticut, USA. 588 p.

Birkhead, T. R. and G. A. Parker. 1997. Sperm competition and mating systems. *In:* Behavioural Ecology: An Evolutionary Approach, 4th edition, p. 121-145. J. R. Krebs and N. B. Davies (eds.). Blackwell Science, Ltd., London, England.

Branstetter, S. and J. A. Musick. 1994. Age and growth estimates for the sand tiger in the northwestern Atlantic Ocean. Transactions of the American Fisheries Society 123: 242-254.

Bres, M. 1993. The behavior of sharks. Reviews in Fish Biology and Fisheries 3: 133-159.

Carrier, J. C., H. L. Pratt, Jr., and L. K. Martin. 1994. Group reproductive behavior in free-living nurse sharks, *Ginglymostoma cirratum*. Copeia 1994: 646-656.

Castro, J. I., P. M. Bubucis, and N. A. Overstrom. 1988. The reproductive biology of the chain dogfish, *Scyliorhinus retifer*. Copeia 3: 740-746.

Castro, J. I., C. M. Woodley, and R. L. Brudek. 1999. A preliminary evaluation of the status of shark species. FAO Fisheries Technical Paper 380. Food and Agriculture Organization of the United Nations, Rome, Italy. 72 p.

Castro, J. I. 2000. The biology of the nurse shark, *Ginglymostoma cirratum*, off the Florida east coast and the Bahama Islands. Environmental Biology of Fishes 58: 1-22.

Chapman, D. D., M. J. Corcoran, G. M. Harvey, S. Malan, and M. Shivji. 2003. Mating behavior of southern stingrays, *Dasyatis americana* (Dasyatidae). Environmental Biology of Fishes 68: 241-245.

Clark, E. 1963. The maintenance of sharks in captivity, with a report on their instrumental conditioning. *In:* Sharks and Survival, p. 115-149. P. W. Gilbert (ed.). D. C. Heath and Co., Boston, Massachusetts, USA.

Cliff, G. 1989. Breeding migration of the sand tiger shark, *Carcharias taurus*, in southern African waters. 68th Annual Meeting of the American Society of Ichthyologists and Herpetologists, 5th Annual Meeting of the American Elasmobranch Society, San Francisco State University, San Francisco, CA, USA, 57-23 June, 1989, Abstract, p. 76.

Compagno, L. J. V. 1984. Sharks of the World. FAO Species catalogue, FAO Fisheries Synopsis 125, Vol. 4, Part 1. Food and Agriculture Organization of the United Nations, Rome, Italy. 655 p.

Compagno, L. J. V. 2001. Sharks of the World. FAO Species Catalogue for Fishery Purposes, No. 1, Vol. 2. Food and Agriculture Organization of the United Nations, Rome, Italy. 269 p.

Davies, N. B. 1992. Dunnock behavior and social evolution. Oxford University Press, Oxford, England.

Dempster, R. P. and E. S. Herald. 1961. Notes on the hornshark, *Heterodontus francisci*, with observations of mating activities. Occasional Papers of the California Academy of Sciences 33: 17.

Demski, L. S. 1990a. Neuroendocrine mechanism controlling sexual development and behavior of sharks and rays. Journal of Aquariculture and Aquatic Sciences 5: 53-67.

Demski, L. S. 1990b. Elasmobranch reproductive biology: implications for captive breeding. Journal of Aquariculture and Aquatic Sciences 5: 84-95.

Dral, A. J. 1980. Reproduction en aquarium du requin du fond tropical, *Chiloscyllium griseum* Müll. et Henle (Orectolobidés). Reviews in French Aquariology 7: 99-104.

Felberbaum, R. E., M. Ludwig, and K. Diedrich. 2000. Clinical applications of GnRH-antagonists. Molecular and Cellular Endocrinology 166: 9-14.

Garner, R. 1997. Shark breeding success. Australasian Regional Association of Zoological Parks and Aquaria Newsletter 35: 11.

Garner, R. 1998. Preliminary report regarding captive breeding observations of the brown-banded bamboo shark, *Chiloscyllium punctatum* Muller and Henle, 1838. Thylacinus 22(1): 31-32.

Garner, R. and B. Mackness. 1998a. Captive breeding of the whitetip reef shark, *Triaenodon obesus*. Thylacinus 22(2): 16-17.

Garner, R. and B. Mackness 1998b. First captive breeding of the blotched fantail ray *Taeniura meyeni* (Müller and Henle, 1841) in Australia. Thylacinus 22(2): 22-24.

Gilmore, R. G., J. W. Dodrill, and P. A. Linley. 1983. Reproduction and embryonic development of the sand tiger shark, *Odontaspis taurus* (Rafinesque). Fishery Bulletin 81(2): 201-225.

Gilmore, R. G. 1993. Reproductive biology of lamnoid sharks. Environmental Biology of Fishes 38: 95-114.

Goldman, K. J. 2002. Aspects of age, growth, demographics and thermal biology of two lamniform shark species. Ph.D. dissertation. Virginia Institute of Marine Science, College of William and Mary, Gloucester Point, VA. 220 p.

Gordon, I. 1993. Pre-copulatory behavior of captive sand tiger sharks, Carcharias taurus. Environmental Biology of Fishes 38: 159-164.

Hagiwara, S. 1990. Reproduction of chondrichthyans in captivity at Shimoda Floating Aquarium. Journal of Aquariculture and Aquatic Sciences 5(4): 119 (abstract).

Hamlett, W. C. and T. J. Koob. 1999. Female reproductive system. *In:* Sharks, Skates, and Rays, p. 398-443. W. C. Hamlett (ed.). Johns Hopkins Press, Baltimore, Maryland, USA.

Henningsen, A. D. 1999. Levels of recirculating reproductively-related steroid hormones in female elasmobranchs. Implications for reproduction in a captive environment. Aquarium Sciences and Conservation 2: 97-116.

Henningsen, A. D. 2000. Notes on reproduction in the southern stingray, *Dasyatis americana* (Chondrichthyes: Dasyatidae) in a captive environment. Copeia 2000(3): 826-828.

Henningsen, A. D. 2002. Age and growth in captive southern stingrays, *Dasyatis americana*. 18th Annual Meeting of the American Elasmobranch Society, 82nd Annual Meeting of the American Society of Ichthyologists and Herpetologists. University of Kansas, Westin Crown Center Hotel, Kansas City, MO, USA, July 3-8, 2002, Abstract, p. 167.

Heupel, M. R., J. M. Whittier, and M. B. Bennett. 1999. Plasma steroid hormone profiles and reproductive biology of the epaulette shark, *Hemiscyllium ocellatum*. Journal of Experimental Zoology 284: 586-594.

Johnson, R. H. and D. R. Nelson. 1978. Copulation and possible olfaction-mediated pair formation in two species of carcharhinid sharks. Copeia 1978: 539-542.

Kajiura, S. M. and T. C. Tricas. 1996. Seasonal dynamics of dental sexual dimorphism in the Atlantic stingray, *Dasyatis sabina*. Journal of Experimental Biology 199: 2297-2306.

Kajiura, S. M., A. P. Sebastian, and T. C. Tricas. 2000. Dermal bite wounds as indicators of reproductive seasonality and behavior in the Atlantic stingray, *Dasyatis sabina*. Environmental Biology of Fishes 58: 23-31.

Klimley, A. P. 1980. Observations of courtship and copulation in the nurse shark, *Ginglymostoma cirratum*. Copeia 1980: 878-882.

Kuenen, M. 2000. A log of captive births by an Atlantic nurse shark, “Sarah”. Drum and Croaker 31: 22-24.

Last, P. R. and J. D. Stevens. 1994. Sharks and Rays of Australia. CSIRO Press, Collingwood, Victoria, Australia. 513 p.

Lucifora, L. O., R. C. Menni, and A. H. Escalante. 2002. Reproductive ecology and abundance of the sand tiger shark, Carcharias taurus, from the southwestern Atlantic. ICES Journal of Marine Science 59: 553-561.

Luer, C. A. and P. W. Gilbert. 1985. Mating behavior, egg deposition, incubation period and hatching in the clearnose skate, *Raja eglanteria*. Environmental Biology of Fishes 13: 161-171.

Luer, C. A. 1989. Elasmobranchs (sharks, skates, and rays) as animal models for biomedical Research. *In:* Nonmammalian Animal Models for Biomedical Research, p. 121-147. A. D. Woodhead and K. Vivrito (eds.). CRC Press, Boca Raton, Florida, USA.

McCourt, R. M. and A. N. Kerstitch. 1980. Mating behavior and sexual dimorphism in dentition in the stingray, *Urolophus concentricus* from the Gulf of California. Copeia 1980: 900-901.

Michael, S. W. 1993. Reef Sharks and Rays of the World. Sea Challengers, Monterey, California, USA. 107 p.

Michael, S. W. 2001. Aquarium Sharks and Rays. Tropical Fish Hobbyist Publications, Neptune City, New Jersey, USA. 254 p.

Mollet, H. F., J. M. Ezcurra, and J. B. O’Sullivan. 2002. Captive biology of the pelagic stingray, *Dasyatis violacea* (Bonaparte, 1832). Marine and Freshwater Research 53: 531-541.

New, J. G. 1994. Electric organ discharge and electrosensory reafference in skates. Biological Bulletin 187: 64-75.

Parker, G. A. 1979. Sexual selection and sexual conflict. *In:* Sexual Selection and Reproductive Competition in Insects, p. 123-166. M. S. Blum and N. A. Blum (eds.). Academic Press, New York, USA.

Parker, P. and D. J. Bucher. 2000. Seasonal variation in abundance and sex ratio of grey nurse (sand tiger) sharks, *Carcharias taurus*, in northern New South Wales, Australia: A survey based on observations of recreational scuba divers. Pacific Conservation Biology 5 (4): 336-346.

Pepperell, J. G. 1992. Trends in the distribution, species composition and size of sharks caught by gamefish anglers off South-eastern Australia, 1961-1990. Australian Journal of Marine and Freshwater Research 43: 213-225.

Pollard, D. A., M. P. Lincoln Smith, and A. K. Smith. 1996. The biology and conservation status of the grey nurse shark (*Carcharias taurus* Rafinesque 1810) in New South Wales, Australia. Aquatic Conservation: Marine and Freshwater Ecosystems 6: 1-20.

Pratt, H. L, Jr. 1979. Reproduction in the blue shark, *Prionace glauca*. Fishery Bulletin 77: 445-470.

Pratt, H. L., Jr. and J. C. Carrier. 2001. A review of elasmobranch reproductive behavior with a case study of the nurse shark, *Ginglymostoma cirratum*. Environmental Biology of Fishes 60: 157-188.

Reid, D. D. and M. Krogh. 1992. Assessment of catches from protective shark meshing off New South Wales beaches between 1950 and 1990. Australian Journal of Marine and Freshwater Research 43: 283-296.

Reynolds, J. D. 1996. Animal breeding systems. Trends in Ecological Evolution 11: 68-72.

Sathyanesan, A. G. 1966. Egg-laying of the chimaeroid fish *Hydrolagus colliei*. Copeia 1966: 132-134.

Schmid, T. H., L. M. Ehrhart, and F. F. Snelson, Jr. 1988. Notes on the occurrence of rays (Elasmobranchii, Batoidea) in the Indian River Lagoon system, Florida. Florida Scientist 51: 121-128.

Schmid, T. H. and F. L. Murru. 1991. Observations on the reproductive biology of bamboo sharks (Hemiscylliidae) maintained in captivity. 71st Annual Meeting of the American Society of Ichthyologists and Herpetologists, 7th Annual Meeting of the American Elasmobranch Society. American Museum of Natural History, New York, USA, June 15-20, 1991, Abstract, p. 33

Simpfendorfer, C. A. 2000. Predicting population recovery rates for endangered western Atlantic sawfishes using demographic analysis. Environmental Biology of Fishes 58: 371-377.

Sisneros, J. A., T. C. Tricas, and C. A. Luer. 1998. Response properties and biological function of the skate electrosensory system during ontogeny. Journal of Comparative Physiology A, 183: 87-99.

Sisneros, J. A. and T. C. Tricas. 2002. Ontogenetic changes in the response properties of the peripheral electrosensory system in the Atlantic stingray (*Dasyatis sabina*). Brain, Behavior and Evolution 59: 130-140.

Sorensen, P. W., A. R. Brash, F. W. Goetz, R. G. Kellner, L. Bowdin, and L. A. Vrieze. 1995. Origins and functions of F prostaglandins as hormones and pheromones in the goldfish. *In:* Proceedings of the Fifth International Symposium on the Reproductive Physiology of Fish, p. 252-254. F. W. Goetz and P. Thomas (eds.). The University of Texas, Austin, USA.

Sorensen, P. W., A. P. Scott, and R. L. Kihslinger. 2000. How common hormonal metabolites function as relatively specific pheromonal signals in the goldfish. *In:* Proceedings of the Sixth International Symposium on the Reproductive Physiology of Fish, p. 125-128. B. Norberg, O. S. Kjesbu, G. L. Taranger, E. Anderson, and S. O. Stefannson (eds.). University of Bergen, Bergen, Norway.

Springer, S. 1967. Social organization of shark populations. *In:* Sharks, Skates, and Rays, p. 149-174. P. W. Gilbert, R. F. Mathewson, and D. P. Rall (eds.). Johns Hopkins Press, Baltimore, Maryland, USA.

Thorson, T. B., J. K. Langhammer, and M. I. Oetinger. 1983. Reproduction and development of the South American freshwater stingrays, *Potamotrygon circularis* and *P. motoro*. Environmental Biology of Fishes 9(1): 3-24.

Tricas, T. C., S. W. Michael, and J. A. Sisneros. 1995. Electrosensory optimization to conspecific phasic signals for mating. Neuroscience Letters 202: 129-131.

Tullis, A., G. M. Peterson, and C. Hull. 1997. Developmental changes in the metabolism and morphology of embryonic white spotted bamboo sharks, *Chiloscyllium plagiosum*. 77th Annual Meeting of the American Society of Ichthyologists and Herpetologists, 13th Annual Meeting of the American Elasmobranch Society. University of Washington, Seattle, USA, June 26-July 2, 1997, Abstract, p. 295

Uchida, S. 1982. Elasmobranch fishes around Ryukyu Island and their cultural status in the big water tank of aquarium. Report of Japanese Group for Elasmobranch Studies, Ocean Research Institute, University of Tokyo, 14: 1-8.

Uchida, S., M. Toda, and Y. Kamei. 1990. Reproduction of elasmobranchs in captivity. *In:* Elasmobranchs as Living Resources: Advances in the Biology, Ecology, Systematics and Status of the Fisheries, p. 211-237., H. L. Pratt, Jr., S. H. Gruber, and. T. Taniuchi (eds.). U. S. Department of Commerce, NOAA Technical Report NMFS 90.

Uchida, S., M. Toda, N. Tanaka, and Y. Kamei. 1997. Reproduction of elasmobranchs in captivity (II). *In:* Proceedings of the Fourth International Aquarium Congress, June 23-27, 1996, Tokyo. p. 99-107. Tokyo, Japan, by the Congress Central Office of IAC '96, Tokyo Sea Life Park.

Van Dykhuizen, G., K. Light, D. Wrobel, and D. Powell. 1997. Ratfish romance at the Monterey Bay Aquarium. 77th Annual Meeting of the American Society of Ichthyologists and Herpetologists, 13th Annual Meeting of the American Elasmobranch Society. University of Washington, Seattle, USA, June 26-July 2, 1997, Abstract, p. 298

Voss, J., L. Berti, and C. Michel. 2001. *Chiloscyllium plagiosum* (Anon., 1830) born in captivity: Hypothesis for gynogenesis. Bulletin of the Institute of Oceanography, Monaco 20(1): 351-353.

West, J. G. and S. Carter. 1990. Observations on the development and growth of the epaulette shark *Hemiscyllium ocellatum* (Bonnaterre) in captivity. Journal of Aquariculture and Aquatic Sciences 5(4): 11-117.

Whitley, G. P. 1940. The fishes of Australia. Part 1. The sharks, rays and devilfish, and other primitive fishes of Australia and New Zealand. Royal Zoological Society of New South Wales, Mosman. 280 p.

Yano, K., F. Sato, and T. Takahashi. 1999. Observations of the mating behavior of the manta ray, *Manta birostris*, at the Ogasawara Islands, Japan. Japanese Ichthyological Research 46: 289-296.

PERSONAL COMMUNICATIONS

Ankley, M. 2000. Long Beach Aquarium of the Pacific, Long Beach, CA 90802, USA.

Areitio, P. 2000. L'Oceanographic, Inc., Grupo Parques Reunidos, s/n. 46013, Valencia, Spain.

Bok, A. 2000. Sea World Aquarium, Durban, South Africa.

Choromanski, J. 1998. Ripley Aquariums, Inc., Orlando, FL 32819, USA.

Davis, R. 2002. SeaWorld Florida, Orlando, FL 32821, USA.

Engelbrecht, M. P. O. 2000. Dillon Beach, CA 94929, USA.

Fischer, A. 2000. Underwater World, Mooloolaba, Queensland, 4557, Australia.

Horton, M. 2000. Sea World Australia, Queensland 4217, Australia.

Kaiser, S. 2000. Atlantis Paradise Island Resort and Casino, New Providence Island, Bahamas.

Kelley, G. 2000. Atlantis Paradise Island Resort and Casino, New Providence Island, Bahamas.

Liu-Ferguson, M. 2000. Atlantis Paradise Island Resort and Casino, New Providence Island, Bahamas.

Martel-Bourbon, H. 2000. New England Aquarium. Central Wharf. Boston, MA 02110, USA.

Pratt, H. L., Jr. 2000. Apex Predators Investigation. Narragansett Laboratory. Narragansett, RI 02882, USA.

Rasmussen, L. E. L. 2000. Department of Biochemistry and Molecular Biology. Oregon Graduate Institute. Beaverton, OR 97006, USA.

Riggles, G. 2000. Indianapolis Zoo, Indianapolis, IN 46222, USA.

Zoller, C. 2000. Moody Gardens. One Hope Boulevard, Galveston, TX 77554, USA.

The Elasmobranch Husbandry Manual: Captive Care of Sharks, Rays and their Relatives, pages 249-259.
© 2004 Ohio Biological Survey

Chapter 18
Elasmobranch Genetics and Captive Management

EDWARD J. HEIST

Fisheries and Illinois Aquaculture Center,
Department of Zoology, Southern Illinois University Carbondale.
Carbondale, IL 62901-6511, USA.
E-mail: edheist@siu.edu

KEVIN A. FELDHEIM

Pritzker Lab for Molecular Systematics and Evolution,
Field Museum.
Chicago, IL 60605, USA.
E-mail: kfeldheim@fieldmuseum.org

Abstract: The advent of the polymerase chain reaction, or PCR, has rapidly changed the field of genetics. Despite this fact, the field of elasmobranch genetics is in its infancy. Several methods exist for examining questions such as population genetic structure, species identification, paternity exclusion, and evolutionary relationships between species. Captive elasmobranchs can provide insight into the study of wild populations through tissue samples collected during routine exams and by shedding light on genetic mating systems of those species reproducing in captivity. Aquarists should try to minimize the loss of genetic variability in captive elasmobranchs to avoid potential inbreeding.

In this chapter, we provide a brief overview of genetic techniques. This chapter is by no means an exhaustive review in methodology. There are many genetic techniques now available for molecular ecologists and geneticists and several published volumes (e.g., Ferraris and Palumbi, 1996; Hillis et al., 1996; Hoelzel, 1998) contain detailed protocols for performing these techniques. As elasmobranch genetics is a relatively young field, we briefly review how these methods have been applied to studies on elasmobranchs. We give proper methods for tissue collection and storage and discuss the genetics of captive populations. Although genetic considerations are perhaps not a priority for captive elasmobranchs, they should not be discarded wholeheartedly. Here, we discuss the potential negative effects of the loss of genetic variability in a captive population and suggest ways to minimize such losses. Finally, we suggest ways captive elasmobranchs can contribute to genetic studies.

PROTEIN-BASED TECHNIQUES

The first molecular technique to gain widespread use in the scoring of polymorphic genetic characters in fishes was allozyme electrophoresis (Utter, 1991). Allozymes are enzymatic proteins that exhibit differential electrophoretic mobility and are generally used to examine genetic structure in populations (Utter et al., 1987), but can also be used for species identification and systematic purposes. As allozymes are proteins, this method is an indirect indicator of genetic variation. Alleles are caused by variation in amino acid sequences reflecting only a fraction of the changes that occur in the nucleotide (DNA) sequence of the protein-coding gene. Allozyme variability is low in sharks when compared to bony fishes (Smith, 1986) and thus allozymes may be of limited use in some elasmobranch species (Lavery and Shaklee, 1989). Because tissue types vary in enzyme expression, a survey

of many loci will require tissue from multiple tissue types (e.g., liver, muscle, nerve, or eye) and hence will typically require lethal sampling. However, for those loci that are expressed in skeletal muscle it is possible to detect sufficient variation from a small biopsy of muscle tissue provided that the tissue is used immediately or frozen at -20 °C or colder (Billington et al., 1996).

Distinct species typically exhibit fixed genetic differences (i.e., no alleles are shared) at one or more allozyme loci. Fixed allelic differences among species are useful for demonstrating the presence of "cryptic" species. For example, Lavery and Shaklee (1991) were able to show that two color morphs of "blacktip" sharks in northern Australia exhibited nearly fixed differences at two allozyme loci and concluded that two species were present. Solé-Cava et al. (1983) demonstrated allozyme differences among two species of angel shark where only a single species had previously been recognized. Solé-Cava and Levy (1987) later demonstrated the presence of a third species. Eitner (1995) suggested the existence of an undescribed species of thresher shark based solely on allozyme data.

DNA-BASED TECHNIQUES

DNA is present in two organelles in animal cells: the nucleus and the mitochondrion. Whereas nuclear DNA is inherited equally from both parents, mitochondrial DNA (mtDNA) in vertebrates is presumed to be maternally inherited. The nuclear genomes of sharks contain in the order of 6-18 picograms of DNA per cell (see Asahida et al., 1995), corresponding to between ~3 x 10^9 and 9 x 10^9 base pairs of DNA per haploid genome. By comparison, the human genome contains 2.91 x 10^9 base pairs of DNA per haploid genome (Venter et al., 2001); hence sharks tend to have nuclear genomes that range from near equal to several times larger than that of humans. In contrast, the mitochondrial genome of animals (including sharks) is very compact, typically 16,000 to 17,000 base pairs in length (Meyer, 1993). Mitochondrial DNA evolves faster than most nuclear DNA regions (for exceptions see "microsatellites" below) and therefore exhibits considerable variation within and among species. The smaller size and high levels of variation make mtDNA a very useful marker for genetic analyses; however, its usefulness may be limited by the fact that it is maternally inherited. For example, it is impossible to determine paternity using mtDNA, and in species in which males and females exhibit different migratory tendencies, mtDNA will only reveal information about female migration.

The development of Polymerase Chain Reaction (PCR) techniques has been largely responsible for the explosion in DNA-based technology over the last two decades. Beginning with a minute quantity of genomic DNA, the PCR process uses a thermostable DNA polymerase and 20 to 40 cycles of DNA synthesis resulting in perhaps a million-fold amplification of the target sequence. The amplified fragment can then be sequenced or subjected to restriction fragment length polymorphism (RFLP) analysis. The source of DNA can be any piece of fresh or ancient nucleated tissue including fin clips, blood, or even a museum specimen (e.g., dried jaw). Tips for preserving and taking tissue samples are provided below.

TYPES OF MOLECULAR MARKERS

Restriction Fragment Length Polymorphisms (RFLPs) use bacterial restriction enzymes that cleave specific (usually 4 to 6 base) sequence motifs in a segment of DNA. Different fragment patterns among individuals are caused by a mutation in the restriction site or a change in the number of nucleotides, through an insertion or deletion, between restriction sites. These different fragment arrays are detected by resolving the fragments on an agarose or polyacrylamide gel that separates the fragments by size. Typically a fragment of DNA is amplified using PCR and then cut with several restriction enzymes. Alternatively, sections of DNA are cut with restriction enzymes, run on a gel and probed with a labeled homologous sequence. The locations of the bands are visualized by the presence of the labeled probe. RFLP analysis of mtDNA has been used to examine the population genetic structure of sharks (Heist et al., 1996a, 1996b).

Numerous "fingerprinting" techniques each produce a large number of bands on a single gel that can be used to infer relatedness among individuals. The multilocus minisatellite technique (Wright, 1993; O'Reilly and Wright, 1995) is a nuclear DNA RFLP approach that uses a probe made from a sequence of DNA known to be present in many copies of the genome of the target organism. When the digested DNA is hybridized to the probe, many fragments are visualized. This technique is commonly used for forensics and paternity-exclusion.

Random Amplified Polymorphic DNA or RAPDs are generated by PCR using short primers (around 10

nucleotides in length) of random sequence. This generates random segments of DNA that are resolved by gel electrophoresis. While this technique is fast and inexpensive (RAPD is an apt acronym), the results are often inconsistent.

A relatively new technique is Amplified Fragment Length Polymorphism or AFLPs (Vos et al., 1995). Like the minisatellite technique, it begins with the use of restriction enzymes to cut genomic DNA. The resultant DNA fragments are then combined with restriction enzyme-specific adapters. Primers complementary to these adapters are then used to amplify fragments using PCR. The use of the adapters removes the inconsistency problem associated with RAPDs and allows the technique to be performed on species for which minisatellite probe sequences have not yet been developed. To date, no elasmobranch study has employed minisatellites, RAPDs, or AFLPs.

What each of these fingerprinting techniques provides is a unique pattern consisting of numerous (typically 20–200) bands on a single gel. Algorithms have been developed to estimate the degree of relatedness among individuals based on the fraction of bands that differ and are shared among individuals. These indices are widely used in captive breeding programs to avoid inbreeding by identifying genetically related individuals. These techniques can be used for paternity-exclusion. Barring a rare mutation, offspring should not possess any alleles not found in either parent. Thus if one parent (typically the mother) can be positively identified, potential sires can be excluded if they lack a band present in the offspring.

Microsatellites are short, tandem repeats of 1-6 base pairs (Ashley and Dow, 1994) that exhibit a high mutation rate (and hence high intraspecific diversity) for the number of repeat units. Primers for these markers must be developed, usually by rather exhaustive and involved protocols (e.g., Dow et al., 1995) for use in PCR. Once primers are developed, PCR is used to amplify microsatellite repeat regions. PCR products are resolved using polyacrylamide gel electrophoresis. Because of their high degrees of polymorphism and hence great utility, microsatellites are rapidly becoming the marker of choice in many studies of population genetics (O'Connell and Wright, 1997). Two studies on Carcharhiniformes (Heist and Gold, 1999a; Feldheim et al., 2001a), one on an orectolobiform (Heist et al., 2003), and one on a lamniform (Pardini et al., 2000) have developed species-specific primers for microsatellite analysis. The utility of these species-specific primers for an array of taxa (Heist and Gold, 1999a; Pardini et al., 2000) may make microsatellite analysis feasible for population genetic studies in many elasmobranch species.

TISSUE COLLECTION AND STORAGE

Prior to the development of PCR, the only suitable tissues for genetic analyses were those that were fresh or freshly frozen and maintained at temperatures of -20 °C (or preferably -80 °C). Allozyme analysis still requires tissue samples of high quality, and DNA-extraction yields, in terms of quantity and quality, from fresh and frozen tissue, are superior to tissues that have been preserved via other means. PCR-based techniques require such small initial amounts of target DNA that nearly any preserved tissue (except that stored in un-buffered formalin) is sufficient. Besides freezing, the two most common methods for preserving tissue for genetic analyses are storage in 95% ethanol and 20% DMSO saturated with sodium chloride (Seutin et al., 1991; Table 18.1 and Table 18.2). Tissues stored in these solutions at room temperature (or preferably refrigerated at 4 °C) can provide adequate DNA for amplification for several years, although 20% DMSO may be a superior buffer for subsequent DNA extractions (Seutin et al., 1991; Dawson et al., 1998). Blood can be stored in Queen's lysis buffer (Seutin et al. 1991) in a ratio of one part whole blood to three parts buffer. Unbuffered formalin, perhaps the most commonly

Table 18.1. Sample collection and appropriate tissue storage for genetic studies. Asterisk denotes tissue that must be frozen if used for an allozyme study.

Sample collection	When collected	Storage	Use
Fin clip	Exam	Frozen or buffer (Table 18.2)	DNA study
Blood sample	Exam	Frozen or Queens lysis buffer (Table 18.2)	DNA study
Muscle biopsy	Necropsy or Exam	Frozen or buffer*	DNA or allozyme study
Internal organs	Necropsy	Frozen or buffer*	DNA or allozyme study
Oviducal gland	Necropsy	Frozen or buffer	Sperm storage study

Table 18.2. Storage buffers for tissue samples used in genetic studies.

TNES urea buffer

6-8 M Urea	**For 1 L (6 M Urea solution):**
0.125 M (125 mM) NaCl	360.4 grams Urea
0.01 M (10 mM) Tris, pH 7.5	7.3 grams NaCl
0.01 M (10 mM) EDTA	1.21 grams Trizma Base
1% SDS	3.72 grams EDTA
pH=7.5	

Long-term storage buffer

0.1 M (100 mM) Trizma Base	**For 1 L:**
0.1 M (100 mM) EDTA	12.2 grams Tris
2% SDS	37.2 grams EDTA
pH=8.0	20 ml of 10% SDS

20% DMSO-salt saturated storage buffer

20% DMSO saturated with 5 M NaCl	**For 1 L:**
optional: EDTA, pH 8.0 (up to .25 M)	243 grams 5 M NaCl
	74.5 grams 0.25 M Na_2EDTA

Dissolve in 400 ml H_2O (800 ml for 1 L). Once EDTA and NaCl dissolved, add DMSO to 20%, 100 ml for 500 ml; 200 ml for 1 L.

Queen's lysis buffer (blood storage)

0.01 M (10 mM) Tris
0.01 M (10 mM) NaCl
0.01 M (10 mM) EDTA
1% n-laurylsarcosine
pH=7.5

95% ethanol or isopropanol can also be used to store tissue samples. If tissues will be used for an allozyme study, they should be frozen at -20°C or colder. Freezing will also work for subsequent DNA studies.

used tissue fixative in museums and aquariums, causes irreversible chemical damage to DNA, rendering tissues all but worthless for subsequent DNA analyses. While protocols exist for extracting DNA from formalin-fixed tissues, the protocols are long and tedious and typically result in DNA that amplifies poorly, if at all. While buffered formalin is less harmful to DNA, an appropriate rule of thumb is: if future genetic analyses of a specimen may be desired, either do not fix the animal in formalin or collect a tissue sample for formalin-free preservation prior to fixing the animal.

Because of the small amount of DNA required for a successful PCR amplification, contamination from other species and other individuals is a serious consideration when collecting samples for genetic analyses. Latex gloves should be worn and either changed or cleaned between samples to prevent the carryover of human DNA or contaminants present on the hands. Instruments should be heat sterilized, not alcohol sterilized, since alcohol will only act to preserve whatever DNA contamination is present on the tools. If heat sterilization is impractical, instruments should be washed vigorously with soap and water to remove foreign tissue, soaked in a 10% bleach solution, and rinsed prior to use on specimens.

SYSTEMATICS AND TAXONOMIC IDENTIFICATION

Molecular markers are proving to be very useful for determining the phylogenetic relationships among elasmobranch species (Dunn and Morrissey, 1995; Kitamura et al., 1996a; Naylor et al., 1997). Molecular genetics has been used to demonstrate the presence of unrecognized or cryptic species (Gleeson et al., 1999; Martin and Birmingham, 2000). Because elasmobranchs are morphologically conserved and because many species are morphologically similar, it is likely that additional species will be recognized and confirmed using molecular genetics. Molecular genetics can be used for species identification (Heist and Gold, 1999b; Shivji et al., 2003) and perhaps even identify the population of origin of a captive elasmobranch.

A few milliliters of blood or a tissue biopsy provide ample DNA for PCR amplification and DNA sequencing. The sequence obtained from the unknown shark must be compared to one from a positively identified specimen. Perhaps the best technique for identifying a single shark is by sequencing all or part of the mitochondrial cytochrome-b (cyt-b) gene. The cyt-b gene is the single most widely-used gene for systematic analyses in vertebrates (Lydeard and Roe, 1997) and hence there is considerable data available for comparison. While cyt-b exhibits very little intraspecific variation within carcharhinid sharks (Heist, 1999), there is considerable divergence among species.

DNA sequence data for comparison are available from the GenBank internet database (www1). By using this service, it is possible to perform taxonomic searches to download sequences from particular species. “Blast” searches can be performed in which a sequence submitted by a user is compared to the entire database and the sequences with the greatest similarities are retrieved. Relatively few shark sequences have been deposited into the GenBank database. Many scientific journals now require deposition of the DNA sequence on the database as a condition of publication; therefore, as more studies are completed and published, the database for elasmobranchs will enlarge considerably.

GENETIC STOCK STRUCTURE

When populations are reproductively isolated, allele frequencies at polymorphic loci diverge due to the stochastic process of random genetic drift within each population. The magnitude of the difference in allele frequencies is represented by various estimates of Wright’s F_{ST}, which can be thought of as the standardized variance in gene frequencies among populations (Wright, 1969). F_{ST} values typically range from 0 to 1, with values <0.05 generally taken to mean that there is little genetic divergence among stocks (Hartl and Clark, 1997). Estimates of F_{ST} are routinely used to determine whether or not a species is divided into multiple stocks (Carvalho and Hauser, 1994).

F_{ST} values are expected to be inversely proportional to the amount of migration (gene flow) among populations. Applying the island model of migration:

$$F_{ST} = \frac{1}{(4Nem + 1)}$$

where Nem is equal to the effective migrants per generation (Wright, 1969). Under this scenario, a single migrant per generation (corresponding to an F_{ST} of 0.2) is considered sufficient to prevent significant genetic divergence. However, if migrants come from nearby populations that are genetically more similar than members of the species as a whole, if some of the migrants are sexually immature, or if migrants have reduced reproductive success relative to natives, F_{ST} may underestimate the number of migrants per generation (Wright, 1969; Mills and Allendorf, 1996). Typically this is not a problem since the number of migrants necessary to reduce F_{ST} to a value very close to zero is so small that a statistically significant value of F_{ST} may be taken as evidence of multiple stocks. However, with the increased statistical power of multiple highly-polymorphic microsatellite loci, a statistically significant (F_{ST}>0) outcome might not represent biologically significant stock structure

(Gold and Richardson, 1999). To further complicate matters, Dizon et al. (1995) argued that in cases in which a type II error (failing to reject the null hypothesis of a single genetic stock when multiple stocks exist) is more deleterious to management practices than a type I error (falsely rejecting the single stock hypothesis when only a single stock is present), prudent risk management practices call for a reduction in the α-level of the test to balance the risks associated with each error type. Thus, a very large value of F_{ST} unambiguously indicates the presence of multiple isolated stocks while small F_{ST} values require careful consideration and judgment. Waples (1998) provides further caution when interpreting small values of F_{ST}, noting that because of the asymptotic shape of the relationship between small F_{ST} values and Nem, a small error in the measurement of F_{ST} will result in a large error in the estimate of Nem.

Previous studies of population genetics in sharks have detected very small values of F_{ST} among continuously distributed sharks, but greater divergences among discrete populations of sharks (Heist, 1999). Allozyme studies of the spottail shark (*Carcharhinus sorrah*) and Australian blacktip shark (*Carcharhinus tilstoni*) found no evidence of multiple stocks within Australian waters (Lavery and Shaklee, 1989).

Populations of gummy shark (*Mustelus antarcticus*) from southern and eastern Australia exhibit significant differences in allozyme and mtDNA profiles (Gardner and Ward, 1998). Within the North Atlantic, studies of the sandbar shark, (*Carcharhinus plumbeus*) and Atlantic sharpnose shark (*Rhizoprionodon terraenovae*) detected no significant differences in mtDNA haplotype frequencies (Heist et al., 1995; Heist et al., 1996b). Heist et al. (1996a) reported small but significant differences in mtDNA haplotype frequencies in shortfin mako (*Isurus oxyrinchus*) between the North Atlantic and other ocean basins; however, there was no evidence of evolutionarily distinct stocks. Feldheim et al. (2001a) found small but statistically significant F_{ST} values in lemon sharks (*Negaprion brevirostris*) from Bimini, Bahamas, and Brazil. Gaida (1997) found significant differences in allozyme allele frequencies among populations of Pacific angel sharks (*Squatina californica*) from different islands in the California Channel Island chain that were isolated by deep channels. Recently Pardini et al. (2001) detected significant differences in mtDNA diversity, but not microsatellite diversity, among white sharks (*Carcharodon carcharias*) from South Africa and Australia/New Zealand, suggesting male-mediated gene flow accompanied by female philopatry.

STOCK TRANSFERS AND RELEASE OF ANIMALS

Effects of stock transfers in fishes, which have generally been viewed as deleterious, can be divided into direct or indirect effects (Waples, 1995; Utter, 1998). Indirect effects include competition and disease transfer. For example, a transferred fish that fails to reproduce may compete for scarce resources with native fishes, or it may be a resistant carrier of a disease organism to which the native stock is susceptible. This last scenario is especially dangerous, and there are numerous examples of native stocks of salmonid fishes whose existence has been threatened through the introduction of diseases carried by introduced stocks (Utter, 1998). Direct effects occur when released fish interbreed with native fishes. Traits gained through domestication selection can be passed on to wild animals (Storfer, 1999). Farm-raised trout have developed a shadow following behavior in which animals follow the shadow of the feed truck (Vrijenhoek, 1998). While this trait may be favorable in captivity, this behavior may lead to an increased risk of mortality in the wild (as animals follow the shadow of a raptor for example).

One of the most serious direct effects of stock transfer is outbreeding depression, which can result from two causes: loss of adaptation and breakup of co-adapted gene complexes (Templeton, 1986; Waples, 1995). The offspring of matings between native and introduced fishes, and subsequent generations of progeny, may not be adapted to the local environment. Hence the gene pool may be disrupted through the presence of foreign maladapted genes. In subsequent generations, genes and chromosomes that have co-evolved as a unit will be shuffled via meiotic reductive division resulting in fish that are maladapted to the environment. Mixing of distinct stocks of fishes may be beneficial where a local stock is suffering from inbreeding depression due to a reduction in population size. However, unless signs of inbreeding depression are apparent, (e.g., fluctuating asymmetry, high occurrence of anatomical or physiological abnormalities), stock transfers should be viewed as potentially dangerous (Vrijenhoek, 1998).

Outbreeding depression will only occur when stock transfers occur among genetically distinct stocks. Because many sharks are highly migratory and many species are pelagic, a single shark stock may range over thousands of kilometers, or there may be a single worldwide stock. If two stocks are reproductively isolated, and therefore have the potential for outbreeding depression, there will be significant frequency differences in polymorphic genetic characters (e.g., allozyme or microsatellite alleles, mtDNA haplotypes). In each of the studies cited in the section on stock structure above, the similarity of gene frequencies between populations indicates that there is sufficient genetic exchange among the surveyed locations so that outbreeding depression would not likely accompany a stock transfer. However, Utter (1998) argued that in pelagic species that have large effective population sizes (e.g., shortfin mako), adaptive differences among stocks can develop even in the presence of considerable gene flow. Many sharks, including species commonly exhibited in aquariums (e.g., sandbar sharks and sand tiger sharks, *Carcharias taurus*), exhibit highly discontinuous distributions. In these cases, there may be significant genetic differences among populations. Heist (1994) observed that sandbar sharks from western Australia and the eastern United States have diagnostically different mtDNA profiles, indicating a long period of isolation and perhaps local adaptation. Populations that are genetically and geographically isolated may exhibit selective differences in terms of physiology, behavior, or disease-resistance, that would make transfers of stocks harmful.

Given the great migratory potential and connectivity of shark populations, coupled with the small numbers of sharks that are likely to be released via aquariums, the likelihood of deleterious results from captive releases of elasmobranchs is small. However, based on the information from captive releases in other fish species, the threats to native stocks outweigh the benefit that would be gained to the population by the addition of captive releases. Thus, captive sharks should not be released into the wild environment, particularly in those situations in which a release will result in a transfer of a shark from one discrete population to another.

GENETIC CONSIDERATIONS OF CAPTIVE BREEDING

For most species, there are genetic detrimental effects associated with captive breeding, including inbreeding and loss of genetic variability through genetic drift (Storfer, 1999). Inbreeding results in an overall loss of heterozygosity as well as an increase in the expression of recessive deleterious traits (Lande, 1988). Ralls et al. (1988) examined captive populations of mammals and found that juveniles from inbred pedigrees suffered higher mortality than non-inbred lines. To decrease inbreeding, careful pedigree analysis should be used to avoid matings by related individuals. If pedigree analysis is not an option, relatedness of individuals either by band sharing or sharing of alleles (see fingerprinting methods and microsatellites above) can be used to identify potentially related animals that should not be bred to one another. On average, full siblings share 50% of their genes, while half siblings share 25% of their genes, above the background sharing of alleles by unrelated individuals.

Captive populations suffer a loss of genetic diversity due to genetic drift, chance fluctuations in allele frequencies over time (Hartl, 1988). Small populations are especially prone to this phenomenon due to few individuals and resulting low overall genetic variability. This can lead to the rapid loss or fixation of alleles. The effects of genetic drift may be reduced in a captive species if several populations are kept in different aquariums. One large captive metapopulation can maintain genetic variability, even though single captive populations may be losing alleles due to drift. For example, if captive populations drift to fixation for different alleles, allelic diversity can still be maintained over the whole captive metapopulation. In cichlids (*Prognathochromis perrieri*), genetic diversity is preserved over several captive populations worldwide (Fiumera et al., 2000).

Other factors may further exacerbate the loss of genetic variation, including unequal family sizes and disproportionate mating of males and females. It is widely accepted that equalizing family sizes will help keep genetic change, over time, to a minimum (Tave, 1993; Falconer and Mackay, 1996), thereby reducing genetic drift. This way, the genes of no single male or female are over-represented in the following generation. Unequal numbers of breeding males and females can reduce genetic variation. If the sex ratio of breeding adults is unequal then the effective population number (Ne), or number of adults contributing genes to the next generation, is actually less than the total number of adults. This is represented by the following equation (from Hartl, 1988):

$$Ne = \frac{4NmNf}{(Nm + Nf)}$$

where Nm is the number of breeding males and Nf is the number of breeding females. Equalization of family sizes and breeding adults may not work for captive animals that do not accept forced breeding or are not amenable to change in breeding structure (Snyder et al., 1996).

GENETIC STUDIES AND CAPTIVE POPULATIONS

Table 18.3 summarizes the types of genetic studies that may be conducted using tissue taken from captive elasmobranchs.

MATING SYSTEMS OF CAPTIVE ELASMOBRANCHS

Little is known about the genetic mating system of most sharks. Nurse sharks (*Ginglymostoma cirratum*), lemon sharks, and blue sharks (*Prionace glauca*) are known to produce litters sired by multiple males (Ohta et al., 2000; Feldheim et al., 2001b, Feldheim et al., 2002a), while most bonnethead (*Sphyrna tiburo*) litters are produced by a single male (Chapman et al., 2000). With the exception of the bonnethead study, these reports are based on one or two litters from each species. Testing the genetic mating system of sharks in captivity may shed some light on what occurs in wild populations. Parental testing will help with the reconstruction of pedigrees. Parental testing is usually best achieved with a co-dominant marker such as microsatellites (Ashley and Dow, 1994), although dominant markers, such as AFLPs, have been used successfully in paternity assignment (Mueller and Wolfenbarger, 1999).

Female elasmobranchs are able to store sperm in their oviducal gland (Pratt, 1979) and stored sperm may be viable for over a year (Castro et al., 1988). This ability to store sperm in some species may lead to multiple males siring a litter of a female. Captive elasmobranchs may shed some light on both the duration sperm remains viable in the oviducal gland (Castro et al., 1988) and how many male ejaculates are stored in the oviducal gland at one time. Microsatellite genotyping of stored sperm would indicate the minimum number of males represented in the sperm sample. For example, if five alleles amplify at a particular microsatellite locus, this would indicate that at least three males had inseminated the female, as each male can have a maximum of two alleles per locus. Oviducal glands should be carefully dissected and stored during any necropsy of a female elasmobranch.

Table 18.3. Types of genetic studies that may be conducted using tissue taken from captive elasmobranchs.

Genetic marker	Study type	Examples
Allozymes	Systematics	Eitner, 1995
	Species designation	Lavery and Shaklee, 1991
	Population genetics	Lavery and Shaklee, 1989; Gaida, 1997
DNA sequencing	Phylogenetics	Naylor, 1992; Martin, 1993; Naylor et al., 1997
	Population genetics	Kitamura et al., 1996b
	Species identification	Heist and Gold, 1999b; Shivji et al., 2003
Fingerprinting	Paternity exclusion	
	Relatedness	
mtDNA RFLPs	Population genetics	Heist et al., 1996a, 1996b
Microsatellites	Genetic tagging	Feldheim et al., 2002b
	Marker development	Pardini et al., 2000; Heist et al., 2003
	Parentage tests	Feldheim et al., 2001a, 2002a
	Population genetics	Heist and Gold, 1999a; Feldheim et al., 2001b
	Relatedness	

CAPTIVE ELASMOBRANCH CONTRIBUTION TO GENETICS

Elasmobranch tissue is relatively difficult to obtain, and the field work involved is often cost-prohibitive. Most genetic studies undertaken would not have been possible if not for collaboration with field researchers, fishermen, etc. Therefore, during routine veterinary examination of captive animals, or during necropsy, extra tissue or blood samples should be taken for genetic studies. Ideally, blood or tissue samples should be kept frozen. Should freezer space be limited and storage at room temperature become necessary, several storage buffers for both tissue and blood samples are available (Seutin et al., 1991; Asahida et al., 1996).

Captive elasmobranchs can contribute a significant aspect to an ongoing or newly developed genetic project. Screening a genomic library for microsatellites only requires genomic DNA from one individual. A captive individual can obviously provide the DNA for this method. In addition, it is often desirable to test both amplification and variability of microsatellite PCR primers across many taxa. Often, sequences flanking a microsatellite repeat are conserved across genera, families, and even orders (Heist and Gold, 1999b; Pardini et al., 2001), and microsatellite primers developed for a particular species may be useful across a suite of species. Testing these primers for amplification and variability only requires a handful of specimens from each species.

For genetic projects not yet underway, having several samples already in storage would be advantageous to researchers worldwide. In addition, stored specimens would increase the sample size of projects already underway. Regional testing of genetic variability based on sequence data is often only comprised of a handful of samples from each population. Stored specimens may provide the researcher an extra area of the species's range to examine and compare to other populations.

REFERENCES

Asahida, T., H. Ida, and K. Hayashizaki. 1995. Karyotypes and cellular DNA contents of some sharks in the Order Carcharhiniformes. Japan Journal of Ichthyology 42: 21-26.

Asahida, T., T. Kobayashi, K. Saitoh, and I. Nakayama. 1996. Tissue preservation and total DNA extraction from fish stored at ambient temperature using buffers containing high concentration of urea. Fisheries Science 62: 727-730.

Ashley, M. V. and B. Dow. 1994. The use of microsatellite analysis in population biology: Background, methods and potential applications. *In:* Molecular Ecology and Evolution: Approaches and Applications, p. 185-210. B. Schierwater, B. Strait, G. P. Wagner, and R. Desalle (eds.). Birkhauser Verlag, Basel, Switzerland.

Billington, N., R. C. Brooks, and R. C. Heidinger. 1996. Use of cellulose acetate electrophoresis to rapidly screen sauger broodstock for sauger-walleye hybrids. Progressive Fish Culturist 58: 248-252.

Carvalho, G. R. and L. Hauser. 1994. Molecular genetics and the stock concept in fisheries. Reviews in Fish Biology and Fisheries 4: 326-350.

Castro, J. I., P. M. Bubucis, and N. A. Overstrom. 1988. The reproductive biology of the chain dogfish, *Scyliorhinus retifer*. Copeia 1988: 740-746.

Chapman, D. D., P. Prodohl, C. A. Manire, and M. S. Shivji. 2000. Microsatellite DNA profiling in the bonnethead shark, *Sphyrna tiburo*: application to mating system and population structure studies. *In:* Proceedings of the 80th Annual Meeting of the American Society of Ichthyologists and Herpetologists, 16th Annual Meeting of the American Elasmobranch Society, June 14-20, 2000, Universidad Autonoma de Baja California Sur, La Paz, B.C.S., Mexico, Abstract. 112 p.

Dawson, M. N., K. A. Raskoff, and D. K. Jacobs. 1998. Field preservation of marine invertebrate tissue for DNA analyses. Molecular Marine Biology and Biotechnology 7: 145-152.

Dizon, A. E., B. L. Taylor, and G. M. O'Corry-Crowe. 1995. Why statistical power is necessary to link analyses of molecular variation to decisions about population structure. *In:* Evolution and the Aquatic Ecosystem, p. 288-294. J. L. Neilson and D. A. Powers (eds.). American Fisheries Society Publisher, Bethesda, Maryland, USA.

Dow, B., M. V. Ashley, and H. F. Howe. 1995. Characterization of highly variable (GA/CT)n microsatellites in the bur oak, *Quercus macrocarpa*. Theoretical Applied Genetics 91: 137-141.

Dunn, K. A. and J. F. Morrissey. 1995. Molecular phylogeny of elasmobranchs. Copeia 1995: 526-531.

Eitner, B. J. 1995. Systematics of the genus *Alopias* (Lamniformes, Alopiidae) with evidence for the existence of an unrecognized species. Copeia 1995: 562-571.

Falconer, D. S. and T. F. C. Mackay. 1996. Introduction to Quantitative Genetics. Longman Group Ltd., Essex, England. 478 p.

Feldheim, K. A., S. H. Gruber, and M. V. Ashley. 2001a. Population genetic structure of the lemon shark (*Negaprion brevirostris*) in the western Atlantic: DNA microsatellite variation. Molecular Ecology 10: 295-303.

Feldheim, K. A., S. H. Gruber, and M. V. Ashley. 2001b. Multiple paternity of a lemon shark litter (Chondrichthyes: Carcharhinidae). Copeia 2001: 781-786.

Feldheim, K. A., S. H. Gruber, and M. V. Ashley. 2002a. The breeding biology of lemon sharks at a tropical nursery lagoon. Proceedings of the Royal Society of London B 269: 1655-1661.

Feldheim, K. A., S. H. Gruber, J. R. C. De Marignac, and M. V. Ashley. 2002b. Genetic tagging to determine passive integrated transponder tag loss in lemon sharks. Journal of Fish Biology 61: 1309-1313.

Ferraris, J. D. and S. R. Palumbi. 1996. Molecular Zoology. Wiley-Liss, New York, USA. 580 p.

Fiumera, A. C., P. G. Parker, and P. A. Fuerst. 2000. Effective population size and maintenance of genetic diversity in captive-bred populations of a Lake Victoria cichlid. Conservation Biology 14: 886-892.

Gaida, I. H. 1997. Population structure of the pacific angel shark, *Squatina californica* (Squatiniformes, Squatinidae), around the California channel islands. Copeia 9: 738-744.

Gardner, M. G. and. R. D. Ward. 1998. Population structure of the Australian gummy shark (*Mustelus antarcticus* Gunther) inferred from allozymes, mitochondrial DNA and vertebrae counts. Marine and Freshwater Research 49: 733-745.

Gleeson, D. M., R. L. J. Howitt, and N. Ling. 1999. Genetic variation, population structure and cryptic species within the black mudfish, *Neochanna diversus*, an endemic galaxiid from New Zealand. Molecular Ecology 8: 47-57.

Gold, J. R. and L. Richardson. 1999. Population structure of two species targeted for marine stock enhancement in the Gulf of Mexico. Aquaculture, Supplement 1: 79-87.

Hartl, D. L. 1988. A Primer of Population Genetics. Sinauer Associates, Inc., Sunderland, MA, USA. 305 p.

Hartl, D. L. and A. G. Clark. 1997. Principles of Population Genetics. Sinauer Associates Inc., Sunderland, MA, USA. 481 p.

Heist, E. J. 1994. Population Genetics of Selected Species of Sharks. Unpublished Ph.D. dissertation. College of William and Mary, Gloucester Point, Virginia, USA.

Heist, E.J. 1999. A review of population genetics in sharks. *In:* Life in the Slow Lane, AFS Symposium 23, p. 161-168. J. A. Musick (ed.). American Fisheries Society, Bethesda, Maryland, USA.

Heist, E. J. and J. R. Gold. 1999a. Microsatellite DNA variation in sandbar sharks (*Carcharhinus plumbeus*) from the Gulf of Mexico and Mid-Atlantic Bight. Copeia 5: 182-186.

Heist, E. J. and J. R. Gold. 1999b. Genetic identification of sharks in the U. S. Atlantic large coastal shark fishery. Fishery Bulletin 97: 53-61.

Heist, E. J., J. E. Graves, and J. A. Musick. 1995. Population genetics of the sandbar shark (*Carcharhinus plumbeus*) in the Gulf of Mexico and Mid-Atlantic Bight. Copeia 18: 555-562.

Heist, E. J., J. A. Musick, and J. E. Graves. 1996a. Genetic population structure of the shortfin mako (*Isurus oxyrinchus*) inferred from restriction fragment length polymorphism analysis of mitochondrial DNA. Canadian Journal of Fisheries and Aquatic Sciences 53: 583-588.

Heist, E. J., J. A. Musick, and J. E. Graves. 1996b. Mitochondrial DNA diversity and divergence among sharpnose sharks, *Rhizoprionodon terraenovae*, from the Gulf of Mexico and Mid-Atlantic Bight. Fishery Bulletin 94: 664-668.

Heist, E. J., J. L. Jenkot, D. B. Keeney, R. L. Lane, G. R. Moyer, B. J. Reading, and N. L. Smith. 2003. Isolation and characterization of polymorphic microsatellite loci in nurse shark (*Ginglymostoma cirratum*). Molecular Ecology Notes 3: 59-61.

Hillis, D. M., C. Moritz, and B. K. Mable. 1996. Molecular Systematics. Sinauer Associates Inc., Sunderland, MA, USA. 655 p.

Hoelzel, A. R. 1998. Molecular Genetic Analysis of Populations, Oxford University Press, Oxford, England. 445 p.

Kitamura, T., A. Takemura, S. Watabe, T. Taniuchi, and M. Shimizu. 1996a. Molecular phylogeny of the sharks and rays of superorder Squalea based on mitochondrial cytochrome b gene. Fisheries Science 62: 340-343.

Kitamura, T., A. Takemura, S. Watabe, T. Taniuchi, and M. Shimizu. 1996b. Mitochondrial DNA analysis for the cytochrome b gene and D-loop region from the bull shark *Carcharhinus leucas.* Fisheries Science 62: 21-27.

Lande, R. 1988. Genetics and demography in biological conservation. Science 2451: 1455-1460.

Lavery, S. and J. B. Shaklee. 1989. Population genetics of two tropical sharks, *Carcharhinus tilstoni* and *C. sorrah*, in northern Australia. Australian Journal of Marine and Freshwater Research 40: 541-557.

Lavery, S. and J. B. Shaklee. 1991. Genetic evidence for separation of two sharks, *Carcharhinus limbatus* and *C. tilstoni*, from northern Australia. Marine Biology 108: 1-4.

Lydeard, C. and K. J. Roe. 1997. The phylogenetic utility of the mitochondrial cytochrome b gene for inferring relationships among Actinopterygian fishes. *In:* Molecular Systematics of Fishes, p. 285-303. T. D. Kocher and C. A. Stepien (eds.). Academic Press, New York, USA.

Martin, A. P. 1993. Hammerhead shark origins. Nature 364: 494.

Martin, A. P. and E. Birmingham. 2000. Regional endemism and cryptic species revealed by molecular and morphological analysis of a widespread species of neotropical catfish. Proceedings of the Royal Society of London, Series B 267: 1135-1141.

Meyer, A. 1993. Evolution of mitochondrial DNA in Fishes. *In:* Biochemistry and Molecular Biology of Fishes, p. 1-38. P. W. Hochachka and T. P. Mommsen (eds.). Elsevier, New York, USA.

Mills, L. S. and F. W. Allendorf. 1996. The one-migrant-per-generation rule in conservation and management. Conservation Biology 10: 1509-1518.

Mueller, U. G. and L. L. Wolfenbarger. 1999. AFLP genotyping and fingerprinting. Trends in Ecology and Evolution 14: 389-394.

Naylor, G. J. P. 1992. The phylogenetic relationships among requiem and hammerhead sharks: Inferring phylogeny when thousands of equally most parsimonious trees result. Cladistics 8: 295-318.

Naylor, G. J. P., A. P. Martin, E. G. Mattison, and W. M. Brown. 1997. Interrelationships of Lamniform sharks: testing phylogenetic hypotheses with sequence data. *In:* Molecular Systematics of Fishes, p. 199-218. T. D. Kocher and C. A. Stepien (eds.). Academic Press, New York, USA.

O'Connell, M. and J. M. Wright. 1997. Microsatellite DNA in fishes. Reviews in Fish Biology and Fisheries 7: 331-363.

Ohta, Y., K. Okamura, E. C. McKinney, S. Bartl, K. Hashimoto, and M. F. Flajnik. 2000. Primitive synteny of vertebrate major histocompatibility complex class I and class II genes. Proceedings of the National Academy of Sciences of the United States of America 97: 4712-4717.

O'Reilly, P. and J. M. Wright. 1995. The evolving technology of DNA fingerprinting and its application to fisheries and aquaculture. Journal of Fish Biology 47: 29-55.

Pardini, A. T., C. S. Jones, M. C. Scholl, and L. R. Noble. 2000. Isolation and characterization of dinucleotide microsatellite loci in the great white shark, *Carcharodon carcharias*. Molecular Ecology 9: 1176-1178.

Pardini, A. T., C. S. Jones, L. R. Noble, B. Kreiser, H. Malcolm, B. D. Bruce, J. D. Stevens, G. Cliff, M. C. Scholl, M. Francis, C. A. J. Duffy, and A. P. Martin. 2001. Sex-biased dispersal of great white sharks - In some respects, these sharks behave more like whales and dolphins than other fish. Nature 412: 139-140.

Pratt, H. L. 1979. Reproduction in the blue shark, *Prionace glauca*. Fishery Bulletin 77: 445-470.

Ralls, K., J. D. Ballou, and A. Templeton. 1988. Estimates of lethal equivalents and the cost of inbreeding in mammals. Conservation Biology 2: 185-193.

Seutin, G., B. N. White, and P. T. Boag. 1991. Preservation of avian blood and tissue samples for DNA analyses. Canadian Journal of Zoology 69: 82-90.

Shivji, M., S. Clarke, M. Pank, L. Natanson, N. Kohler, and M. Stanhope. 2003. Genetic identification of pelagic shark body parts for conservation and trade monitoring. Conservation Biology 16: 1036-1047.

Smith, P. J. 1986. Low genetic variation in sharks (Chondrichthyes). Copeia 1986: 202-207.

Snyder, N. F. R., S. R. Derrickson, S. R. Beissinger, J. W. Wiley, T. B. Smith, W. D. Toone, and B. Miller. 1996. Limitations of captive breeding in endangered species recovery. Conservation Biology 10: 338-348.

Solé-Cava, A. M. and J. A. Levy. 1987. Biochemical evidence for a third species of angel shark off the east coast of South America. Biochemical Systematics and Ecology 15: 139-144.

Solé-Cava, A. M., C. M. Voreen, and J. A. Levy. 1983. Isozymic differentiation of two sibling species of Squatina (Chondrichthyes) in South Brazil. Comparative Biochemistry and Physiology 75B: 355-358.

Storfer, A. 1999. Gene flow and endangered species translocations: a topic revisited. Biological Conservation 87: 173-180.

Tave, D. 1993. Genetics for Fish Hatchery Managers. AVI Publishing Company, Inc., Westport, Connecticut, USA. 415 p.

Templeton, A. 1986. Coadaptation and outbreeding depression. *In:* Conservation Biology: The Science of Scarcity and Diversity, p. 105-116. M. E. Soule (ed.). Sinauer Associates Inc., Sunderland, Massachusetts, USA.

Utter, F. 1991. Biochemical genetics and fishery management: An historical perspective. Journal of Fish Biology 39: 1-20.

Utter, F. 1998. Genetic problems of hatchery-reared progeny released into the wild and how to deal with them. Bulletin of Marine Science 62: 623-640.

Utter, F., P. Aebersold, and G. Winans. 1987. Interpreting genetic variation detected by electrophoresis. *In:* Population Genetics and Fishery Management, p. 21-45. N. Ryman and F. Utter (eds.). University of Washington Press, Seattle, USA.

Venter, J. C., M. D. Adams, E. W. Myers, P. W. Li, R. J. Mural, G. G. Sutton, H. O. Smith, M. Yandell, C. A. Evans, R. A. Holt, J. D. Gocayne, P. Amanatides, R. M. Ballew, D. H. Huson, J. R. Wortman, Q. Zhang, C. D. Kodira, X. Q. H. Zheng, L. Chen, M. Skupski, G. Subramanian, P. D. Thomas, J. H. Zhang,G. L. G. Miklos, and C Nelson. 2001. The sequence of the human genome. Science 291: 1304-1351.

Vos, P., R. Hogers, M. Bleeker, M. Reijans, T. van de Lee, M. Hornes, A. Frijters, J. Pot, J. Peleman, M. Kuiper, and M. Zabeau. 1995. AFLP: A new technique for DNA fingerprinting. Nucleic Acids Research 23: 4407-4414.

Vrijenhoek, R.C. 1998. Conservation genetics of freshwater fish. Journal of Fish Biology 53 (Supplement A): 394-412.

Waples, R. S. 1995. Genetic effects of stock transfers in fish. *In:* Protection of Aquatic Biodiversity: Proceedings of the World Fisheries Congress, Theme 3, May 5-8 1992, Athens, Greece. p. 51-69. D. P. Philipp, J. M. Epifanio, J. E. Marsden, J. E. Claussen, and , R. J. J. Wolotira (eds.). Science Publishers Inc., Lebanon, New Hampshire, USA.

Waples, R. S. 1998. Separating the wheat from the chaff - Patterns of genetic differentiation in high gene flow species. Journal of Heredity 89: 438-450.

Wright, S. 1969. Evolution and the Genetics of Populations. Volume 2. The Theory of Gene Frequencies. University of Chicago Press, Chicago, Illinois, USA. 520 p.

Wright, J. M. 1993. DNA Fingerprinting of Fishes. *In:* Biochemistry and Molecular Biology of Fishes, p. 58-91. P. W. Hochachka and T. P. Mommsen (eds.). Elsevier, New York, USA.

INTERNET RESOURCES

www1. http://www.ncbi.n1m.nih.gov/Entrez/

The Elasmobranch Husbandry Manual: Captive Care of Sharks, Rays and their Relatives, pages 261-270.
© 2004 Ohio Biological Survey

Chapter 19
Physiological and Behavioral Changes to Elasmobranchs in Controlled Environments

GREG CHARBENEAU

Mystic Aquarium Institute for Exploration,
Mystic, CT 06355, USA.

New Jersey State Aquarium,
1 Riverside Drive,
Camden, NJ 08103, USA.
E-mail: gcharbeneau@njaquarium.org

Abstract: Stress is defined as a stimulus acting on a biological system and the subsequent behavioral and physiologic reaction of that system (Pickering, 1981). Minimizing potential stress to elasmobranchs in a controlled environment will increase their survivorship. Parameters that can indicate the presence of stress include inappetence and anorexia, evasive or avoidance behavior, and changes to any of the following: skin coloration, ventilation, swimming behavior, feeding behavior, blood parameters, and steroid titers. Stressors may be divided into two basic categories, abiotic and biotic. Abiotic stressors include spatial constraints, transport and handling, compromised water quality, lighting, electromagnetic fields, and vibrations. Biotic stressors include species compatibility, sexual conflict, interactions with divers, inappropriate nutrition, and disease-producing pathogens. Multiple observations of animals throughout the day will allow an understanding of baseline parameters, facilitating comparison to unusual behaviors or changes in physical appearance that may indicate the presence of stressors. Careful assessment of "stressful" situations is recommended as stress responses may be of a generic nature and "snap" judgments can result in ill-informed husbandry decisions. Once a stress stimulus has been positively identified, every effort should be made to modify or eliminate it quickly. Chemico-therapeutic intervention may be required if an animal has been injured or is immunosuppressed.

The concept of biological stress has many definitions in the scientific literature and it is difficult to establish a single, comprehensive definition. All definitions of stress, however, share the common premise of a stimulus acting on a biological system and a subsequent behavioral and physiologic reaction of that system (Pickering, 1981). Stress is regarded as any stimulus that is sufficient to unbalance the internal environment (homeostasis) of an organism. Stressors can include hyperactivity, physical injury, and modification of the external environment (Smith, 1992), as well as diving activities and interaction with humans during capture, transport, and other husbandry procedures.

This chapter will examine behavioral and physiological parameters that may used as indicators of stress, comment on pre-disposing abiotic and biotic factors, and suggest techniques to moderate the effects of described stressors.

CLINICAL SIGNS

Minimizing potential stress to elasmobranchs in a controlled environment will increase their survivorship. To control stress it is important to first quantify natural behaviors and physiology in the wild, and compare these norms to observations of reactions in captive situations.

Murru (1990) states that it is important for animals maintained in controlled environments to exhibit as close to "normal" activity as possible. One must therefore understand the natural ecology and sociobiology of the species concerned to better understand clinical signs indicative of stress.

Parameters that may be used as potential indicators of stress include skin coloration, ventilation, swimming behavior, evasion or avoidance, feeding behavior, anorexia, and physiological changes. This list highlights the importance of monitoring elasmobranch behavior and of recognizing how it may be affected by environmental changes. Further, it points to the importance of documenting behavioral and clinical observations prior to, and during, changes to controlled environments. This information will be useful in developing a course of action to avoid stress, mitigate reactions, and prompt appropriate corrective treatments, where required.

Skin coloration

Epidermal hyper- or hypo-coloration (i.e., acute darkening or fading of the skin) is symptomatic of stress and may be characterized by general color changes to the entire body or manifest as a blotchiness of the skin. Rajiformes exhibit similar responses but, in addition, often display dark lines that run sagittally from the spine to the distal tip of the pectoral fins.

Hypo-coloration of the epidermis may be attributed to the effect of increased levels of catecholamines on melanocytes and the vasoconstriction of peripheral blood circulation (Cliff and Thurman, 1984). An additional biochemical reaction to catecholamines is the mobilization of glucose. Changing skin color may therefore indicate to what degree glucose redistribution has occurred and, indirectly, the depth of stress experienced by the animal (Smith, 1992).

Ventilation

There are two basic methods of breathing, or ventilation, used by sharks: obligate ram ventilation and active ventilation. Some species of sharks, such as nurse (*Ginglymostoma cirratum*), sand tiger (*Carcharias taurus*), whitetip reef (*Triaenodon obesus*), and leopard (*Triakis semifasciata*) sharks have demonstrated the ability to use both modes of ventilation. Ventilation rates may vary both inter- and intra-specifically under different environmental conditions.

Abnormal ventilation rates can be used as an indicator that some physiological or environmental stressor exists. It is important to understand the ventilation technique used by a species and to document ventilation rates under different circumstances. Examples of different circumstances include: at rest, actively swimming (including cruising, rest-glide, and recovery phases), the presence of divers, during feeding, during courtship, post-acclimatization, post-physical exam, and throughout transport. Information documented during different conditions will provide baseline data against which changed ventilation rates can be compared.

Changes to ventilation during stress will depend on the mode of ventilation normally employed by an elasmobranch. For active ventilators, movements should be relaxed and fluid, and at a reasonably constant rate. Obligate ram ventilators should have their mouth slightly open, and the gill slits partially flared, with minimal movement of the jaw or gills. Changes to ventilation may be indicated by the rate, orientation, and/or degree to which the mouth, gill slits, and spiracles open and close.

The degree of increase or decrease in ventilation rate may be slight or profound, depending on the condition of the animal and/or the stress stimulus. Stressed sharks have been observed ram ventilating with their mouth agape and gill slits flared more than normal. This behavior may be coupled with an increased swimming speed. Heavy or forced ventilation is evidenced by a stronger pumping of the gill slits or spiracles (i.e., a profound, "squeezing" of the gill slits or spiracles is observed). A pumping of the mouth and an increased ventilation rate may also be observed. Shallow or weak ventilation is characterized by a decrease in the magnitude that gill slits and spiracles open during ventilation cycles. Shallow or weak ventilation is usually associated with a decreased ventilation rate; however, it may occur independently or with an increased ventilation rate.

Changes in environmental conditions (e.g., a pulse of poor water quality) may cause an animal to temporarily minimize the water contacting its gills by "protecting" them. Protection of the gills is indicated by a severe decrease in ventilation rate, the mouth, gill slits, and spiracles remaining closed. Another stress response is ram ventilation accompanied by "coughing" or "jaw popping" (i.e., moving the jaw forward in the same manner adopted during feeding), often followed by several forced ventilations.

Swimming behavior

Swimming behavior can indicate stress in elasmobranchs and should be regularly monitored, especially when any change in environmental conditions occurs. Swimming behavior indicative of stress can include any of the following: constant, rapid swimming; quick or "jerky" maneuvers; slow, labored swimming with exaggerated lateral head movements; head above and tail below the horizontal plane, in some cases with the head out of the water; poor navigation (i.e., colliding with exhibit decoration, walls, or other animals); quick movements up through the water column followed by powerless gliding to the bottom of the exhibit; and swimming in tight circles or "looping".

Swimming behavior can be influenced by buoyancy, especially in the sand tiger shark. This species is unique in that it retains air in its stomach to regulate buoyancy. Sand tigers are normally neutrally buoyant, able to hang almost motionless in the water column. When stressed, the ability of a sand tiger to regulate the amount of air in its stomach can be compromised. This reaction can be caused by the physical trauma associated with invasive husbandry activities. If there is too much air in its stomach, the sand tiger may be observed near the surface struggling to swim down through the water column. Alternatively, the shark may be observed floating "belly up" at the surface, or occasionally upside down on the bottom of the aquarium. Too little air in the stomach will cause the shark to be negatively buoyant and sink to the bottom. The shark may be observed swimming laboriously, body angled, with the head up and tail down. This type of swimming behavior may be followed by periods where the shark "rests" on the bottom. If fatigue is extreme, the shark may remain on the bottom for long periods of time, dorsal, lateral, or even ventral side up.

Swimming behavior can be influenced by poor water quality. For example, sand tiger sharks exposed to toxic volatile organic compounds exhibited erratic swimming behavior, swimming slowly and resting intermittently on the bottom of the exhibit (Rasmussen et al., 2000).

Evasion or avoidance

Another indicator of stress in captive elasmobranchs is their evasion or avoidance of specific areas or animals, whereby the subject actively swims away from the stimulus in question. In the case of normally conspicuous benthic species, they may suddenly go into hiding indicating the presence of a disagreeable stimulus.

Over time, many animals fall into repetitive swimming patterns. If a change in the environment occurs, this may cause the animal to change its pattern or avoid a particular area within the exhibit. This behavior is usually caused by an array of factors that can be broken into two basic categories, biotic and abiotic. Examples of biotic factors include changes to social structure and species composition, changes to husbandry practices, and the presence of sick or injured animals. Examples of abiotic factors include changes to exhibit décor, changes to water flow, mechanical vibrations, etc. Animals affected by any one of these stimuli may actively avoid the source of stress. Careful observation of evasive behavior will provide clues as to the problem and how it may be remedied.

Evasive behavior may be observed during feeding sessions, whereby aggressive species are the cause of a stressful environment and other animals avoid the feeding station or are inhibited from taking food. Some animals may avoid a feeding station if activities outside the exhibit produce a lot of visual or auditory stimuli. Improper feeding techniques (e.g., the method of presenting food items) can be a source of stress for elasmobranchs and cause them to avoid the feeding station. For example, attempting to feed a smalltooth sawfish (*Pristis pectinata*) with "snake tongs" can create a stressful situation as the animal cannot readily access the food. The animal may therefore subsequently avoid the feeding station and displace other animals in their quest for more readily available food.

During mating season, females of some species of elasmobranch may be observed with bite wounds and lesions on their pectoral fins, on their body adjacent to the pectoral fins, or near the gill slits. Females experiencing these injuries have been known to avoid males and/or areas frequented by males, and may even avoid feeding stations, becoming temporarily anorexic.

Anorexia

A decrease in food intake, total loss of appetite, and other changes to normal feeding behavior

may be an indicator of stress in some shark species. Feeding behavior can be characterized in a number of different ways including pre-feeding, feeding technique, post-feeding, amount and type of food consumed, and time required to consume a normal ration. Behavior during feeding sessions should be well understood and documented for each specimen so that meaningful comparisons can be made if and when changes occur.

Fish in controlled environments become conditioned to normal operating regimes within and around their exhibit. Animals will frequently alter their swimming pattern and speed just prior to and/or during feeding sessions. If an elasmobranch is less excited than usual at the beginning of a feeding session, it may be suffering from some form of stress. Stressed animals may not take food as readily as usual or may require more time to come to a feeding station. Dropping food items, decreased or increased consumption rates, deviations in the way food is accepted, and refusal to consume normal daily ration are all possible indicators of stress.

If anorexia is not addressed quickly, changes in body form may occur (e.g., concave abdomen, head wider than axial girth, prominent pelvic girdle, etc.). In the case of Rajiformes the dorsal surface above the coelomic cavity, proximal to the spine, may become concave.

Some elasmobranchs exhibit seasonal changes to diet and daily ration, which could be misdiagnosed as stress. Food intake should be well documented so that such seasonal trends are not misunderstood.

Physiological responses

Different conditions or stimuli can cause physiological responses indicative of stress. These conditions include: capture, restraint, transport, confinement, exposure to heavy metals or volatile organic compounds, and changes to water quality parameters (e.g., temperature, salinity, etc.). Physiological responses to stress can be identified, and to some degree quantified, using blood pictures (i.e., hemograms and serum chemistry) (Stoskopf, 1993), as well as measurement of the corticoid 1 α-hydroxy-corticosterone (Idler et al., 1969; Kime, 1977; Manire et al., 1999; Manire et al., 2001).

Capture and transport

Cliff and Thurman (1984) studied the effects of stress, during capture and transport, on the blood of juvenile dusky sharks (*Carcharhinus obscurus*). Samples were taken within two minutes of capture on hook and line, 10 minutes post-hyperactivity, 30 minutes post-transport (i.e., 60 minutes post-capture), and three, six, and 24 hours post-capture. Potassium (principally an intracellular cation) rose significantly but returned to baseline levels within 24 hours. Total serum magnesium increased and remained high during the post-stress period, and total and ionized serum calcium levels rose and returned to baseline levels within 24 hours. Although variable, creatinine kinase concentrations generally remained high during post-stress periods. Blood lactate, blood glucose, and serum osmolality were elevated, while pH declined.

In addition to changes in blood chemistry, stress associated with capture and restraint may elicit a complex group of physiological responses involving circulatory, respiratory, endocrine, and muscular systems. Examples include hypoxia, respiratory and/or metabolic acidosis, cellular damage, etc. (Manire et al., 2001). In extreme cases mortality can result.

Heavy metals

Torres et al. (1986) examined blood chemistry in the smallspotted catshark (*Scyliorhinus canicula*) during both confinement stress and exposure to zinc. Significant decreases in erythrocyte counts (RBCC), hematocrit (Hct), hemoglobin (Hb), leucocrit (Lt), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentrations (MCHC) were observed in "stressed" sharks. Mean corpuscular volume (MCV) did not change and a significant increase in glucose was observed. Animals exposed to 80 mg l^{-1} zinc for 24 hours exhibited significant decreases in Hb, MCH, MCHC, and plasma glucose, and elevated Lt and RBCC, suggesting that exposure to heavy metals can influence blood chemistry.

An additional study examined the affects of copper exposure on smallspotted catsharks. Decreased RBCC and Hct was observed at the lowest concentration (i.e., 2 mg l^{-1} copper II). At higher concentrations (i.e., 4 mg l^{-1}, 6 mg l^{-1}, 8 mg l^{-1}, and in particular, the near-lethal concentration of 16 mg l^{-1}) a general reduction of all blood parameters

was observed (Tort et al., 1986). Liver composition (i.e., protein, glycogen, and lipid levels) was not affected by copper exposure over the duration of the experiment.

Volatile organic compounds

Volatile organic compounds can infiltrate water and cause clinical and physiological signs of stress, and in some extreme cases even death. Rasmussen et al. (2000) recently examined a situation where two moribund sand tiger sharks exhibited stressed swimming behavior. Liver samples taken from stressed bony fish (i.e., swimming frantically and jumping from the water surface) from within the same exhibit revealed the presence of a number of volatile organic compounds. Water samples taken shortly after the observed stressed behavior revealed the presence of many volatile organic compounds (e.g., acetone, tetrahydrofuran, 2-butanone, 1,1,1-trichloroethane, methyl isobutyl ketone, toluene, and 1,2,3-trichloro-propane). All other tested water parameters were within normal limits. It is believed that the volatile organic compounds damaged the gills of the sand tiger sharks and ultimately resulted in their death. The most likely source of these toxic compounds was fumes produced during the application of a waterproofing compound on the walls of a nearby exhibit (Rasmussen et al., 2000).

Water quality

Temperature (Spotte, 1992) and salinity fluctuations (Claiborne, 1998) should be minimized as they can disrupt acid-base homeostasis and osmotic pressure in elasmobranchs. Maintenance of internal pH (acid-base homeostasis) occurs through two processes: internally between blood and tissue, and externally by transference between the animal and its surrounding environment. Nursehound (*Scyliorhinus stellaris*) subjected to a sudden water temperature increase of 10°C displayed a rapid decline in pH, a rise in physiologic carbon dioxide (CO_2), and an elevated concentration of blood bicarbonates (HCO_3^-). Bicarbonate was simultaneously released into the surrounding seawater (Spotte, 1992). Physiological reactions were inversed when nursehound were subjected to a water temperature decrease of 10°C.

Salinity fluctuations can potentially cause stress in elasmobranchs. Plasma sodium chloride (NaCl) concentrations are normally lower than that of seawater in chondrichthyan fishes. However, the presence of urea and trimethylamine oxide (TMAO) means that elasmobranch plasma is hyperosmotic to the environment. Urea aids the osmotic challenge that sharks would otherwise face in the marine environment, while TMAO counteracts the potentially toxic effects of high blood urea (Karnaky, 1998).

Odor of urea

Some elasmobranchs produce a strong odor, reminiscent of urea, when subjected to stress, whether they are in or out of the water. Evans and Kormanik (1985) found that stress, associated with handling and anesthesia, was followed by a significant and transient increase in the efflux of urea across the branchial epithelium of spiny dogfish (*Squalus acanthias*). In small aquariums the urea odor may be more easily identified, due to lack of dilution. Examples of this phenomenon have been observed in small exhibits containing yellow (*Urobatis jamaicensis*), southern (*Dasyatis americana*), and Atlantic (*Dasyatis sabina*) stingrays. During handling, the urea odor may be produced rapidly as has been observed in lemon (*Negaprion brevirostris*) and bull (*Carcharhinus leucas*) sharks. A sandbar shark (*Carcharhinus plumbeus*), recently bitten by another shark, was observed to reek of urea and was assumed to be stressed, as other stress indicators were observed (i.e., hypo-coloration and anorexia). The urea odor will usually dissipate once the causative stress stimulus has been removed; this may be rapid, or may take several days.

Corticosteroids

Early studies into elasmobranch blood worked on assays to identify and measure the principal corticoid 1α-hydroxycorticosterone (1α-OH-B) (Kime, 1977; Idler et al., 1969). Idler et al. (1969) studied the corticoid 1α-OH-B in 15 species of elasmobranch because initial experiments indicated that cortisol and/or corticosterone were the principal plasmatic corticosteroids. More recent studies with bonnethead sharks (*Sphyrna tiburo*) found no change in corticosterone concentrations during acute or chronic stress (Manire et al., 1999).

Manire et al. (1999) examined the possible role of 1α-OH-B in reproduction of bonnethead sharks and the Atlantic stingray. A significant difference

in corticosterone concentrations was observed between male and female bonnethead sharks, but no difference was observed between immature and mature sharks. Additionally, significant differences in corticosterone concentrations were observed during various reproductive stages in mature males and females of both bonnethead sharks and Atlantic stingrays.

Recent attempts at producing 1α-OH-B in elasmobranchs have been unsuccessful and assays for this steroid have not been developed. This is an area that merits further investigation (Manire, 2001).

PREDISPOSING FACTORS

Successful husbandry and increased survivorship of animals in aquariums must be built on their environmental and physiological requirements (Murru, 1990). Stress factors that affect these requirements may be divided into two basic categories, abiotic and biotic. Abiotic stressors are characterized by the absence of life or non-biological factors independent of living organisms and biotic stressors pertains to life or ecological factors due to the interactions of living organisms (Wallace et al., 1981). Abiotic factors include spatial constraints, transport and handling, water quality, lighting, electromagnetic fields, and vibrations. Biotic factors include species compatibility, sexual aggression, interactions with divers, nutrition, and pathogens.

Abiotic factors

Spatial constraints

Spatial constraints in controlled environments have the potential to be stressful, particularly for pelagic elasmobranchs. The size and shape of an exhibit has a direct impact on the behavior of animals therein. If an exhibit is too small it has the potential to limit swim patterns, restrict courtship behavior, and increase aggression between and within species. Within an elasmobranch exhibit, corners having an angle of $\leq 90°$ are considered dead space to a swimming shark, making navigation difficult, consuming valuable energy reserves, and creating unnecessary distress (Murru, 1990). Similarly, excessive currents within an exhibit may provoke overexertion, elevating metabolic rates and resulting in anaerobic respiration. Prolonged periods of anaerobic respiration will ultimately become stressful for an elasmobranch.

Transport and handling

Many elasmobranchs have succumbed to stress induced during transportation. Careful handling on capture, proper pre-transport staging (Murru, 1990), a good transport regime, and a swift acclimatization period with minimum stress (Smith, 1992) are all important components of a successful transport. Likewise, manipulation of elasmobranchs during physical examinations should be swift and impose the least possible stress to the animal under scrutiny.

Water quality

The most important environmental stressor appears to be exposure to poor water chemistry, or sudden changes thereof (Spotte, 1992). Poorly designed life support systems (LSSs) may not adequately remove particulates and toxic metabolic byproducts from the water, or achieve suitable gas balance (in particular oxygen concentrations), resulting in physiological stress to the animals within an exhibit.

Sudden changes to salinity, temperature, pH, oxygen concentrations, and environmental hypercapnia (increased CO_2) will all affect acid-base homeostasis (Eckert and Randall, 1983; Spotte ,1992; Claiborne, 1998), as well as causing other types of stress responses in elasmobranchs. Nitrogenous compounds (ammonia, nitrite, and nitrate) are toxic to elasmobranchs (Spotte, 1979). A buildup of nitrogenous wastes can result in signs of a neurological challenge (Stoskopf, 1993).

Lighting

Lighting levels may present a potential stress to elasmobranchs in aquariums. Light intensity, light quality, and photoperiod influence the ability of a fish to make vitamins, navigate throughout its surroundings, and reproduce (Moe, 1992). Although there is no scientific study to support this claim, it is possible that inappropriate photoperiods, and/or a lack of crepuscular periods of low illumination, may cause some degree of stress in elasmobranchs. The sudden lighting of an exhibit from complete darkness to high illumination, or vice versa, is certainly not recommended as elasmobranchs react suddenly and erratically to such changes. A “night”-light employed during nocturnal periods, to simulate the moon, will decrease predation of smaller fishes and sharks by larger sharks, reducing stress to the former.

Electromagnetic fields

The electrical fixtures within an aquarium building produce electromagnetic fields that may stress elasmobranchs and ultimately impact animal health. Exposure to excess electromagnetic fields has been hypothesized as a contributor to head and lateral line erosion (HLLE) and general poor health (Goertz, pers. com.). Spiny dogfish, and to a lesser extent, the dusky smooth-hound (*Mustelus canis*), have been observed swimming with their head out of the water when stressed. It has been hypothesized that low levels of electricity within the exhibit were responsible for this behavior.

Vibration and acoustics

The immediate environment surrounding an aquarium may be exposed to vibration and noise (high-frequency vibration) from LSS equipment and husbandry activities. These vibrations may be conducted into an exhibit and cause stress to the elasmobranchs therein. Swimming behavior consistent with stress has been observed in elasmobranchs during periods of underwater maintenance, while restarting LSSs, and during instances of sudden loud noises from outside an exhibit.

Biotic factors

Species compatibility and sexual aggression

Species compatibility is an important part of the successful husbandry of elasmobranchs. Inter- and intraspecific species selection, animal size, and population density must be considered when determining the composition of an exhibit's population. It is important to ensure that growth rate and maximum size of a species is well understood to avoid placing animals in a confined and stressful environment. Courtship, often involving behavior where an elasmobranch bites and holds another with its teeth, may create stress in restricted environments. Lacerations of the pectoral fins and gill covers, resulting from sexual aggression and copulation, should be monitored closely to ensure they are healing without complication (Uchida et al., 1990).

Interaction with divers

Diving activities within an exhibit have the potential to cause stress responses in elasmobranchs, mostly related to navigation and swimming behavior. Divers, and occasionally bubble streams and noises created by the divers, represent an obstacle for sharks to negotiate, sometimes eliciting "flight responses". As the shark attempts to evade the stimuli presented by divers it can swim into décor, other divers, walls, etc., and potentially damage itself or others (refer to Chapter 12 of this manual for more information about diving with elasmobranchs).

Nutrition

If elasmobranchs are over- or underweight it can cause physiological and behavioral stress. Underfed animals may be more aggressive and prey on cohabitants, making the environment stressful for smaller or less dominant animals.

Inappropriate food types, sizes, and feeding techniques can cause stress. It is therefore important to understand how each animal normally obtains its food and, where possible, to attempt to simulate this during feeding sessions. Having animals take food quickly can prevent aggressive animals from competing for the same food item. Food items that are too large may necessitate the animal to tear the food into smaller pieces, creating an opportunity for aggressive animals to compete for the same food item. In an extreme case, bull sharks have been observed "ramming" the stomach of sand tiger sharks, coercing them to spit out food fish and leave it available for the bull sharks to consume. Minimizing the work required to consume its daily ration, without excess competition with co-habitants, will alleviate potential stress. In elasmobranch exhibits containing different species, it may be necessary to set up several feeding stations where more than one person can feed the animals simultaneously. In this way, different groups of animals can be fed at specified locations, cutting down aggression and competition.

Goiter has been observed in a number of different elasmobranchs. Goiter is a physiological condition, usually related to nutrition, which may cause a compounding stress reaction in an elasmobranch. Goiter is described as a thyroid enlargement, due to hyperplasia and hypertrophy, caused by low aquatic iodine concentrations or goitrogenic agents that block the release of iodine from the thyroid gland (Crow et al., 2001). Goiter in elasmobranchs is usually characterized by a swelling of the posterior portion of the lower jaw.

Goiter can be seen as a round swelling within the buccal cavity and, if severe, externally on the ventral surface of the lower jaw. Profound goiter-induced changes to the jaw have been known to cause stress responses (i.e., changes to swimming patterns, changes to ventilation rate and depth, and anorexia).

Pathogens

Disease can cause stress responses in sharks and rays (e.g., changes to ventilation rate and depth, swimming behavior, skin coloration, and feeding behavior). An infestation of the gills by monogeneans may cause a change in ventilation rate and depth, mimicking similar responses to other adverse environmental conditions. Internal parasites such as coccidia (*Eimeria southwelli*) have been known to cause skin discoloration, emaciation, coelomic cavity distention, and ultimately death in cownose rays (*Rhinoptera bonasus*) (Stamper and Lewbart, 1998). Other chapters, detailing different disease-producing organisms (refer to Chapters 24, 25, and 26 of this manual), provide more information about clinical signs that may be observed. From this information it is possible to interpret signs of disease-induced stress and develop appropriate management strategies.

TECHNIQUE TO ALLEVIATE POTENTIAL STRESSORS

A proactive approach to animal management is the key to successfully maintaining elasmobranchs. This approach requires planning during exhibit and LSS design; considered species selection; and, a careful acquisition, transport, and acclimatization process. Once animals have been acclimatized to their new environment, detailed record-keeping and strong communication skills are essential tools for keeping colleagues apprised of animal status, developing husbandry regimes, and thus increasing specimen survivorship. Multiple observations of animals throughout the day allow an understanding of baseline parameters, facilitating comparison to unusual behaviors or changes in physical appearance. Table 19.1 summarizes a number of behavioral, biochemical, and physiological changes in elasmobranchs that may be attributable to an exposure to stressors.

If an observed change to baseline parameters is attributed to stress, the next step is to determine causative stimuli. A careful assessment of any given situation is advised, as stress responses may be of a generic nature and “snap” judgments may result in ill-informed husbandry intervention. For example, parasitic infestations of the gills may elicit the same stress response as low dissolved oxygen concentrations. Regardless, observed stress responses should be investigated quickly. Often the determination of a stressor may require the observation of several different stress responses and other physical changes to an exhibit, piecing together clues somewhat like a detective investigating a crime scene. Once a stress stimulus has been positively identified, every effort should be made to modify or eliminate it. The course of action taken will be dictated by the source of stress. Chemico-therapeutic treatment may be required if an animal has been injured and/or immunosuppressed.

REFERENCES

Claiborne, J. B. 1998. Acid-base regulation. *In:* The Physiology of Fishes, p. 177-192. D. H. Evans (ed.). CRC Press, Boca Raton, Florida, USA.

Cliff, G. and G. D. Thurman. 1984. Pathological and physiological effects of stress during capture and transport in the juvenile dusky shark, *Carcharhinus obscurus*. Comparative Biochemistry and Physiology 78: 167-173.

Crow, G. L., W. H. Luer, and J. C. Harshbarger. 2001. Histological assessment of goiters in elasmobranch fishes. Journal of Aquatic Animal Health 13(1): 1-7.

Eckert, R. and D. Randall. 1983. Animal Physiology Mechanisms and Adaptations. 2nd Edition. W. H. Freeman and Company, New York, USA. 765 p.

Evans, D. H. and G. A. Kormanik. 1985. Urea efflux from the *Squalus acanthias* pup: The effects of stress. Journal of Experimental Biology 119: 375-379.

Idler, D. R., B. Truscott, and M. McMenemy. 1969. Production of 1 á-hydroxycorticosterone in vivo and in vitro by elasmobranchs. General and Comparative Endocrinology, Supplement 2: 325-330.

Karnaky, K. J. Jr. 1998. Osmotic and ionic regulation. *In:* The Physiology of Fishes, p. 157-172. D. H. Evans (ed.). CRC Press, Boca Raton, Florida, USA.

Kime, D. E. 1977. Measurement of 1 á-hydroxycorticosterone and other corticosteroids in elasmobranch plasma by radioimmunoassay. General and Comparative Endocrinology 33: 344-351.

Manire, C. A., R. Hueter, E. Hull, and R. Spieler. 2001. Serological changes associated with gill-net capture and restraint in three species of sharks. Transactions of the American Fisheries Society 130: 1038-1048.

Manire, C. A., L. E. L. Rasmussen, and T. Tricas. 1999. Elasmobranch corticosterone concentrations: Related to stress or sex or what? *In:* Proceedings of the 15th annual meeting of the American Elasmobranch Society conference, State College, Pennsylvania, USA, June 24-30, 1999, Abstract, p. 21-22.

Moe, M. A. Jr. 1992. The Marine Aquarium Handbook Beginner to Breeder. Green Turtle Publications, Plantation, Florida, USA. 521 p.

Murru, F. L. 1990. The care and maintenance of elasmobranchs in controlled environments. *In:*

Table 19.1. Behavioral, biochemical, and physiological changes observed in captive elasmobranchs that may indicate stress, showing possible stressors. I = parameter increases; D = parameter decreases; I+D = parameter may both increase or decrease; CO = specified condition observed; NC = no discernable change observed; UN = result unknown.

Observed change	Spatial constraints	Transport + handling	Temp. increase	Temp. decrease	Water quality changes	Heavy metals	Electro-magnetic field	Vibration	Courtship	Diver presence	Nutrition problems	Disease
Skin Coloration	CO	CO	CO	CO	CO	CO	CO	CO	CO	CO	CO	CO
Ventilation	CO	CO	CO	CO	CO	CO	CO	CO	CO	CO	CO	CO
Swimming Behavior	CO	CO	CO	CO	CO	CO	CO	CO	CO	CO	CO	CO
Evasion or Avoidance	CO	CO	CO	CO	CO	CO	CO	CO	CO	CO	CO	CO
Anorexia	CO	CO	CO	CO	CO	CO	CO	CO	CO	CO	CO	CO
Urea Odor	CO	CO	UN	UN	UN	UN	UN	UN	UN	UN	UN	UN
Potassium		I										
Magnesium		I										
Total and Ionized Serum Calcium		I										
Creatinine Kinase		I										
Blood Lactate		I										
Blood Glucose	I	I				D						
Serum Osmolality		I										
Acid - Base Homeostasis					CO							
pH		D	D	I								
Hypoxia		CO										
Hypercapnia				CO								
Respiratory and/or Metabolic acidosis												
Acidemia/Acidosis		CO	CO									
Alkalosis			I	D								
Cellular Damage		CO										
Erythrocyte Counts	D					I+D						
Hematocrit	D					I+D						
Hemoglobin	D					I+D						
Leucocrit	D					I+D						
Mean Corpuscular Hemoglobin	D					I+D						
Mean Corpuscular Hemoglobin Concentration	D					I+D						
Mean Corpuscular Volume	NC											

Elasmobranchs as Living Resources: Advances in Biology, Ecology, Systematics and the Status of Fisheries, p. 203-209. H. R. Pratt, S. H. Gruber, and T. Taniuchi (eds.). NOAA Technical Report, 90.

Pickering, A. D. 1981. Stress and Fish. Academic Press, New York, USA. 367 p.

Rasmussen, J. M., M. M. Garner, and K. R. Petrini. 2000. Presumptive volatile organic compound intoxication of sharks and teleost fish in a newly constructed aquarium: Why fish and paint fumes just don't mix. *In:* Proceedings AAZV and IAAAM Joint Conference, September 17-21, 2000, New Orleans, Louisiana, p. 367-368. New Orleans, Louisiana, USA. 577 p.

Smith, M. F. L. 1992. Capture and transportations of elasmobranchs with emphasis on the grey nurse shark Carcharias taurus. Australian Journal of Marine and Freshwater Research 43: 325-343.

Spotte, S. 1979. Fish and Invertebrate Culture. John Wiley and Sons Inc., New York, USA. 112 p.

Spotte, S. 1992. Captive Seawater Fishes. Science and Technology. John Wiley and Sons Inc., New York, USA. 527 p.

Stamper, A. M. and G. A. Lewbart, 1998. *Eimeria southwelli* infection associated with high mortality of cownose rays. Journal of Aquatic Animal Health 10: 264-270.

Stoskopf, M. K. 1993. Clinical pathology of sharks, skates, and rays. *In:* Fish Medicine, p. 754-775. M. K. Stoskopf (ed.). W. B. Saunders Company, Harcourt Brace Jovanovich, Inc. Philadelphia, Pennsylvania, USA.

Torres, P., L. Tort, J. Planas, and R. Flos. 1986. Effects of confinement stress and additional zinc treatment on some blood parameters in the dogfish *Scyliorhinus canicula*. Comparative Biochemistry and Physiology. Vol. 83C(1): 89-92.

Tort, L., P. Torres, and R. Flos. 1987. Effects on dogfish hematology and liver composition after acute copper exposure. Comparative Biochemistry and Physiology. Vol. 87C(2): 349-353.

Uchida, S., T. Minoru, and Y. Kamei. 1990. Reproduction of elasmobranchs in captivity. *In:* Elasmobranchs as Living Resources: Advances in Biology, Ecology, Systematics and the Status of Fisheries, p. 211-237. H. R. Pratt, S. H. Gruber, and T. Taniuchi (eds.). NOAA Technical Report, 90.

Wallace, R. A., J. L. King, G. P. Sanders. 1981. Biology: The Science of Life. Scott Foresman and Company, Glenview, Illinois, USA. 1074 p.

PERSONAL COMMUNICATIONS

Goertz, C. E. 2001. Wise Laboratory of Environmental and Genetic Toxicology, Portland, ME 04104-9300, USA.

The Elasmobranch Husbandry Manual: Captive Care of Sharks, Rays and their Relatives, pages 271-280.
© 2004 Ohio Biological Survey

Chapter 20

Physical Examination of Elasmobranchs

GARY VIOLETTA

SeaWorld Orlando
7007 SeaWorld Dr,
Orlando, FL 32821, USA.
E-mail: Gary.Violetta@seaworld.com

Abstract: Physical examinations are a powerful husbandry tool for maintaining sharks in a controlled environment. When combined with blood analyses and medical procedures, physical exams remove some of the guesswork, allowing a less ad hoc approach to shark husbandry. Physical examinations include the following basic steps: (1) staff scheduling and equipment preparation; (2) maneuvering the specimen to a confined area; (3) evaluating and restraining the animal; (4) the examination itself, either physical, medical, or both; and finally (5) releasing the specimen back into the exhibit. Safety for both staff members and animal(s) throughout the procedure is paramount.

Routine physical examinations are performed on sharks for a variety of husbandry reasons including specimen measurement, medical evaluation, blood analysis, and data collection for research projects. Measurements or morphometrics present us with growth rate information on different species and provide valuable insight into the growth of individual specimens. As an example, when compared to wild populations, the growth rate of captive sand tiger sharks (*Carcharias taurus*) could contribute to the problem of spinal curvature observed in some captive specimens. One theory suggests that the muscular and skeletal systems grow at different rates, resulting in inadequate skeletal support for large muscle masses. By monitoring and controlling dietary intake, the risk of spinal curvature may be reduced (Berzins et al., 1999, Berzins and Walsh, 2000). Evaluation of blood profiles may reveal organ dysfunction, microbial infections, or anemia. Regular physical examinations provide an opportunity to sample blood and diagnose medical conditions before they become critical. Research conducted on captive elasmobranchs has included, among others, studies of dietary composition, age and growth, bioenergetics, physiology, pathology, and behavior (please refer to Chapter 39 of this manual for a more comprehensive list of research projects conducted on captive elasmobranch populations).

To obtain an estimate of the number of facilities that perform routine physical examinations on their elasmobranchs, a survey was sent to 45 institutions. Of the queried institutions, 33 responded. Sharks and rays were displayed by 32 and 30 institutions, respectively. Routine physical examinations (defined as yearly, quarterly, or monthly, where measurements were taken and/or blood was extracted, etc.) were performed at nine (27.3%) of the facilities. Of the 24 institutions not performing routine physical examinations, five reported taking measurements when the animal was restrained for medical reasons or during a transport.

When designing an exhibit, a holding or medical pool, to perform routine physical examinations and medical procedures, should be considered. The proper shape and dimensions of the pool will obviously vary depending on the species and size of specimens displayed. However, the pool should be sufficient to allow sharks to swim without duress during long-term observation. Care must be taken to eliminate obstructions and unnavigable corners. The pool should be deep enough to allow the animals normal swimming

patterns, but shallow enough to permit aquarium staff to interact safely with the animals (please refer to Chapters 4 and 5 of this manual for more information about tank design).

The size of the shark will dictate the dimensions and type of restraint and transport equipment used. However, independent of animal size, the overall procedure is similar for all physical exams and includes the following basic steps:

1. Staff scheduling + equipment preparation.
2. Maneuvering specimen to a confined area.
3. Evaluating and restraining the animal.
4. The examination: (a) physical; (b) medical.
5. Releasing the animal to the exhibit.

Each of these steps will be discussed in greater detail below.

STAFF SCHEDULING + EQUIPMENT PREPARATION

Before physical examinations can begin, personnel scheduling and equipment checks must be completed. Depending on animal size and the scope of the examination, staff numbers can range from a single person to ten individuals. One person would normally be required to examine small sharks (e.g., juvenile whitespotted bamboosharks, *Chiloscyllium plagiosum*), while as many as 10 personnel may be required to examine a group of larger carcharhinid sharks. Other individuals from within the company may be required (e.g., Veterinary Services for blood and medical procedures, Water Quality Services for water analyses, etc.). At SeaWorld Orlando, Florida, USA, an emergency medical technician (EMT) from Health Services is required to be present in the event of staff injury. As a courtesy, the Operations and Education Departments are notified in advance so they can respond to questions from the guests during examinations.

Equipment preparation can be simple. Physicals on whitespotted bamboosharks only require a tape measure, an analytical balance, a 20 cm standard aquarium hand-net, and a small plastic container. Procedures involving larger sharks can be more complicated, and equipment requirements for larger specimens have been summarized in Table 20.1. Before starting any procedure, perform an equipment check, using a comprehensive list, to ensure that all required equipment is present and in proper working condition.

MANEUVERING A SPECIMEN TO A CONFINED AREA

Physical examinations usually imply confining the animal at one end of the exhibit tank or moving the animal from the exhibit into a holding pool. In either case, the intent is to confine the shark in a smaller, more manageable area. If possible, water depth should be at a level where the aquarium staff can work safely but the animal can swim normally. At SeaWorld Orlando, and SeaWorld San Antonio, Texas, USA, water depth in the holding pools has been maintained at ~90-100 cm. In cases where examinations are performed within an exhibit, lowering the water to an appropriate level should be seriously considered.

In large exhibits, a barrier net can be used to progressively reduce the swimming area and maneuver sharks into the holding pool. If water depth is going to be reduced, this procedure should be completed prior to setting the barrier net. If the bottom of the exhibit is flat, the net can easily be dragged through the water until the desired containment area is achieved. If the exhibit contains obstructions, such as artificial coral structures, the net must be maneuvered over the coral structures in a manner that will still contain the sharks, without damaging the coral. At SeaWorld Orlando, two methods have been developed. The first method requires several SCUBA divers on the “shark-less” side of the net. As the net is pulled through the water, the SCUBA divers manually maneuver the net around and over obstructions. The second method employs the use of a “back and forth” motion as the net is pulled through the water. The barrier net’s float line is positioned on a movable catwalk or bridge suspended above the water. When the sharks are in a position where they cannot swim to the other side of the net, the catwalk and net are moved forward quickly, causing the lead line to rise from the aquarium bottom and over obstructions. As sharks swim toward the barrier net, the catwalk is pushed backwards causing the lead line to sink. This procedure is repeated until the net is in its proper position and sharks are confined. SCUBA divers then enter the water to anchor the lead line with additional weight.

Nurse sharks (*Ginglymostoma cirratum*) have been known to push their way under a barrier net and escape. In order to save time and effort during physical examinations, nurse sharks are systematically moved to the holding pool during the weeks prior to examination day. In this way, the nurse sharks do not disrupt the capture of other

Table 20.1. Equipment and preparation required for the physical and medical examination of large sharks.

Equipment	Preparations
Shark stretcher	The stretcher should be of correct size. The shark should fit comfortably in the stretcher. The caudal fin and especially the head should fit within the confines of the stretcher's length. The stretcher's width should completely enclose the shark. The stretcher should be free of any tears, holes, or worn ropes resulting from previous examinations.
Scale battery	The battery for the digital scale should be properly charged and extra batteries should be available. Prior to the start of the examination, the scale should be tested and, if possible, zeroed (i.e., using the tare function) with the weight of the stretcher.
Blood equipment	The correct type and number of syringes, needles, and blood tubes should be available in the medical box.
Oxygenation equipment	Artificial ram ventilation equipment must be available, including a working oxygen regulator, full oxygen cylinder with air line and diffuser, clear vinyl tubing, and working bilge pump.
Marking equipment	If the shark is to be marked for identification, silver nitrate sticks, freshwater, and dry towels should be available. If PIT tags are to be used, the reader should be checked and extra batteries available.
Tag and weigh lines	Stretcher tag lines and weighing lines should be available if weighing is anticipated.
Catch net(s)	The catch net(s) should be in good repair and easy to deploy.
Holding pool gate	If it is necessary to isolate an animal from the main exhibit, the holding pool gate should be easy to access.
Maneuvering poles	There should be shark maneuvering poles for everyone in the water.
Protective gloves	There should be protective gloves for everyone in water.
SCUBA fin	A SCUBA fin may be necessary to hold the specimen's head down during medical procedures.
Equipment check list	An equipment check list can be used to ensure that all equipment is present and in proper working condition.

species. During examination day, it is common to have 10 or more nurse sharks already in the holding pool. An alternative method to prevent nurse sharks from escaping, is to position a second barrier net ~3 m behind the first barrier net. Thus, if a nurse shark escapes the first barrier net, SCUBA divers can enter the water and maneuver the shark to the correct side of the first barrier net.

Once sharks are restricted to a confined area, they can be maneuvered into the holding pool. In large exhibits this has been accomplished by using either two box-shaped nets or one large rectangular catch net. Box nets measure ~120 cm x 120 cm x 180 cm (deep) and are constructed of a 2.5 cm x 2.5 cm mesh netting. The net opening is attached to a square, 5 cm diameter, polyvinyl chloride (PVC) pipe frame. On opposite sides of the frame, two 15 m guide ropes are attached. An additional 20 m guide rope is attached to the bottom of the net (note: the length of guide ropes is dependent on the length, width, and depth of the exhibit). Individual staff members control each of the guide ropes. To control the shape and movement of the net, the bottom guide rope is lightly pulled against the two “frame” guide ropes, extending the bag of the net (Figure 20.1). Guided by staff members, two box nets are used in

concert until the target shark is netted. With sand tiger sharks and sawfishes (*Pristis* spp.) caution should be exercised as teeth can become entangled in the net. Once the box net is pulled into the holding pool, the gate can be closed and the shark released.

Figure 20.1. A box net used to capture sharks. The box net is constructed of a 5 cm diameter PVC pipe frame (**a**), two guide ropes on opposite sides of the frame (**b**), an additional guide rope attached to the bottom of net (not seen), and 2.5 cm x 2.5 cm mesh netting (**c**).

Another technique used to maneuver specimens into the holding pool employs a shallow rectangular catch net (~180 cm x 240 cm x 5 cm deep) constructed of 5 cm x 5 cm mesh netting attached to a 5 cm diameter PVC pipe frame. Four guide ropes (~6 m in length, depending on pool depth) are attached to each corner of the net. Individual staff members control each of the guide ropes. The net is lowered toward the bottom of the exhibit, with one side of the PVC frame in contact with the side of the pool. The target shark is maneuvered over the catch net, using two or more 6 m long x 2.5 cm diameter PVC poles, and the net is raised when the shark is in position. The side of the catch net furthest from the exhibit wall is raised more quickly than the side adjacent to the exhibit wall, confining the shark slightly. As the far side of the catch net breaks the surface of the water, the near side of the net, in contact with the wall, is secured to the floor by placing the net frame in a trough at the entrance of the holding pool. The far side of the net is raised further, until the entire net is perpendicular to the floor, the animal has been maneuvered into the holding pool, and the gate has been closed. (Figure 20.2).

Figure 20.2. An example of the technique required to maneuver a shark, within a box net, into a holding pool. The frame of the box net is placed into a trough on the pool bottom, at the mouth of the holding pool, while the opposite side of the frame is raised until the net is perpendicular to the pool bottom.

EVALUATING AND RESTRAINING THE ANIMAL

Once an animal is in the holding pool, it must be evaluated before a physical examination can begin. Ventilation rate, swimming patterns, and ability to negotiate the holding pool should all be evaluated. Abnormalities, or deviations, from baseline parameters can occur during the post-capture period. If abnormalities are observed, curatorial and medical staff need to consider the risks associated with a given procedure and decide whether it should be terminated and the specimen released back into the exhibit. If the specimen is reacting normally, physical examination can begin by restraining the animal in an appropriate apparatus, dependent on specimen size.

For neonate whitespotted bamboosharks (<25 cm total length (TL)) a small, 10 cm x 25 cm x 5 cm (deep) plastic container may be used. The sharks are easily removed from the aquarium and placed into the container using a hand net. The sharks are immediately ready for examination.

For medium-size sharks (< 100 cm TL), such as juvenile scalloped hammerheads (*Sphyrna lewini*), the procedure is similar. Sharks are removed from the containment area using a 75 cm diameter hoop net made of 0.6 cm x 0.6 cm knotless mesh netting. The sharks are placed in a 120 cm x 60 cm x 30 cm (deep) Styrofoam™ shipping box, half filled with water and lined with a white cotton bath towel to prevent abrasion.

Sharks are manually restrained so that physical examination can begin immediately. To reduce stress, another wet towel may be placed over the head of the shark during the examination.

For large sharks (>100 cm TL), a 1.8-2.7 m long x 1.5 m wide shark stretcher is used, depending on the shark's total length. Stretchers can be made from various types of waterproof canvas. Two removable aluminum or stainless steel poles are inserted along the edges of the stretcher's length, acting as handles. A guide rope attaches to a metal clip ~15 cm from each end of the aluminum or stainless steel poles. An additional 1.8 m rope is woven through grommets along the edge of the end of the stretcher, to purse the stretcher as required. Holes (~6 x 5 cm diameter) are located along the mid line of the stretcher to allow for drainage when an animal is lifted clear of the water. The shark should fit comfortably in the stretcher. When the aluminum or stainless steel poles are together and the anterior end of the stretcher has been pursed, the shark's head should be confined. When the caudal end of the stretcher is pursed, the entire caudal fin should be located within the stretcher, although, in the case of very large elasmobranchs (e.g., smalltooth sawfish, *Pristis pectinata*), the caudal fin may have to extend beyond the end of the stretcher.

SeaWorld (Orlando and San Antonio) have adopted various methods to restrain large sharks. The first technique requires two staff members to be in the water, each holding one of the two stretcher poles at a ~30° incline. The stretcher is then maneuvered through the holding pool until the shark swims head-first into the "mouth" of the stretcher. Poolside staff members guide the shark toward the stretcher using 2.5 cm diameter PVC poles. When the target shark is within the stretcher the two stretcher poles are quickly brought together and both purse lines pulled tight, cinching the ends of the stretcher shut (Figures 20.3a and 20.3b). Two or more poolside staff may then assist with final shark restraint. Once the stretcher poles are together and the purse lines taut, each line may be wrapped around the poles to secure them. The stretcher may then be carefully moved to the physical examination area, normally poolside. Table 20.2 summarizes anecdotal reports of stretcher restraint times for different shark species.

The second restraining method requires four staff members to be in the water (wet), and two more poolside (dry). Guide ropes are attached to the ends of each stretcher pole and the stretcher positioned flat on the pool bottom. The two dry staff, positioned poolside, are responsible for pulling the purse lines and the stretcher lines nearest the pool wall. Two of the wet staff, positioned opposite the dry staff, are responsible for pulling the other stretcher lines. The remaining two wet staff guide the shark toward the stretcher using white PVC poles (2.5 cm diameter x 120 cm long). Once the shark is over the stretcher, the dry staff simultaneously pull their stretcher ropes and tighten the purse ropes. At the same time, wet staff pull up their stretcher ropes and walk towards the side of the pool (Figures 20.4a and 20.4b). Once the stretcher poles are together and the purse lines taut, each line may be wrapped around the poles to secure them. The

Figure 20.3. An example of a technique used to restrain a shark in a stretcher (**20.3a**), whereby the two poles are brought together, the purse lines are pulled tight, cinching the ends shut, and the lines are wrapped around the poles (**20.3b**).

Table 20.2. Successful stretcher restraint times for different shark species. All observations by author except where indicated by pers. com.

Species name	Common name	Restraint time (minutes)	Source
Carcharias taurus	sand tiger shark	<30	Choromanski, pers. com.
		30	
		45	Henningsen, pers. com.
Carcharhinus acronotus	blacknose shark	5	Henningsen, pers. com.
Carcharhinus leucas	bull shark	20	
Carcharhinus limbatus (<120 cm TL)	blacktip shark	<5	
Carcharhinus melanopterus	blacktip reef shark	30	
Carcharhinus plumbeus	sandbar shark	15-20	Henningsen, pers. com.
		<30	
Chiloscyllium plagiosum	whitespotted bamboo shark	60+	
Eucrossorhinus dasypogon	Tasseled wobbegong	30+	
Ginglymostoma cirratum	nurse shark	120	Henningsen, pers. com.
		120+	
Negaprion brevirostris	lemon shark	30	
		60	Henningsen, pers. com.
Orectolobus japonicus	Japanese wobbegong	30+	
Pristis pectinata	smalltooth sawfish	1	
Sphyrna lewini (<92 cm TL)	scalloped hammerhead shark	10	
Sphyrna lewini (>92 cm TL)	scalloped hammerhead shark	0.5	
Sphyrna tiburo	bonnethead shark	5-10	Henningsen, pers. com.
		10	
Stegostoma fasciatum	zebra shark	40	Henningsen, pers. com.
		60+	
Triaenodon obesus	whitetip reef shark	120+	

shark is now restrained and ready for the examination.

The third restraining method requires the shark to be target trained. One week prior to the examination, a stretcher (without guide ropes) is placed in position on the bottom of the pool. Using feeding tongs, the target shark is enticed to a position over the stretcher and fed. This process is repeated each day, until the day of examination. On the day of examination, ropes are attached to the stretcher. Using the same feeding procedure, the shark is guided over the stretcher, the ropes are pulled taut, and the shark is restrained (as above). The shark must be restrained on the first or second attempt, or it will quickly learn to avoid the stretcher.

A final restraining method, used on delicate or highly-strung sharks (e.g., the blacktip shark, *Carcharhinus limbatus*), uses low doses of anesthetic to sedate the shark. The principal objective is to slow the animal down so it can be maneuvered readily into a stretcher without excessive harassment. Commonly used anesthetics include a combination of ketamine HCl (2.0-15.0 mg kg^{-1}) and xylazine HCl (5.8-6.0 mg kg^{-1}) administered intramuscularly via pole syringe, and aqueous MS-222 (80-100 mg l^{-1}) administered by inhalation or irrigation (please refer to Chapter 21 of this manual for more information about anesthetics).

THE EXAMINATION

Examinations may be physical, medical, or a combination of the two (Table 20.3).

Physical examination

Once the animal is restrained, a vinyl ram ventilation tube (2.5 cm diameter) is pushed through the tied stretcher end and positioned in front of the shark's mouth. The ram ventilation tube is connected to a battery-powered bilge pump, submerged in the holding pool. An oxygen line is connected to the intake of the bilge pump, allowing small amounts of oxygen to be mixed with the pumped water.

Once the shark relaxes, a 243 cm x 60 cm x 1.9 cm (thick) plywood board is positioned under the stretcher, so the submerged shark is resting on the plywood. The plywood reduces dorsal-ventral flexing, resulting in more accurate measurements.

Table 20.3. Basic parameters measured during physical examinations and potential samples taken during medical examinations.

Physical examinations:	
1. Morphometrics	
Total Length (TL)	Measured in cm from the anterior tip of the snout to the posterior tip of the caudal fin.
Fork Length (FL)	Measured in cm from the anterior tip of the snout to the posterior notch of the caudal fin.
Precaudal Length (PCL)	Measured in cm from the anterior tip of the snout to the precaudal pit.
Girth (G)	Measured in cm around the girth at the insertion of the pectoral fins.
Eye-to-eye (ETE)	The external distance between the eyes (e.g. *Sphyrna* spp.) measured in cm.
Weight (WT)	Measured in kg.
2. Blood samples	Refer to Chapter 23 of this manual for sampling techniques.
3. Macroscopic exam	Examination for any abnormalities such as lesions, abrasions, protrusions, etc.
Medical examinations:	Types of examinations can include eye exams, tissue biopsies (including gill biopsies), dermal scrapes, microbiological samples, radiology, ultrasound, endoscopy, and surgical procedures.
	A trained veterinarian performs the majority of medical procedures listed. Procedures such as CAT scans and MRIs may be performed at a local hospital when required.

The purse rope on the caudal end of the stretcher is untied and the stretcher opened. If required, the shark is straightened. Using a cloth measuring tape, the total length (TL), fork length (FL), and precaudal length (PCL) are taken. To ensure accurate measurements, the end of the measuring tape must be on the tip of the snout and the measuring tape should lay flat against the shark's body. In some cases, the girth (G), eye to eye (ETE), and other measurements can be taken.

For sharks >90 cm TL, a 10-12 ml blood sample is taken. Samples can be taken adjacent to the

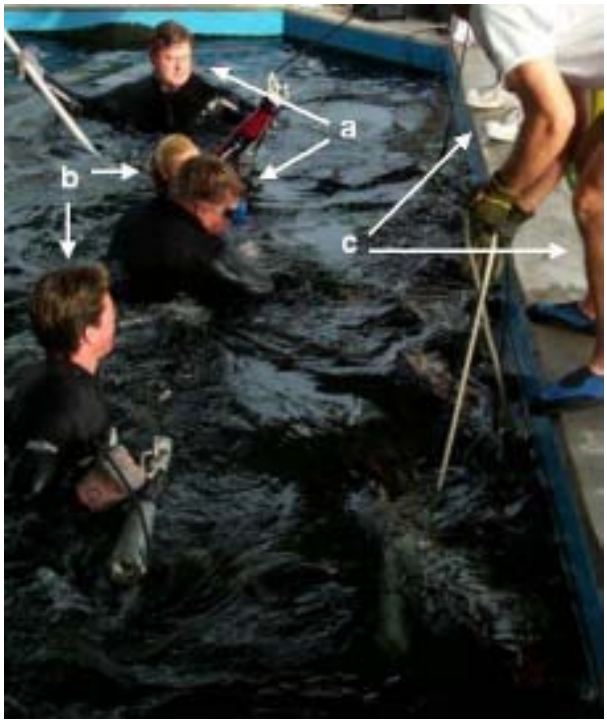


Figure 20.4. An example of a different technique used to restrain a shark in a stretcher. In this case two staff members (**a**) guide the shark into the stretcher using PVC poles. At the appropriate moment, two other staff members (**b**) close the stretcher by walking to the wall, while an additional two staff members poolside (**c**) pull the stretcher ropes and the purse lines (**20.4a**). When the stretcher poles are together, the purse lines are tightened and secured (**20.4b**).

insertion of either dorsal fin or from a site on the ventral midline. For dorsal samples, a 12 ml syringe with an 18-gauge butterfly needle is used. The needle is inserted on either side of the midline, under the dorsal fin, at an angle of ~30° and ~1.0 cm from the dorsal fin insertion. For ventral samples, the ventral surface of the shark is exposed by lifting the caudal fin out of the stretcher. Using an 18-gauge needle (3.8 cm long) and a 12 ml syringe, the needle is inserted at a 90° angle to the latitudinal and longitudinal planes of the ventral midline, between the anal fin and the caudal fin. The vein is located ventral to the spine. When using this method, it is important that there is little or no lateral flexion or rotation in the precaudal area. When taking samples from larger sand tiger sharks, an 8.9 cm long spinal needle replaces the 18-gauge needle.

After blood is drawn, it is immediately transferred to an EDTA tube (or a heparin tube in the case of sawfishes), for complete blood counts, and a clot tube with thrombin, for blood chemistries. EDTA is preferred as heparin causes white cell agglutination resulting in non-preserved cellular morphology (Perry, pers. com.). For institutions that cannot afford thrombin tubes, a less expensive no-additive clot tube may be used. Blood samples should be immediately taken to the laboratory for analysis (please refer to Chapter 23 of this manual for more information about blood sampling and blood analysis techniques).

While measuring or sampling, staff should also macroscopically examine the shark. A systematic approach is most effective, starting with head, nostrils, mouth, eyes, and gills, and proceeding posteriorly. If an abnormality is observed, options should be discussed and a course of action decided and carried out. If required, examinations represent a good time to apply identification tags (e.g., silver nitrate markings, PIT tags, etc.).

Once measurements, sampling, and other procedures are complete, the caudal end of the stretcher is closed and secured. Nylon ropes (2 x 2.1 m long), knotted both at the mid point and 20 cm either side of the mid point, are clipped to each end of both stretcher poles. The knotted ropes are then hooked to a digital scale suspended from the ceiling by two pulleys. Depending on the position of the shark within the stretcher, the rope is hooked to the scale between two of the knots, keeping the stretcher parallel to the surface when it is raised clear of the water (Figure 20.5). Excess water is allowed to drain. Once stabilized, the weight is recorded and the shark and stretcher are lowered back into the water.

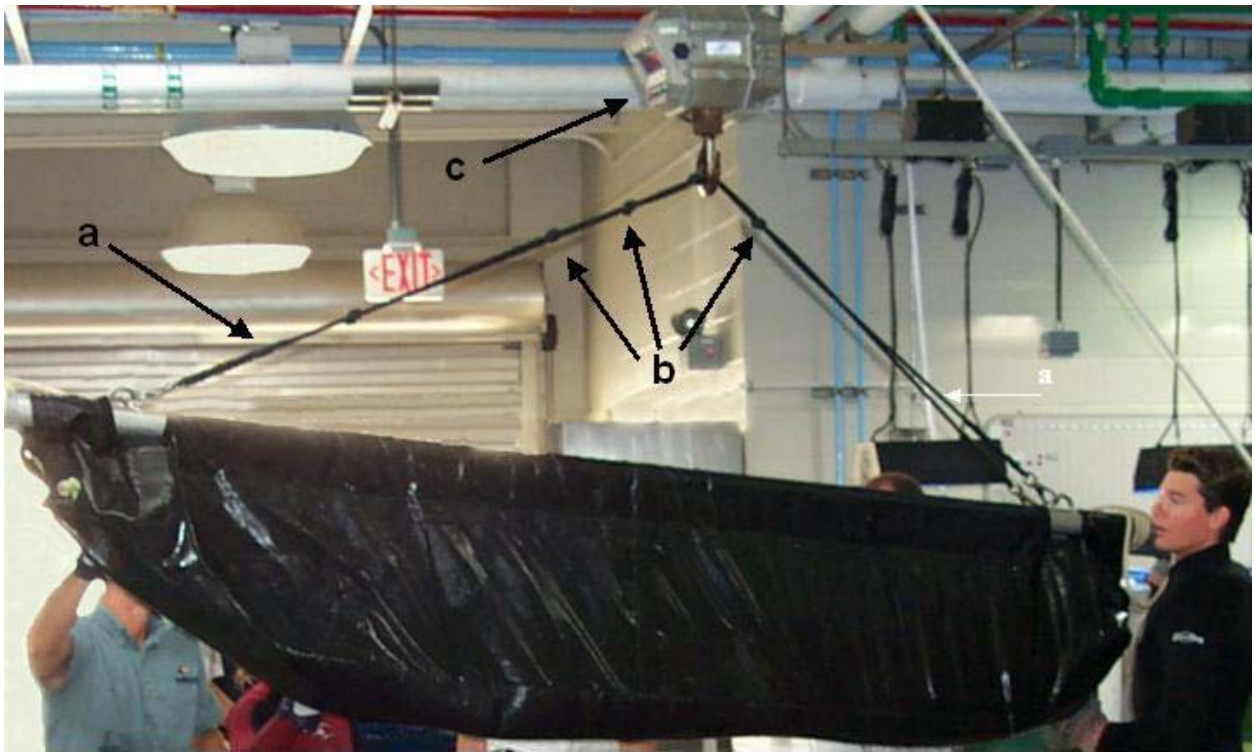


Figure 20.5. Weighing a shark using a stretcher suspended from the ceiling by a knotted rope (**a**), with knots approximately 20 cm apart (**b**), and a digital scale (**c**).

Medical examination

Restraining techniques for medical examinations are the same as for physical examinations. For tissue biopsies, dermal scrapes, microbiological cultures, certain surgical procedures, and ultrasonography, the affected area of the shark must be exposed for examination. If the affected area is located on or near the mouth, safety precautions are taken to prevent injury. Precautions may be as simple as protecting the clinician by placing a SCUBA fin next to the shark's mouth. Other medical procedures (e.g., radiology, endoscopy, invasive surgery, etc.) may require the shark to be anesthetized. MS-222 has been successfully used for this purpose (refer to Chapter 21 of this manual for more information about anesthetics). To anesthetize the shark, it is removed from the pool and placed in a vessel containing a known volume of water. A pre-dissolved 50 mg l^{-1} dose of MS-222 is then added to the container. Ventilation is monitored. If required, more MS-222 is added to the shark container, in 10 mg l^{-1} increments, until the animal is immobilized. For radiographs and certain surgical procedures, the shark is removed from the container and placed on a wet, padded surface. While out of the water, it is extremely important to moisten the skin and gills periodically, using a 60 ml syringe filled with water. If the shark must remain sedated, water containing the anesthetic (e.g., water from the anesthetic induction vessel) is used. When the procedure is almost complete, clean water (i.e., without the anesthetic) is applied. Upon completion of the procedure, the animal is again restrained in the stretcher and moved to another container filled with clean water or back into the holding pool. In order to help reverse anesthesia, a ram ventilation tube (as per above) should be placed in or near the mouth of the shark. If possible, blood should be drawn to measure lactic acid levels. The shark should be constantly monitored until normal ventilation is observed or the animal demonstrates a normal swimming pattern.

RELEASE INTO THE EXHIBIT

Following examinations, sharks may be returned to their exhibit. When more than one shark is being examined, it is important to release examined sharks to the area that is not confined by the barrier net. More specifically, examined sharks should not be mixed with animals awaiting capture and examination. For example, when a restrained shark is ready for release, the stretcher may be maneuvered between the poolside and the barrier net until it is on the unconfined side of the net. Both purse ropes on the outer stretcher pole are then released, allowing the animal to swim into the exhibit. The shark's swimming pattern should be monitored for any abnormalities.

SAFETY CONCERNS

During examinations, safety for both animals and personnel is of primary concern. The safety protocol begins with a staff meeting prior to the procedure. It is important to review the entire procedure to ensure that all personnel understand their individual responsibilities. In addition, everyone should review specific skills (e.g., knot tying techniques, etc.). Personnel should be reminded of critical safety concerns, including:

1. Pay attention to all instructions from the team leader.
2. Constantly monitor the location of all sharks and know where the target shark is at all times.
3. While in the water, do not corner, nor get trapped in a corner with, a shark.
4. While in the water, keep your feet on the pool bottom and keep your hands close to your body.
5. While in the water, always have a PVC pole in your hand and keep it between yourself and the sharks (unless you are responsible for operating a stretcher and are ready to catch a shark).
6. While in the water, always wear a full wet suit and gloves.
7. Poolside staff must wear protective clothing to prevent "shark burn" (i.e., abrasions from the shark's skin).

Safety for the animal is critical, key areas of which include correct restraining techniques, monitoring of ventilation rates, and appropriate times of restraint for different species. Past observations indicate that safe restraint times (i.e., time from the animal entering the stretcher to its eventual release) vary from species to species (Table 20.2).

CONCLUSION

Physical examinations are a powerful husbandry tool for maintaining sharks in a controlled environment. When combined with blood analyses and medical procedures, examinations remove some of guesswork, preventing an ad hoc approach to shark husbandry. It is the

responsibility of the curator, veterinarian, and/or director to decide on the frequency and profundity of routine physical examinations, and the associated commitment of financial support.

ACKNOWLEDGEMENTS

I would like to thank the entire Aquarium Department staff members of SeaWorld Orlando and SeaWorld San Antonio. It is through their hands-on hard work, dedication, and valuable suggestions that these physical examination procedures were developed. I would like to thank all those individuals who participated in the survey and contributed information toward this chapter. I would like to thank the SeaWorld Zoology Corporate staff for their editorial review and comments. This manuscript was written under SeaWorld Orlando's technical contribution number 2002-02-F.

REFERENCES

Berzins, I. K., K. Jeselson, M. Walsh, F. Murru, B. Chittick, S. Mumford, H. Martel-Bourbon, S.B. Snyder, M.J. Richard, H. Lane, and R. Lerner. 1999. Spinal problems in captive sand tiger sharks, *Carcharias taurus*. *In:* AZA Annual Conference Proceedings, September 24-28, 1999, Minneapolis, MN, p. 23. American Zoo and Aquarium Association, Silver Spring, Maryland, USA.

Berzins, I. K. and M. Walsh. 2000. Sand tiger shark, *Carcharias taurus*, scoliosis data and sample collection guidelines. *In:* Proceedings of the American Association of Zoo Veterinarians and International Association for Aquatic Animal Medicine Joint Conference, September 17-21, 2000, New Orleans, Louisiana, USA. p. 184-187.

PERSONAL COMMUNICATIONS

Choromanski, J. 1998. Ripley Aquariums, Inc., Orlando, FL 32819, USA.

Henningsen, A. 2001. National Aquarium in Baltimore, Baltimore, MD 21202, USA.

Perry, C. 2002. SeaWorld Orlando, Orlando, FL 32821, USA.

The Elasmobranch Husbandry Manual: Captive Care of Sharks, Rays and their Relatives, pages 281-295.
© 2004 Ohio Biological Survey

Chapter 21
Immobilization of Elasmobranchs

M. Andrew Stamper

The Living Seas
Walt Disney World Resorts
2020 North Avenue of the Stars
Lake Buena Vista, Fl 32830, USA.
E-mail: andy.stamper@disney.com

Abstract: Anesthesia is a procedure that may be required during the husbandry and clinical care of elasmobranchs. Elasmobranchs may be anesthetized using tonic immobility or a suitable immersion or injectable anesthetic. Combinations of anesthetic drugs can yield a smoother anesthesia, and reversal agents can ease the process of recovery or prematurely end anesthesia if required. Variability of reaction to an anesthetic depends on many factors including body temperature, liver function, kidney function, reaction with non-target tissues, drug binding sites, injection site, specimen size and weight, seasonal variation in specimen body composition, gill area to body weight ratio, specimen age, nutritional status, lipid content, stress, and disease status. Monitoring a specimen before, during, and after anesthesia is critical to success. In particular, ventilation rate, heart rate, and, if possible, blood gas and lactate values should be monitored and recorded. The pharmacology of anesthetics used in elasmobranchs is a developing area of study and merits a great deal more research.

There is much debate about the question of whether fish experience "pain" or not. Recent philosophical and physiological endeavors have determined that fish do not have the required neural structures (or alternative neural systems) for producing the "pain" experience (Rose, 2002). Yet fish display physiological stress responses to noxious stimuli. For this reason alone, appropriate anesthetic regimes should be given when handling fish in stressful situations or performing invasive sampling or surgical procedures. Much of the information for safe and effective anesthetic procedures in many aquatic species, including elasmobranchs, is still anecdotal. Furthermore, with so many species of elasmobranchs, each with its own physiological niche, potentially wide variations in environmental conditions, as well as changes in physiological status, the outcome of an anesthetic procedure can be difficult to manage. The relatively recent alliance of veterinary science and fish biology can greatly widen the current state of elasmobranch medicine, with the veterinary community providing a wider breadth of regulated anesthetics and clinical skills. This chapter highlights issues to consider when anesthetizing elasmobranchs and identifies areas to be investigated in the realm of aquatic animal anesthesia.

VARIABILITY OF DRUG INTERACTIONS

Variability of reaction to a drug depends on many factors:

1. The body temperature of most elasmobranchs is dependent on ambient temperatures (poikilothermic), but some species do have the ability to elicit an endothermic (internal heat generation) response (e.g., the porbeagle shark, *Lamna nasus*, the mako shark, *Isurus oxyrinchus*, and the white shark, *Carcharodon carcharias*) (Muñóz-Chápuli and Satchell, 1999). This endothermic response could theoretically affect enzyme activity and may affect biochemical reactions and drug interactions.

2. The liver metabolizes many drugs to either active or inactive forms. The weight and composition of the liver varies between elasmobranch species, but can account for up to 23% body weight of which as much as 80% may be fat (Holmgren and Nilsson, 1999). The elasmobranch liver contains a greater percentage of oils than other vertebrate species. The amount of oil in elasmobranchs will affect chemical reactions since the pharmacodynamics of drugs depends on their lipophilic ("fat-loving") and lipophobic ("fat-avoiding") solubility.

3. The kidneys of sharks are different to those of mammals, having a higher filtration rate and differing selectivity (Lacy and Reale, 1999), influencing the elimination rate of drugs. Drug elimination is dependant on blood flow. The renal-portal system is an anatomical adaptation that allows blood from the caudal half of the animal to drain directly into the kidneys. This arrangement may have direct consequences for drugs that are nephrotoxic or are eliminated directly by the kidneys. Although a concern in reptiles, recent studies indicate that this risk may not be as critical as at first thought (Beck et al., 1995) and may not pose a threat to elasmobranchs. Regardless, it would be prudent to err on the side of caution when using potentially nephrotoxic drugs.

4. In any species of animal, drugs can react with blood proteins or bind with non-target tissue, thus altering the amount of drug available and, therefore, the effect on target tissue.

5. Drug binding sites are the molecular locations on target tissue that bind the drug to produce the desired effect. Sharks are different biochemically from other vertebrates, and drugs developed for mammals may result in several possible outcomes in elasmobranchs: (a) they may bind to the same active binding site and produce the desired effect; (b) the binding site may be slightly different or there may be a lower number of binding sites, resulting in a weakened response; (c) there may be no binding sites at all, resulting in no effect; or (d) there may be a chemically similar binding site but one that triggers a different physiological function, resulting in a completely different response.

6. Injection site may result in a variable reaction to drugs. Not only is there the concern of getting the drug into the muscle or vasculature, but some species have regional heterothermy (Bernal and Graham, 2001; Totland et al., 1981) and thus a variable density of vasculature which may result in unpredictable drug uptake.

The protective nature of shark denticles and epidermis requires the use of heavy needles (16-18 gauge) to penetrate the tough integument. Additional problems may occur because the skin of the shark does not have a great degree of contractibility and muscle is at a positive resting potential, resulting in possible leakage of the drug from the injection site. The author recently designed a device to collect fluids leaking from injection sites and noted considerable drug loss during initial trials. Three relatively tranquil sand tiger sharks (*Carcharias taurus*) lost 3-16% of a drug (0.9% saline) when injected with 5.0 ml, at a depth of 25 mm, using an 18 gauge needle. These losses could account, at least in part, for observed variation in reactions to drugs within a species. To minimize drug leakage, it may be possible to angle the needle either anteriorly or posteriorly, depositing the drug away from the injection site to reduce leakage. Recently the author has used a Teleinject dart (Teleinject USA Inc., Aqua Dulce, California, USA) and left the dart in the animal until it has become sedated. This technique appears to reduce leakage of the drug.

Other factors that may influence an effective anesthetic protocol in elasmobranchs include: size and weight (body condition), seasonal variation (body composition), gill area to body weight ratio, age (sexual maturity and variation in body composition), nutritional status, lipid content, stress, and disease status. These factors should all be considered when attempting to determine safe and effective anesthetic drugs for elasmobranchs.

STAGES OF ANESTHESIA

The different stages of anesthesia have been summarized in Table 21.1. It should be noted that different animals do not exhibit anesthetic stages equally, nor do all drugs elicit all of the indicated stages in an equivalent manner.

MONITORING

Before a drug is administered, several parameters should be monitored for reference purposes. A

Table 21.1. The different stages of anesthesia. Please note that all animals will not necessarily exhibit stages equally, nor will drugs elicit all of the stages as described. Modified from Soma (1971) and Stoskopf (1993).

Stage	Plane	Description	Corresponding Behavioural response
Stage 0		Normal	Swimming actively, reactive to external stimuli, muscle tone and equilibrium normal.
Stage 1			Subjective in nature. Disorientation.
	Plane 1	Light sedation	Voluntary swimming continues; slight loss of reactivity to visual and tactile stimuli; respiratory rate, equilibrium, and muscle tone normal.
	Plane 2	Deep sedation	Voluntary swimming stopped; total loss of reactivity to visual and tactile stimuli; slight decrease in respiratory rate; equilibrium normal; muscle tone slightly decreased; still responds to positional changes.
Stage 2	Plane 1	Light narcosis	This stage is also known as the excitement phase. There is a loss of consciousness and subsequent excitement (uninhibited action, uncoordinated movements, struggling, exaggerated response to painful stimuli, and spinal reflexes). Efforts to right self, muscle tone decreased, and still weakly responds to positional changes. Respirations can be irregular.
	Plane 2	Deep narcosis	Ceases to respond to positional changes; decrease in respiratory rate to approximately normal; total loss of equilibrium; no efforts to right self; muscle tone decreased; some reactivity to strong tactile and vibration stimuli; suitable for external sampling (e.g., gill biopsy).
Stage 3			Four planes with increasing depression of respiration, circulation, protective reflexes, and muscle tone.
	Plane 1	Light anesthesia	Total loss of muscle tone; responds to deep pressure; further decrease in respiratory rate; suitable for minor surgical procedures.
	Plane 2-4	Surgical anesthesia	Respiratory rate very low; heart rate slow.
Stage 4		Medullary collapse	Represents complete respiratory arrest. Cardiac arrest will ensue unless anesthetic regime is not modified.

safe and quiet environment should be available to the animal since other sharks in the tank may disturb or prey on immobilized animals. Ventilation rates should be taken if the animal is actively pumping water through the gills and not ram-ventilating (i.e., using forward motion to force water through the gills). Caudal fin strokes can be measured to gauge the initial effects of the drug, as this activity is usually the first to be reduced when drugs take effect. After the animal becomes recumbent (i.e., lying down), respiration rates and righting reflex should be closely monitored. If respiration ceases, heart rate should be monitored using an ultrasound or Doppler unit placed over the region of the heart. Monitoring can be done with the animal in or out of the water.

If the animal is stable, respiratory and cardiac parameters should be monitored every 2-5 minutes and trends such as changes in rhythm or speed noted. Although animals may be ventilating well, a lowered heart rate and increased resistance to circulatory flow through the gill capillaries, as erythrocytes accumulate within the capillary bed and become swollen, can cause hypoxia (Tyler and Hawkins, 1981). A better way to monitor respiratory efficiency is to obtain periodic blood gas samples. These samples enable the worker to determine blood O_2, CO_2, and pH, as well as lactic acid levels. Blood gas analyses are usually based on arterial samples, but these are difficult to obtain from sharks. Venous samples are not true indicators of the

animal's current physiological status, but they do give useful information about trends. Handheld analyzers are expensive but offer the most rapid monitoring system. Blood gas units are frequently built to accommodate mammalian body temperatures, but many units allow the observer to calibrate for lower body temperatures (e.g., ambient water temperature). Lactic acid is often a good parameter to determine an animal's physiological state; upward trends are of concern, but further investigations need to be performed to determine what levels indicate that an animal is at risk.

VARIOUS TECHNIQUES OF IMMOBILIZATION

Physical methods

The following have been described as methods of anesthesia used for non-invasive procedures. The first two methods are not recommended due to possible subclinical pathophysiologic (clinically unnoticeable but physiologically damaging) effects.

Electronarcosis

Electronarcosis or galvanonarcosis is performed by the application of an uninterrupted direct electric current that induces immobility of the animal (Harthoorn, 1976).

Hypothermia

Submerging animals in cold water can induce immobility. This process has been done alone, or in combination with chemical anesthesia (Schoettger, 1967).

Tonic Immobility (TI)

Tonic immobility, also known as hypnosis, is immobility induced by turning an animal upside-down for a period of time. Both batoids and shark species such as leopard sharks (*Triakis semifasciata*), whitetip reef sharks (*Triaenodon obesus*), blacktip reef sharks (*Carcharhinus melanopterus*), Caribbean reef sharks (*Carcharhinus perezi*), swellsharks (*Cephaloscyllium ventriosum*), shovelnose guitarfish (*Rhinobatos lentiginosus*), clearnose skates (*Raja eglanteria*), cownose rays (*Rhinoptera bonasus*), and southern stingrays (*Dasyatis americana*) have been restrained using tonic immobility, though variation is great between species. For a list of potential variations refer to Henningsen (1994).

Chemical methods

Immersion anesthesia

Immersion or inhalation anesthesia has the advantage of being safe to deliver. The drugs can be modified through addition or dilution. However, a major disadvantage, especially with large sharks, is the large amount of drug needed to accomplish the task. Furthermore, the use of immersion drugs in large bodies of water is usually not practical or economically feasible.

Injectable anesthesia

Injectable anesthesia can have several advantages over immersion anesthesia. If performed carefully, injection anesthesia can allow animals to be captured in large exhibits without the expense of excessive employee time and capital. Delivery can be achieved by hand injection, pole syringe, Hawaiian sling, or remotely, through an underwater dart gun (Harvey et al., 1988). Several injectable drugs have been investigated in sharks, but use is currently experimental. Unfortunately, at the time of writing, no injectable anesthesia on batoids has been reported.

ROUTES USED FOR INJECTABLE ANESTHETICS

Intravenous (IV)

Intravenous injection is the fastest and most reliable method of anesthetic delivery, producing a rapid induction and often short duration of anesthesia. The disadvantage of IV application is that the animal has to be appropriately restrained in order to deliver the drug. One of the most suitable blood vessels for IV injection in sharks and rays is the vein that lies along the midline, just ventral to the vertebral column. Locating this vein can be done by placing the needle just posterior to the trailing edge of the first or second anal fins and holding it at an angle of 30-90^{o} relative to the length of the shark, directed anteriorly (refer to Figure 29.1 in Chapter 29). When the needle is inserted ~4 cm, for a 10 kg shark, it should penetrate the vein. This

technique is suitable for catheterization and drug administration (Stoskopf et al., 1984).

Catheterization refers to the introduction of a catheter into a vessel, allowing direct access to the vasculature and thus permitting medications to be given in a direct and consistent manner, and possibly over a long period of time, if the catheter is secured in place. This process can be accomplished through the use of a Tuohy needle.

In larger sharks, needles have the potential to become plugged with cartilage since they have to penetrate a cartilage wall protecting the vessel. In this case, a spinal needle (with a removable stylet protecting the needle aperture) can be used. Intravenous injections can be given in the lateral portal vessel, the dorsal lymph vessel, and even the heart itself by direct cardiac puncture (Tyler and Hawkins, 1981).

A study by Walker (1972) using indigo cyanine green found a circulation time of 1-2 minutes when the marker was injected into the caudal vein of the tail. Slower circulation has been further reflected in cases where intravenous drug onset times were correspondingly slow.

Intraperitoneal (IP)

Intraperitoneal (into the body cavity) injection is another avenue for drug administration. In this case, the sedative must pass through the serosal membrane that lines all the organs of the coelomic cavity, making anesthetic induction time erratic. Inserting the needle at an acute angle directed anteriorly to the pelvic girdle, on the right side of the specimen, minimizes the possibility of puncturing any internal organs and causing unnecessary damage to the specimen (refer to Figure 29.1 in Chapter 29).

Intramuscular (IM)

Intramuscular injection is possible to deliver by hand, to slow-swimming sharks, or by remote-injection devices. Regardless of the avenue of IM administration, injection time is minimal, reducing handling times. The bulk of muscle tissue in sharks has a poor blood supply, limiting the number of effective injection sites. The best region for IM administration is the dorsal saddle, an area of musculature surrounding the first dorsal fin, extending laterally to just above the lateral line, and longitudinally from the posterior gill slit to a point halfway between the first and second dorsal fins (Stoskopf et al., 1984) (refer to Figure 29.1 in Chapter 29).

Skeletal-muscular movement helps circulate blood and lymph (Gruber and Keyes, 1981). This movement has a direct impact on drugs that are delivered IM, since they may not be circulated to the appropriate tissues if the animal is sedentary. Consequently, anesthetic induction time may be erratic or delayed and the injection of large volumes of drugs may form a sterile abscess in the musculature (Tyler and Hawkins 1981).

ANESTHETIC AGENTS

The following drug information is a composite of documented anesthetic protocols in peer-reviewed venues. Table 21.2 provides additional anecdotal drug information for various species of elasmobranchs. The author does not take responsibility for doses or protocols presented in this formulary. Drug dose experiences will be added periodically to the web-based version of this manual and such contributions, which should conform to the format established within the manual, are encouraged.

Immersion anesthetics

Benzocaine

Most work with benzocaine has been performed on teleosts. Benzocaine is similar in action to MS-222 (see below), but is much less soluble in water unless first dissolved in acetone or ethanol. Advantages include its high potency, quick onset of effect, relatively high margin of safety, and its relatively low cost (Larid and Oswald, 1975; Tyler and Hawkins, 1981). As with MS-222, Tyler and Hawkins (1981) report some subsequent physiological changes, most likely due to hypoxia resulting from anesthetic-induced respiratory suppression, decreased cardiac rate, increased resistance to circulatory flow through gill capillaries, and a swelling of erythrocytes resulting in their accumulation within the gill capillaries.

Etomidate or metomidate

Etomidate and metomidate (Aquacalm®, Syndel International, Inc., Canada) provide a more rapid induction and recovery time than MS-222. In sandbar sharks (*Carcharhinus plumbeus*), 10 mg

Table 21.2. Elasmobranch anesthetic drug formulary showing anecdotal information for various species of elasmobranch.

Drug name	Species name	Common name	Body weight (kg)	Body length (cm)	Water temperature (°C)	Dose
Tricaine methanesulfonate	*Carcharhinus melanopterus*	blacktip reef shark	-	-	24.0-25.5	50-125 mg l^{-1} buffered
	Carcharhinus plumbeus	sandbar shark	-	-	24.0-25.5	50-125 mg l^{-1} buffered
	Cephaloscyllium ventriosum	swellshark	-	-	14.0	50-125 mg l^{-1} buffered
	Heterodontus francisci	horn shark	-	-	14.0	50-125 mg l^{-1} buffered
	Pteroplatytrygon violacea	pelagic stingray	50.0 kg	100 cm DW	20.0	80-100 mg l^{-1}
	Squatina californica	Pacific angelshark	-	-	10-14	80-100 mg l^{-1}
	Stegostoma fasciatum	zebra shark	-	-	24.0-25.5	50-125 mg l^{-1} buffered
	Torpedo californica	Pacific electric ray	0.5 kg	50 cm DW	10-14	80-100 mg l^{-1}
	Triaenodon obesus	whitetip reef shark	-	-	24.0-25.5	50-125 mg l^{-1} buffered
	Triakis semifasciata	leopard shark	-	-	14.0	50-125 mg l^{-1} buffered
Diazepam	*Carcharhinus plumbeus*	sandbar shark	-	-	-	1.2-1.6 mg kg^{-1}
	Carcharias taurus	sand tiger shark	-	-	-	0.1 mg kg^{-1}
Butorphanol	*Taeniura lymma*	bluespotted ribbontail ray	-	-	-	0.5 mg kg^{-1}
Detomidine + Ketamine	*Carcharhinus leucas*	bull shark	-	-	-	0.2 mg kg^{-1}
			85.7 kg	-	23.0	128 µg kg^{-1}

of either of these drugs provides stage 2 induction in approximately 2-4 minutes. Increasing to 20 mg reduces the induction time to less than a minute but anesthetic depth is much more difficult to control. Etomidate is considerably more potent than metomidate in freshwater teleosts, but no noticeable differences were observed in sandbar sharks. Recovery from these drugs is as rapid as the induction. Recovery from stage 2 plane 2 is approximately 3-5 minutes for metomidate. Recovery from deeper planes can be considerably prolonged (e.g., >1 hour) which might be due to decreased cardiac output (Stoskopf, 1986).

Halothane-oxygen-nitrous oxide

Anesthesia is achieved by using a medical vaporizer to mix the three gasses and subsequently introduce them into the water via an aeration bubbler. Dunn and Koester (1990) have reported an initial administration of 1.5% halothane, 100-200 ml

Please note that the author does not take responsibility for doses or protocols presented in this formulary.

Dose of combination drug	Route	Times used	Comments	Reference
-	Immersion	n = ~50	Time to handling: 5 minutes. Induction: 5-10 minutes. Maintenance: good if kept at 50-75 mg l^{-1} for 10-60 minutes (*Carcharhinus melanopterus*: 45 minutes). Recovery: good. Observations: with some ram ventilators, start with high dose (usually 50-100 mg l^{-1}) in a round transport tank until they go down, then switch to a manageable regime for physical examinations, work ups, etc. Make sure oxygenated water is running through the gills. 50% of blacktip sharks will stop breathing and are prone to capture myopathy, so method of capture critical. Animals suffering from capture myopathy may recover slowly and exhibit increased LDHs and CPKs.	Mylniczenko, pers. com.
-	Immersion	n = ~50	Time to handling: 5 minutes. Induction: 5-10 minutes. Maintenance: good if kept at 50-75 mg l^{-1} for 10-60 minutes (*Carcharhinus plumbeus*: 45 minutes). Recovery: good.	Mylniczenko, pers. com.
-	Immersion	n = ~50	Time to handling: 15 minutes. Induction: 15 minutes. Maintenance: good if kept at 50-75 mg l^{-1} for 10-60 minutes. Recovery: good, 15-20 minutes.	Mylniczenko, pers. com.
-	Immersion	n = ~50	Time to handling: 15 minutes. Induction: 15 minutes. Maintenance: good if kept at 50-75 mg l^{-1} for 10-60 minutes. Recovery: good, 15-20 minutes.	Mylniczenko, pers. com.
-	Immersion	n = 20	Time to handling: up to 10-15 minutes at 100 mg l^{-1} (most other species succumb quicker). Maintenance: good, preferable to maintain anesthesia for <5 minutes to avoid problems.	Ezcurra, pers. com.
-	Immersion	-		Ezcurra, pers. com.
-	Immersion	n = ~50	Time to handling: 5 minutes. Induction: 5-10 minutes. Maintenance: good if kept at 50-75 mg l^{-1} for 10-60 minutes (*Stegostoma fasciatum*: 1 hour). Recovery: good.	Mylniczenko, pers. com.
-	Immersion	-		Ezcurra, pers. com.
-	Immersion	n = ~50	Time to handling: 5 minutes. Induction: 5-10 minutes. Maintenance: good if kept at 50-75 mg l^{-1} for 10-60 minutes (*Triaenodon obesus*: 1 hour). Recovery: good.	Mylniczenko, pers. com.
-	Immersion	n = ~50	Time to handling: 15 minutes. Induction: 15 minutes. Maintenance: Good if kept at 50-75 mg l^{-1} for 10-60 minutes. Recovery: good, 15-20 minutes.	Mylniczenko, pers. com.
See comments	PO	-	Supplementation: Initial dose followed by MS-222 staged anesthesia. Observations: oxygenated water should be pumped over the gills regardless of species.	Mylniczenko, pers. com.
-	IM	n = 20	Time to handling: 20 minutes. Maintenance: good for 5 hours. Observation: each animal had a different reaction and therefore required dosage. Assess level of activity, following the initial dose, by manipulating the caudal and dorsal fin after 20 minutes of post-dosage swimming. If activity is low then basic husbandry procedures may be performed. If activity is still too high after 30 minutes post-dosage, supplement the induction dose with an additional 20% of the anesthetic.	McEwan, pers. com.
-	IM	n = 2	Observations: bradycardia and cessation of respiration. Reversed: Naloxone IV at 0.01 ml kg^{-1}.	Mylniczenko, pers. com.
-	IM	-		Mylniczenko, pers. com.
5 mg kg^{-1}	IM	n = 1	Time to handling: no affect. Induction: poor. Maintenance: poor. Recovery: no affect. Observations: injected while free-swimming with pole syringe. Injection believed to be complete. Reversal: Yohimbine given despite no anesthesia observed.	Walsh, pers. com.; Stamper, personal observation.

minute^{-1} nitrous oxide, and 200-300 ml minute^{-1} oxygen. Maintenance levels were reduced to 0.5-0.8% halothane, 100-200 ml minute^{-1} nitrous oxide, and 200-300 ml minute^{-1} oxygen. Reportedly, easy control over anesthetic depth, shorter recovery times, and a high survival rate are some of the advantages of this regime. A disadvantage is that the vapors will contaminate the room, so precautions must be taken to protect personnel.

Oxygen

Oxygen has a sedative effect on some species. Oxygenated water is flushed across the gills of the elasmobranch by bubbling 100% oxygen in front of a water current directed into the mouth of the animal. Elevated dissolved oxygen levels (concentration dependent on temperature and height above sea level) will usually have a sedative effect. Caution must be exercised as prolonged exposure to elevated oxygen can result

Table 21.2 (continued). Elasmobranch anesthetic drug formulary showing anecdotal information for various species

Drug name	Species name	Common name	Body weight (kg)	Body length (cm)	Water temperature (°C)	Dose
Medetomidine + Ketamine	*Carcharhinus acronotus*	blacknose shark	-	-	25.5	59.2-70.4 µg kg^{-1}
	Carcharhinus leucas	bull shark	85.7 kg	-	23.0	90 µg kg^{-1}
	Carcharhinus plumbeus	sandbar shark	-	-	24.5	87 µg kg^{-1}
	Carcharias taurus	sand tiger shark	-	-	24.0	70.0-80.0 µg kg^{-1}
			-	-	21.0-22.0	60.0-80.0 µg kg^{-1}
	Chiloscyllium plagiosum	whitespotted bambooshark	-	-	23.4	60 µg kg^{-1}
	Ginglymostoma cirratum	nurse shark	20.0 kg	-	-	75 µg kg^{-1}
			68.0 kg	-	-	90 µg kg^{-1}
			-	-	-	70-100 µg kg^{-1}
	Negaprion brevirostris	lemon shark	-	-	26.0	90 µg kg^{-1}
	Triaenodon obesus	whitetip reef shark	-	-	27.0	90 µg kg^{-1}

in toxicity through depressed ventilation and an associated rise in blood CO_2 concentrations. If left unchecked, acidosis will ensue resulting in potentially life-threatening acid-base imbalances (Spotte, 1992). Signs of oxygen narcosis include depressed respiratory effort, behavioral changes, loss of equilibrium, and eventually death if the animal is not carefully monitored and the regime regulated.

Quinaldine

Quinaldine (e.g., Quinaldine, Synergy, India) has been successfully used in the past (Gruber and Keyes, 1981), although specifics have not been given.

Tricaine Methane Sulfonate (MS-222)

MS-222 (Finquel®, Argent Laboratories, USA) is a water-soluble narcotic that is a derivative to p-aminobenzoic acid. Both sharks and batoids can be anesthetized using 75-95 mg l^{-1} of MS-222. MS-222 may be added slowly to evaluate effect. If greater doses are used, then the solution should be buffered with sodium bicarbonate, especially when inducing animals in open systems with a hand pump, as water containing high concentrations of MS-222 can become extremely acidic. Gilbert and Kritzler (1960) found that 1.0 g l^{-1} of MS-222 delivered via a hand sprayer could be used to anesthetize large sharks and rays. Gilbert and Wood (1957) describe a technique of first

of elasmobranch. Please note that the author does not take responsibility for doses or protocols presented in this formulary.

Dose of combination drug	Route	Times used	Comments	Reference
2.82 mg kg^{-1}	IM	-	Time to handling: 10-11 minutes. Induction: excellent. Maintenance: excellent. Recovery: excellent, within 5 minutes. Observations: injected while manually restrained. Reversal: full dose IV.	Stamper, personal observation.
4.5 mg kg^{-1}	IM	n = 1	Time to handling: 69 minutes. Induction: poor. Maintenance: good. Recovery: prolonged, 1 hour and 26 minutes. Observations: Injected with pole syringe. Specimen considered to be abnormal. Specimen became recumbent after handling; re-dosed with half supplemental dose. Reversal: 2x reversal dose (equivalent doses given IM and IV) at 54 minutes.	Walsh, pers. com.; Stamper, personal observation.
5.4 mg kg^{-1}	IM	-	Time to handling: 20 minutes. Induction: excellent. Maintenance: good. Recovery: good. Observations: injected while free-swimming with pole syringe. Reversal: full dose IV.	Author's experience
4 mg kg^{-1}	IM	n = 2	Time to handling: 20 minutes. Induction: excellent. Maintenance: excellent. Recovery: good, 20 minutes. Observations: injected while free-swimming with pole syringe. Reversal: full dose IV.	Author's experience
5.0-10.0 mg kg^{-1}	IM	-	Time to handling: 4-18 minutes. Induction: excellent. Maintenance: excellent. Recovery: poor to good, 12-20 minutes. Observations: injected while manually restrained (a single specimen was injected while free-swimming). Specimens had scoliosis and lordosis. One animal was euthanazed for medical reasons. Reversal: specimen 1 (anesthetized for 20 minutes) was given atipamezole IM and doxapram IV after 15 minutes; specimen 2 was given doxapram; specimen 3 was given atapamizole (50% IM and 50% IV) and swam away 18 minutes later with no noticeable side effects.	Stamper, personal observation.
3 mg kg^{-1}	IM	-	Time to handling: 20 minutes. Induction: fair. Maintenance: poor. Recovery: good. Observations: injected while manually restrained. Reversal: full dose IV.	Author's experience
7.5 mg kg^{-1}	IM	n = 1	Time to handling: 10 minutes. Induction: good. Maintenance: good, surgical, but some movement. Recovery: good. Observations: injected while manually restrained. Supplemented with MS-222	Mulican, pers. com.
9 mg kg^{-1}		n = 1	Time to handling: 50 minutes. Induction: fair, still active. Maintenance: fair, still moving. Recovery: good, ~1 hour. Observations: injected with pole syringe.	Walsh, pers. com.; Stamper, personal observation.
5-7 mg kg^{-1}	IM	-	Observations: poor to mild sedation. Medetomidine often repeated at 5 mg kg^{-1} and ketamine at 30 µg kg^{-1}.	Mylniczenko, pers. mom.
4.5 mg kg^{-1}	IM	n = 2	Time to handling: 30 minutes. Induction: poor. Maintenance: undetermined. Recovery: prolonged, >24 hours. Observations: injected while manually restrained. Both specimens "blanched" after drug administration. Leakage noted from injection site (amount undetermined). Specimen became recumbent after handling. Reversal: full dose IV.	Walsh, pers. com.; Stamper, personal observation.
4.5 mg kg^{-1}	IM	n = 1	Time to handling: 30 minutes. Induction: fair to good. Maintenance: fair to good. Recovery: good, 20 minutes. Observations: injected while manually restrained. Specimen had scoliosis and infection; considered to be abnormal. Specimen stopped gilling unless touched. Reversal: 2x reversal dose (equivalent doses given IM and IV).	Walsh, pers. com.; Stamper, personal observation.

bringing large sharks up to the surface of the water with a hook and line and then applying a high concentration of 1.0 g l^{-1} MS-222 using a hand sprayer. Affects were noted within 10 seconds and the animals were anesthetized within a minute. It is recommended that the head of the patient remain out of water and the MS-222 should be buffered when applied directly to the gills. A direct linear relationship exists between the concentration of MS-222 and the time required to achieve muscular relaxation (Dunn and Koester, 1990). Dunn and Koester (1990) found that a large number of elasmobranch species can be anesthetized for surgery (i.e., stage 3) using 75-95 mg l^{-1} MS-222, but that species-specific responses were common. Many sharks have been anesthetized using a low dose of 50 mg l^{-1} MS-222 as a “pre-anesthetic” dose, followed by doses of up to 85 mg l^{-1} MS-222 (Davis, pers. com.). This “pre-anesthetic” dose appears to reduce the excitement phase and lower the overall maintenance level of MS-222.

MS-222 excretion in the spiny dogfish (*Squalus acanthias*) was primarily through the gills and excretion rate was a function of cardiac output (Maren, et al., 1968). Elimination of MS-222 into the water can result in a positive feedback of increasing anesthetic concentration, if the heart slows, resulting in a possible overdose if animals are not closely monitored.

Injectable anesthetics

Alfaxalone-alfadolone

Alfaxalone-alfadolone (Saffan®, Pitman-Moore, Inc., USA.) is a chemical that can be administered intramuscularly via dart gun. Alfaxalone-alfadolone has been administered to the spiny dogfish at 1.5 ml kg^{-1} (stage 3 anesthesia; n=2), the brown ray (*Raja miraletus*) at 0.2-0.3 ml kg^{-1} (stage 2 anesthesia; n=2), the skate (*Dipturus batis*) at 0.2 ml kg^{-1} (stage 2 anesthesia; n=1), the black tip shark (*Carcharhinus limbatus*) at 0.4 ml kg^{-1} (stage 2 anesthesia; n=1), and the spotted eagle ray (*Aetobatus narinari*) at 0.3 ml kg^{-1} (stage 1 anesthesia; n=1) (Harvey et al., 1988), demonstrating the great variability of this drug between species.

Azaperone

Azaperone (Stresnil, Pitman-Moore, Inc., USA) is a butyrophenone tranquilizer that reduces response to the environment without motor impairment or sedation. Preliminary studies in spiny dogfish showed the most efficacious application of azaperone is directly over the gills rather than by injection. No effect was noted when animals were injected with the drug intramuscularly; however, when 4 mg kg^{-1} of the drug were deposited on the gill filaments, and the animal held out of water for several seconds, an effect was observed (Latas, 1987). Following dosing with the drug or placebo, both exposed and control animals were left undisturbed for 4 hours. Thereafter, both groups were caught for blood sampling. Drugged animals showed no flight response when compared to control animals. Blood glucose levels were not depressed in animals exposed to azaperone and they fed the next day, compared to several days of anorexia in control animals. Drugged animals were capable of negotiating tank walls and returned to normal behavior within 24 hours. The advantages of using this drug include uninterrupted swimming patterns, normal gill ventilation, and normal cardiovascular function. Azaperone may be useful for animals that are prone to panic, aggression, and self-induced trauma (Latas, 1987).

Carfentanil citrate

Carfentanil citrate (Wildnil®, Wildlife Pharmaceuticals, Inc., Canada) is a potent narcotic analogue of fentanyl, an agent commonly used in veterinary medicine. Carfentanil citrate failed to achieve any effect when given at 0.25 mg kg^{-1} to a nurse shark (*Ginglymostoma cirratum*) and a lemon shark (*Negaprion brevirostris*). Even when administered at massive doses no effect was observed (Stoskopf, 1986; Stoskopf, 1993).

Detomidine hydrochloride

Detomidine hydrochloride (Dormosadan®, Pfizer, Inc., USA) is an alpha-2 adrenergic and is of the same family of drugs as xylazine, although more potent. Detomidine can be used with ketamine (refer to injectable anesthetic combinations which follow), and is reversed with yohimbine and/or atipamezole.

Ethanol

Larger sharks have been injected intraperitonealy with 47.5% ethanol (Sudak, 1966). For animals weighing up to 113 kg, 1.1 ml kg^{-1} were used, whereas larger animals received 0.55 ml kg^{-1}. Animals were visually unaffected for 50 minutes post-injection, but could be in dorsal recumbency for up to an hour (due to a lack of control animals, it is not clear whether this was a state of tonic immobility). Animals were reported to show effects of the alcohol after 3-4 hours, but were fine after 24 hours. The types of effects were not stated. Sudak's (1966) study indicated that a dusky shark (*Carcharhinus obscurus*) died during anesthesia. Having lacerations to its snout, the shark may have impacted an obstruction due to its decreased ability to maneuver.

Ketamine hydrochloride

Ketamine hydrochloride (Ketaset®, Fort Dodge Animal Health, USA) is an analgesic and cataleptic cyclohexamine. Ketamine provides good peripheral analgesia (pain relief) in mammals through suppression of dorsal horn cell activity in the spinal cord, but provides little visceral analgesia. In addition, seizure-like muscle spasms due to spinal reflex firing are occasionally noted (Stoskopf, 1993). [Refer to injectable anesthetic combinations which follow.]

Medetomidine

Medetomidine (Dormitor®, Pfizer Inc., USA) is an alpha-2 adrenergic of the same family of drugs

as xylazine and detomidine, but much more potent. Medetomidine has been used in combination with ketamine in several shark species (refer to injectable anesthetic combinations which follow).

Propofol

Propofol (Diprivan®, AstraZeneca Pharmaceuticals, USA) is a sedative-hypnotic that is a relatively new drug in exotic animal medicine. The advantage of using propofol is quick induction time and rapid metabolism, achieving surgical plane relatively quickly. Propofol is easily titrated (i.e., small incremental doses using a drip system), with non-cumulative effects, and recovery is swift once drug supply has been discontinued. The disadvantages of propofol is that it causes respiratory depression, it must be given intravenously, and it is expensive with a limited shelf life. Mitchell et al. (2001) gave 2.5 mg kg^{-1} propofol to whitespotted bamboo sharks (*Chiloscyllium plagiosum*) over 30 seconds and the sharks achieved a surgical plane of anesthesia after 5 minutes. Righting response returned within 60 minutes in four of the sharks, and 75 minutes in the other two. No changes in respiration or cardiac effects were noted throughout the procedure.

Teletamine

Teletamine (Telazol®, Fort Dodge Animal Health, USA) is chemically related to ketamine and generally more potent in mammalian species when given in combination with zolazepam, a relative of diazepam (refer to injectable anesthetic combinations which follow).

Sodium pentobarbital

Sodium pentobarbital (Dibutal® (60 mg ml^{-1}) Diamond Laboratories, Des Moines, Iowa, USA) has been used for a satisfactory general surgical anesthesia in nurse sharks (n=9) when given as a rapid IV injection at 10 mg kg^{-1} or less (Walker, 1972). Slow injections resulted in erratic responses. Intraperitoneal delivery was shown to be the slowest and most unreliable, while intramuscular injection resulted in only slightly improved responses unacceptable for sedation. Serum half-life is approximately 15 seconds with a second half-life of several days, due to an inability of the animals to excrete the drug through their gills or kidneys. An intravenous dose of 10 mg kg^{-1} resulted in a loss of gilling or ventilatory movements within a minute of injection. Gilling returned after 10 minutes and a weak righting response was observed at 3 hours. An intravenous dose of 20 mg kg^{-1} resulted in a loss of gilling within a minute. Gilling returned after 3 hours and a weak righting response was observed after 5 hours. A high intravenous dose of 60 mg kg^{-1} resulted in animal death. It appears that larger, more active sharks require smaller doses per kilogram than smaller, sedentary specimens. Specifically, sandbar sharks and bull sharks responded similarly to the nurse shark (at 10 mg kg^{-1}) when given 6 mg kg^{-1} IV (Walker, 1972).

Xylazine

Xylazine (Rompun®, Bayer, Inc., Germany) is a thiazine derivative, distantly related to the phenothiazine tranquilizers. It is a convulsant in teleosts and causes major changes in the electrocardiogram (Oswald, 1978). [Refer to injectable anesthetic combinations which follow.]

Other injectable agents

Alternative, less well understood, barbiturates include pentobarbitone sodium (Nembutal®, Abbott Laboratories, Inc., USA), pentothal sodium (Pentothal®, Abbott Laboratories, Inc., USA), and tubcurare (Curare®, Abbott Laboratories, Inc., USA). Each of these drugs has been used to anesthetize elasmobranchs, although Gruber and Keyes (1981) claim that MS-222 resulted in a better overall anesthetic event.

Injectable anesthetic combinations

Several combinations of drugs have been or are currently being investigated. Xylazine has been used in combination with ketamine in several shark species to ameliorate the muscle spasms that can occur with ketamine alone, although individual and species variation has been noted (Stoskopf, 1993). Stoskopf (1986) found 12 mg kg^{-1} ketamine and 6 mg kg^{-1} xylazine to be an effective anesthetic combination. Andrews and Jones (1990) found 16.5 mg kg^{-1} ketamine and 7.5 mg kg^{-1} xylazine to be a safe regime for two male and five female adult sandbar sharks during a 4-hour transport. During an 8-hour simulated transport, an additional four mature female

sandbar sharks were immobilized safely using the same protocol. These animals reached a stage 1 plane 2 anesthesia.

Teletamine / zolazepam was tested unsuccessfully on a lemon shark, when dosed at 12 mg kg^{-1}, and a sand tiger shark, at an unstated dosage. The animals displayed irritability, rapid swimming, and unrestrained biting (Stoskopf, 1986; Stoskopf, 1993).

Medetomidine has been used in combination with ketamine in several shark species to ameliorate muscle spasms that can occur with ketamine alone (Snyder et al., 1998). However, there appears to be a great variation of reaction between species when given under similar conditions and doses (author's experience). For future investigations a starting dose of 0.09-0.10 mg kg^{-1} medetomidine and 4-5 mg kg^{-1} ketamine should be employed.

The efficacy of medetomidine in elasmobranchs is unknown. A combination of detomidine and ketamine has been tried in a single bull shark (*Carcharhinus leucas*), on two separate occassions, with little to no effect (author's experience).

CANDIDATE ANESTHETIC AGENTS

Drugs that have not been documented in the scientific literature but should be investigated either alone or in combination with other anesthetics include the following:

Eugenol (clove oil)

Eugenol is an over-the-counter drug that has been used in teleosts, but is yet to be formally described in elasmobranchs (Sladky et al., 2001).

Diazepam

Diazepam (Valium®, F. Hoffmann-La Roche Ltd., Switzerland) is a benzodiazepine. Diazepam, having an injectable and oral form, causes sedation in many species of animals, and has been used as an anticonvulsant. Diazepam is often used with ketamine to prevent seizures and provides a synergistic response (i.e. less of each drug is required), however, there are anecdotal reports of erratic responses.

Midazolam hydrochloride

Midazolam hydrochloride (Versed®, F. Hoffmann-La Roche Ltd., Switzerland) is a benzodiazepine only found in injectable form. In mammals, midazolam is shorter acting than diazepam and is reportedly amnestic (i.e., causes loss of memory) when given to humans (Connor, 2001).

REVERSAL AGENTS

Reversal agents are those that reverse or ameliorate the effects of anesthetic agents.

Atipemazole

Atipemazole (Antisedan®, Pfizer, Inc., USA) is a reversal agent for medetomidine and is given in equal volumes to medetomidine (equating to 5 times the microgram dose). The present recommendation for sharks is to give a full reversal dose intravenously and a full induction dose intramuscularly.

Doxapram hydrochloride

Doxapram hydrochloride (Dopram®, A. H. Robins Company, USA) has been touted as an anesthetic reversal agent; however, it has been noted to produce dramatic arousal in elasmobranchs (Stoskopf, 1986) and should be considered more as a stimulant. Doxapram does not competitively bind to the anesthetic's binding site, but rather causes stimulation of an unknown origin and should be used with caution because animals can be extremely excitatory and dangerous under the influence of this drug.

Yohimbine hydrochloride

Yohimbine hydrochloride (Watson Laboratories, Inc., USA) has been used to reverse alpha-2 adrenergics, predominantly xylazine. Yohimbine has been administered intravenously to a nurse shark and was noted to cause arousal after the animal had been previously sedated using a combination of ketamine and xylazine (Stoskopf, 1986).

Flumazenil

Flumazenil (Romazicon®, F. Hoffmann-La Roche Ltd., Switzerland) has been used to reverse the effects of the benzodiazepines such as diazepam

and midazolam. Flumazenil is most effective in mammals when given intravenously, but can be given intramuscularly.

SUPPORTIVE CARE AND EMERGENCY DRUGS

Sharks and rays under anesthesia should be carefully monitored. Animals exhibiting slowing respiration, and especially slowing heart rate, should be placed in fresh seawater or seawater with lower concentrations of anesthetic. If the animal is anesthetized with an injectable anesthetic, the reversal counterpart should be given in a partial or full dose depending on the deterioration of vital signs. Animals not responding to these tactics can be given fluids. When applied, fluid therapy needs to take into account the osmotic balance of the animal and the three major plasma components which account for osmoregulation in elasmobranchs: urea, NaCl, and trimethylamine oxide. An elasmobranch balanced salt solution can be made by adding 8.0 g l^{-1} NaCl and 21.02 g l^{-1} urea to phenol red-free Hank's balanced salt solution (Andrews and Jones, 1990). Freshwater given orally at 1-3% body weight can be beneficial.

If anesthetized animals continue to decline, doxapram can be given (see above). Elasmobranchs tend to be sensitive to doxapram and may respond with explosive excitement so caution should be exercised when giving this drug. Other traditional mammalian emergency drugs, such as epinephrine or corticosteroids, can be given in the case of physiological collapse, but the effects are not well understood (for more information about emergency drugs and shock therapies please refer to Chapter 29 of this manual).

FUTURE STUDIES

Drug anesthetic and pharmacokinetic studies are desperately needed in elasmobranch medicine. For nonprofit organizations it is often possible to co-publish a study by partnering with the anesthesia department of a university veterinary or medical school. Usually the aquarium can be responsible for providing the study animals and the collection of samples, whereas the university can provide expertise in regard to anesthetic protocols and laboratory analyses.

A model of a typical anesthetic study is outlined below. It is imperative that the reader recognize the following to be an example only. An anesthesiologist should be contacted prior to any study to critique methodology. Depending on the institution, you may need an institutional review before experiments can proceed. Research institutions may need an animal research permit (e.g., USDA permit) as well.

Model of an anesthesia research project

A minimum of seven animals (animals can serve as their own controls) should be used for the pilot study, but more animals may have to be examined if variability is great. Sex and age should be considered since this may influence results. Prior to a drug trial each animal should be weighed (kg), examined visually, and have blood drawn for a complete blood count, using Natt-Herrick's stain techniques (Campbell, 1988), and serum chemistry (including lactate) analysis.

The elasmobranchs should be held individually in identical recirculating systems. Water parameters such as salinity, temperature, ammonia, nitrite, nitrate, calcium, etc., should be the same in all systems. Details of the recirculating system components should be documented, including flow rate, pump type, size of tanks and their configuration, and use of heating or cooling elements. Manufacturer addresses should be noted for all components.

Animals should be assigned blindly drawn numbers to randomly divide them into treatment groups of equal number. Each animal should receive a single dose of a known amount (mg kg^{-1}) of drug. Drug name, manufacturer name and address, route of administration (IM, IV, IP, or immersion), size of needles, size of syringes, and rate of delivery should all be noted. A description of how it was assured that the drug was properly administered should be included.

Prior to administering the anesthetic, the following need to be documented: date; environmental temperature; specimen weight; specimen health status; pre-dosage fasting time; initial ventilation (gilling) if the animals are not ram ventilating; activity level of the animal just prior to induction (calm, active, or excited); demeanor of the animal just prior to induction (depressed, alert, aggressive, or apprehensive); physical status (healthy or status of illness); the immobilizing conditions (single animal or school, etc.); environmental conditions (large enclosure or small enclosure); manual restraint or free swimming; and body condition (obese, good, fair, thin, poor, emaciated, etc.).

During the immobilization the following should be noted: drug and dose (amount in mg or percentage); route; time given; delivery success; effect of the drug (i.e. no effect, mild sedation, heavy sedation, light anesthesia, surgical anesthesia, excessively deep, or death); time until initial effect; time of animal recumbency; and the time from discontinuation or reversal (amount, placement, and time of drug administration).

Once the animal is in hand, the following should be monitored and documented at predetermined time intervals: ventilation rates (if it is not being ram ventilated); heart rate and rhythm (either through ultrasound or Doppler probe); and core temperature (note: take care not to damage the spiral colon).

Fresh blood may be analyzed for blood O_2, CO_2, and lactic acid levels with a hand-held blood gas unit. Ambient water temperature and supplemental O_2 (added to the water) need to be documented and considered when analyzing blood gases. Note whether intra-operative fluids have been given and at what rate. Finally, note whether the animal was kept in dorsal or ventral recumbency.

After anesthesia the following should be rated and recorded: induction time; muscle relaxation; anesthesia (e.g. poor, fair, good, excellent); complications (e.g. none, minor, major, fatal); recovery time; recovery process (e.g. normal, abnormal, prolonged, or stormy); and overall anesthesia success (e.g. complete, partial, or none).

ACKNOWLEDGEMENTS

The author would like to thank Mike Walsh for initiating this project, as well as access to his literature collection. I would like to thank Ilze Berzins for her editorial review.

REFERENCES

Andrews, J. C. and R. T. Jones. 1990. A method for the transport of sharks for captivity. Journal of Aquariculture and Aquatic Sciences 5: 70-72.

Beck, K., M. Loomis, G. Lewbart, L. Spelman, and M. Papich. 1995. Preliminary comparison of plasma concentrations of gentamycin injected into the cranial and caudal limb musculature of the eastern box turtle (*Terrapene carolina carolina*). Journal of Zoo and Wildlife Medicine 26: 265-268.

Bernal, S. D. C. and J. B. Graham. 2001. Water-tunnel studies of heat balance in swimming mako sharks. Journal of Experimental Biology 204: 4043-4054.

Campbell, T. 1988. Avian Hematology and Cytology. Iowa State University Press, Ames, IA, USA. 104 p.

Connor, E. P. (ed.). 2001. Physicians' Desk Reference. Medical Economics Company, Inc. Montvale, NJ, USA. 3506 p.

Dunn, R. F. and D. M. Koester. 1990. Anesthetics in elasmobranchs: A review with emphasis on halothane-oxygen-nitrous oxide. Journal of Aquariculture and Aquatic Sciences 5(3): 44-52.

Gilbert, P. W. and H. Kritzler. 1960. Experimental shark pens at the Lerner Marine Laboratory. Science 140: 424.

Gilbert, P. W. and F. G. Wood. 1957. Method of anesthetizing large sharks and rays safely and rapidly. Science 126: 212-213.

Gruber S. H. and R. S. Keyes. 1981. Keeping sharks for research. *In:* Aquarium Systems, p. 373-402. A. D. Hawkins (ed.). Academic Press, Harcourt Brace Jovanovich, London, United Kingdom. 452 p.

Harthoorn, A. M. 1976. The chemical capture of animals. A guide to the chemical restraint of wild and captive animals. Baillier Tindall, London, United Kingdom, 416 p.

Harvey, D., C. Denny, S. Kaiser, and J. Young. 1988. Remote intramuscular injection of immobilizing drugs into fish using a laser-aimed underwater dart gun. Veterinary Record 122(8): 174-177.

Henningsen, A. 1994. Tonic immobility in 12 elasmobranchs: Use as an aid in captive husbandry. Zoo Biology 13: 325-332.

Holmgren, S. and S. Nilsson. 1999. Digestive System. *In:* Sharks, Skates, and Rays. The Biology of Elasmobranch Fishes, p. 144-173. W. C. Hamlett (ed.). Johns Hopkins University Press. Baltimore, Maryland, USA. 515 p.

Lacy, E. and E. Reale. 1999. Urinary System. *In:* Sharks, Skates, and Rays. The Biology of Elasmobranch Fishes, p. 353-397. W. C. Hamlett (ed.). Johns Hopkins University Press, Baltimore, Maryland, USA.

Larid, L. M. and R. L. Oswald. 1975. Benzocaine (ethyl p-aminobenzoate) as a fish anesthetic. Fisheries Management 64: 92-93.

Latas, P. J. 1987. The use of azaperone in the spiny dogfish (*Squalus acanthias*). *In:* International Association for Aquatic Animal Medicine Annual Proceedings. p. 157-165. May 10-14, 1987, Monterey California. Veterinary Software Publishing Inc., O'Fallon, IL, USA.

Maren, T. H., R. Embry, and L. E. Broder. 1968. The excretion of drugs across the gill of the dogfish, *Squalus acanthias*. Comparative Biochemistry and Physiology 26: 853-864.

Mitchell, M. A., S. M. Miller, J. J. Heatley, T. Wolf, F. Lapuz, and J. A. Smith. 2001. Clinical and cardiorespiratory effects of propofol in the white spotted bamboo shark (*Chiloscyllium plagiosum*). *In:* Proceedings of the 26th annual meeting of the American College of Veterinary Anesthesiologists, October 11-12, New Orleans, Louisiana. Veterinary Anaesthesia and Analgesia Vol. 29(2) pp. 97-112. Blackwell Syngery Malden, MA, USA.

Muñóz-Chápuli, R. and Satchell G. H. 1999. Circulatory System: Anatomy of the Peripheral Circulatory System. *In:* Sharks, Skates, and Rays. The Biology of Elasmobranch Fishes, p. 198-218. W. C. Hamlett (ed.). Johns Hopkins University Press, Baltimore, Maryland, USA. 515 p.

Oswald, R. L. 1978. Injection anesthesia for experimental studies in fish. Comparative Biochemistry and Physiology, 60C: 19-26.

Rose, J. D. 2002. The neurobehavioral nature of fishes and the question of awareness and pain. Reviews in Fisheries Science 10(1): 1-38.

Schoettger, R. A. 1967. MS-222 as an anesthetic for channel catfish; Its toxicity, efficacy, and muscle residues. Investigations in fish control. U.S. Dept. Interior, Bureau of Sport Fisheries and Wildlife, Technical Paper, pp 1-14.

Sladky, K. K., C. R. Swanson, M. K. Stoskopf, M. R. Loomis, and G. A. Lewbart. 2001. Comparative efficacy of tricaine methanesulfonate and clove oil for use as anesthetics in red pacu (*Piaractus brachypomus*). American Journal of Veterinary Research 62(3): 337-342.

Snyder, S. M. J. Richard, I. K. Berzins, and M. A. Stamper. 1998. Immobilization of sand tiger sharks (*Odontaspis taurus*). *In*: Proceedings of the International Association for Aquatic Animal Medicine, May 2-6, San Diego, CA. Volume 29: 120-121 p. Veterinary Software Publishing Inc., O'Fallon, IL, USA.

Soma, L. R. (ed.). 1971. Textbook of Veterinary Anesthesia. Williams and Wilkins Company, Baltimore, MD, USA. 621 p.

Spotte, S. 1992. Captive Seawater Fishes. John Wiley & Sons, New York, USA. 977 p.

Stoskopf, M. K. 1986. Preliminary notes on the immobilization and anesthesia of captive sharks. Erkrankungen Der Zootiere. Akademie-Verlag, Berlin 28: 145-151.

Stoskopf, M. K. 1993. Shark Pharmacology and Toxicology. *In:* Fish Medicine, p. 809-816. M. K. Stoskopf (ed.). W. B. Saunders, Inc., Philadelphia, Pennsylvania, USA. 882 p.

Stoskopf, M. K., B. Smith, and G. Klay. 1984. Clinical note; Blood sampling of captive sharks. Journal of Zoo and Wild Animal Medicine 15: 116-117.

Sudak, F. N. 1966. Immobilization of large sharks by means of ethanol. Copeia 3: 611-612.

Totland, G. K., H. Kryvi, Q. Bone, and P. R. Flood. 1981. Vascularization of the lateral muscle of some elamobranchiomorph fishes. Journal of Fish Biology 18(2): 223-234.

Tyler, P. and A. D. Hawkins. 1981. Vivisections, anaesthetics and minor surgery. *In:* Aquarium Systems, p. 248-278. A. D. Hawkins (ed.). Academic Press, Harcourt Brace Jovanovich, London, United Kingdom. 452 p.

Walker, M. D. 1972. Physiologic and pharmacologic aspects of barbiturates in elasmobranchs. Comparative Biochemistry and Physiology 42(A): 213-221 pp.

PERSONAL COMMUNICATIONS

Davis, R. 2002. Sea World, Orlando, FL 32821, USA.

Ezcurra, M. 2001. Monterey Bay Aquarium, Monterey, CA 93940, USA.

McEwan, T. 2002. The Scientific Centre, Salmiya, 22036, Kuwait.

Mulican, T. 2002. The Newport Aquarium, Newport, KY 41071, USA.

Mylniczenko, N. D. 2002. The John G. Shedd Aquarium, Chicago, IL 60605, USA.

Walsh, M. 2002. Sea World, Orlando, FL 32821, USA.

The Elasmobranch Husbandry Manual: Captive Care of Sharks, Rays and their Relatives, pages 297-306.
© 2004 Ohio Biological Survey

Chapter 22
Diagnostic Imaging of Elasmobranchs

MARK D. STETTER

Disney's Animal Programs
P.O. Box 10,000
Lake Buena Vista, FL 32830, USA.
E-mail: mark.stetter@disney.com

Abstract: Diagnostic imaging is a term used to group together a variety of different medical technologies that allow the health professional to "peer" inside a patient. These diagnostic tests have become one of the primary tools in research and medicine for both humans and veterinary science. Radiographs may be used for routine health screening, diagnostic evaluation of ill animals, reproductive evaluation, and research. Radiographs of sharks and rays provide excellent images of skeletal anatomy, although identification of individual soft tissue organs (e.g., heart, liver, kidneys, etc.) is difficult and rare. The use of contrast medium placed within the gastrointestinal tract (GI) can help identify the size and location of certain organs. To aid radiographic interpretation it is always best to acquire two views, whenever possible. This allows the clinician to form a multi-dimensional image and helps interpret potential artifacts. Usually these views include a dorsoventral (DV) view and a lateral view. Ultrasonography is an excellent complement to radiography. While radiographs provide outstanding images of skeletal anatomy, ultrasonography provides useful information about soft tissue structures, organ location, organ size, and pathological changes. In general, a 7.5 MHz transducer is used for medium to small sharks and rays, while a 5.0 or 3.5 MHz probe is better suited for large sharks. For small or dorsally compressed animals, a 10.0 MHz transducer will provide the best image. Computerized Tomography (CT) and Magnetic Resonance Imaging (MRI) provide thin, detailed anatomical slices (images) of a selected area of interest. Radiographs and ultrasound are more appropriate for routine scanning, while CT and MRI are reserved for times when a more detailed understanding is required for a specific lesion or anatomical area. Because CT and MRI require animals to be sedated and transported for imaging, they will be less commonly used than other methods of imaging.

Diagnostic imaging is a term used to group together a variety of different medical technologies that allow the health professional to peer inside an individual without having to actually "open up" the patient. These diagnostic tests have become one of the primary tools in medicine and clinical research for both humans and veterinary science. There is a tremendous variety of medical imaging techniques. In this chapter we will focus on those commonly used in veterinary medicine, including: radiography, ultrasonography, computerized tomography (CT), and magnetic resonance imaging (MRI). In general, the same equipment and techniques used by veterinarians on domestic animals can be used when working with elasmobranchs. Because of their cost and availability, CT and MRI are less commonly used than radiology and ultrasonography, but, when available, both provide outstanding images of anatomical structures. CT and MRI equipment is currently being incorporated into many veterinary schools and referral centers. It is anticipated that they will be more commonly used in aquatic animal medicine in the near future.

RADIOLOGY

Indications for radiographs in elasmobranchs include routine health screening, diagnostic evaluation of ill animals, reproductive evaluation, and research. Radiographs of sharks and rays provide excellent images of skeletal anatomy. For this reason radiology is the imaging tool of choice

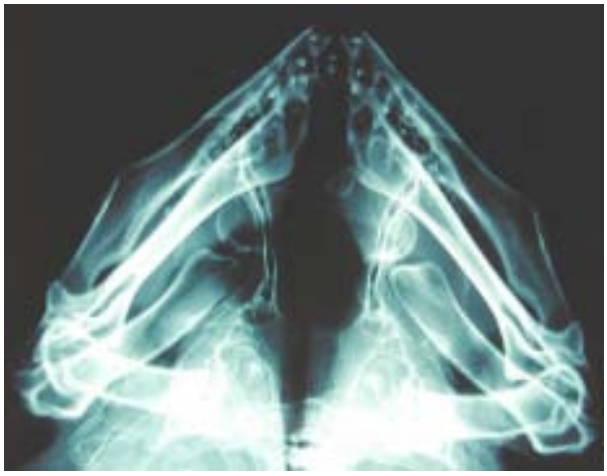


Figure 22.1. Skull radiograph of a wobbegong shark (*Orectolobus* sp.). This image was taken using mammography film. Note the exceptional detail acquired of the skeletal anatomy.

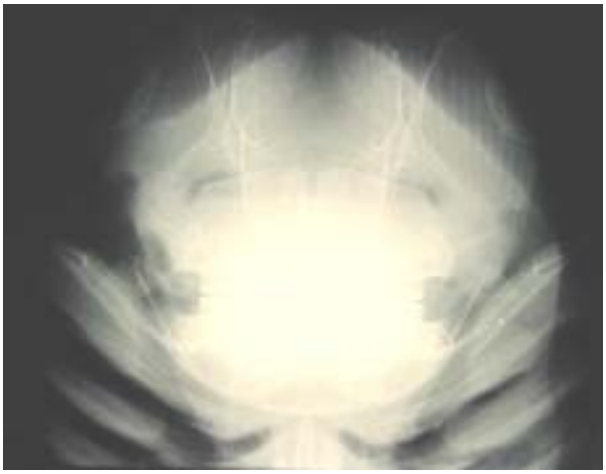


Figure 22.2. Skull radiograph of a cownose ray (*Rhinoptera bonasus*). The partially calcified orbital structures, gill arches, and dental plates are well visualized.

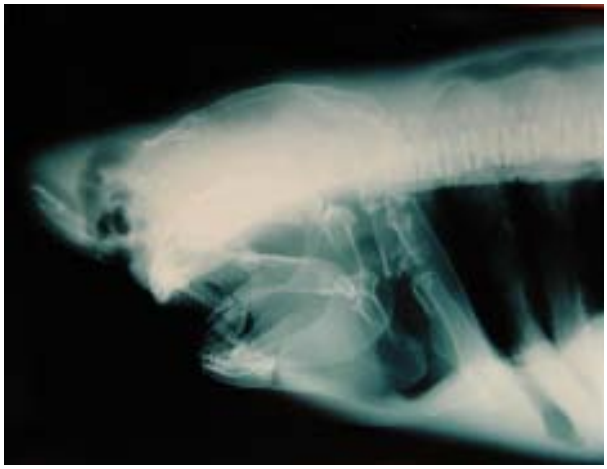


Figure 22.3. Lateral skull radiograph of a leopard shark (*Triakis semifasciata*). Note the excellent detail of the mandible, maxilla, and associated structures.

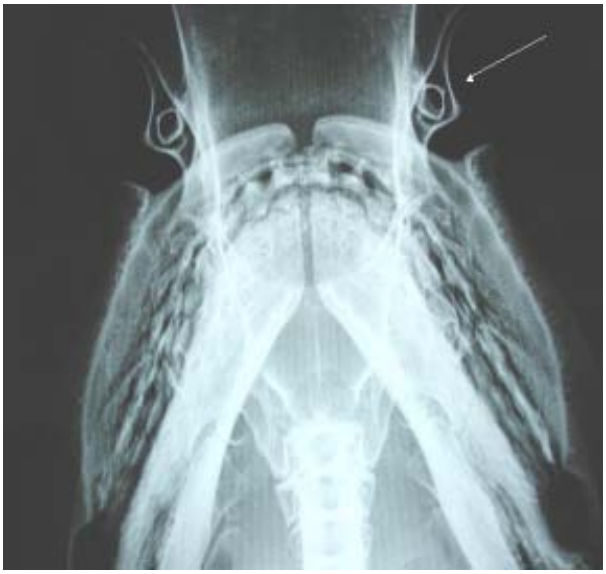


Figure 22.4. Dorsoventral skull radiograph of a leopard shark (*Triakis semifasciata*). Note the dental anatomy and nasal openings (arrow).

Figure 22.5. Dorsoventral radiograph of a cownose ray (*Rhinoptera bonasus*). This image captures the skeletal anatomy of the wing. Note the numerous skeletal articulations.

for evaluating skeletal abnormality. Images of sharks and rays demonstrate that even with cartilaginous bones, radiographic detail is excellent (Figures 22.1-22.5) and areas of bone degradation and loss can be observed (Figures 22.6 and 22.7).

Radiographs of mammals usually allow visualization of individual soft tissue organs (e.g., heart, liver, kidneys, etc.); however, this is not true in elasmobranchs, where individual organs can rarely be identified. The use of contrast medium placed within the gastrointestinal tract (GI) can help identify the size and location of certain organs. The author has commonly used barium or iohexol as GI contrast agents (Figure 22.8). The barium is delivered via a soft rubber tube into the stomach. Radiographs should be taken prior to administration, immediately after administration, and then at routine intervals to document the contrast agent's passage through the GI tract. In general, rays and skates have a fast GI transit time. When these animals are stressed during examination, they may pass contrast media in their feces in less than fifteen minutes. Sharks have longer transit times, which will vary greatly depending upon species, diet, and metabolic status.

A variety of different radiographic machines is available and can be used with aquatic animals.

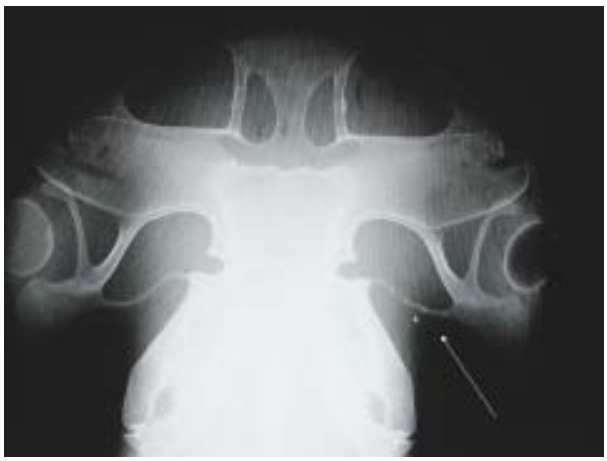


Figure 22.6. Dorsoventral view of a skull radiograph of a bonnethead shark (*Sphyrna tiburo*). This animal had a severe fungal infection (*Fusarium solani*) which had invaded the skull (arrow). There is evidence of deep infection with bone lysis.

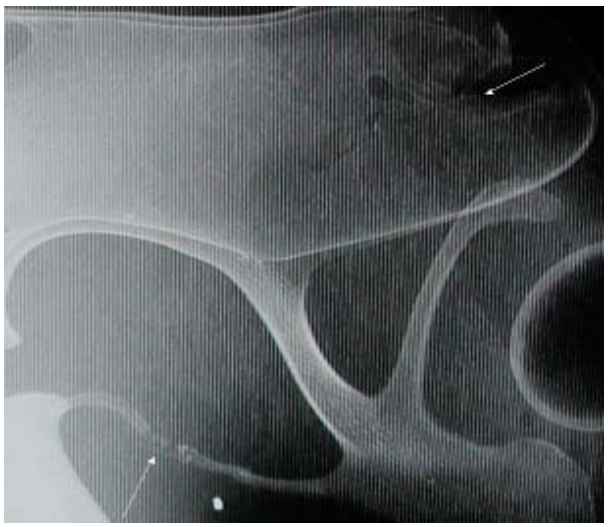


Figure 22.7. Enlarged view of the bonnethead shark (*Sphyrna tiburo*) skull from Figure 22.6. Note the severe osteomyelitis in several areas of the skull (arrows).

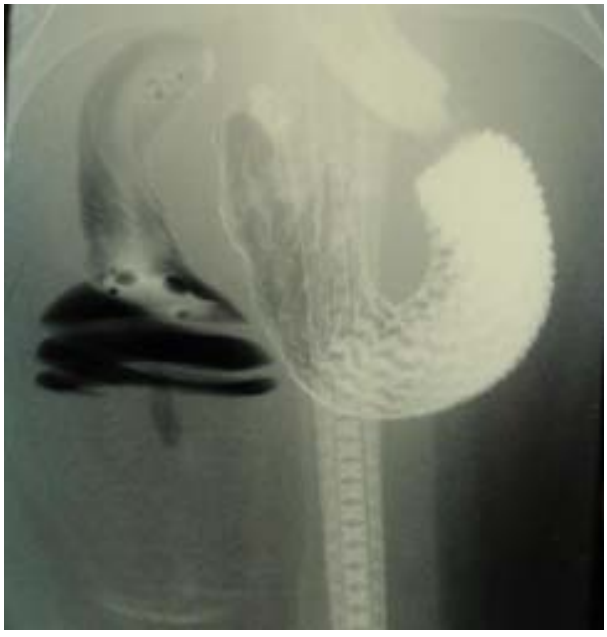


Figure 22.8. Dorsoventral view of a cownose ray (*Rhinoptera bonasus*) undergoing a gastrointestinal contrast study. Note the barium in the stomach and air within the spiral colon. The mucosal ruggae are well delineated by the contrast agent, and gas helps delineate the lumen of the spiral colon.

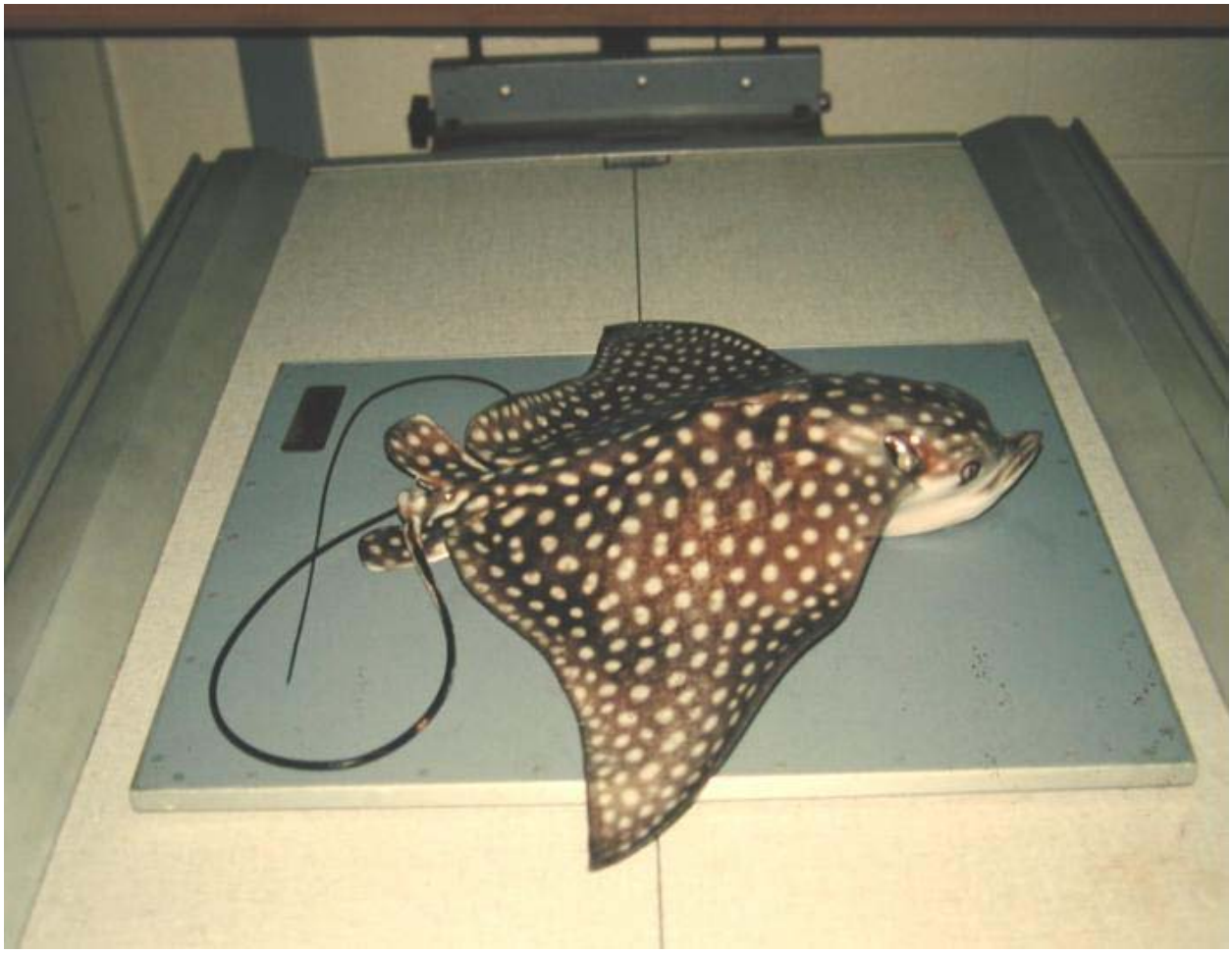


Figure 22.9. An anesthetized eagle ray (*Aetobatus narinari*) positioned for a DV radiograph taken directly on top of the radiograph cassette.

Standard small-animal radiology machines have been commonly used. These machines provide excellent images but are stationary and require the patient be brought to the machine (Figures 22.9 and 22.10). Table top cassette placement is commonly used for smaller animals. As patient size and thickness increases, a bucky or grid system should be used to decrease scatter radiation. For larger elasmobranchs, a portable radiographic unit is commonly used. These x-ray units, typically used in equine medicine, provide the flexibility of bringing the unit to the animal (Figures 22.11 and 22.12). Rare-earth screen cassettes and associated high definition film provide the most detailed images. The author has placed these cassettes in sealed plastic bags to prevent water damage when used with aquatic animals.

Figure 22.10. A bonnethead shark (*Sphyrna tiburo*) positioned for a DV radiograph. Proper positioning is critical to image interpretation. Markers should be used to identify right from left. (Photograph courtesy of Jane Capobianco, The Living Seas Pavilion, Orlando, USA).

Figure 22.11. A large, anesthetized sand tiger shark (*Carcharias taurus*) positioned for radiographs with a portable radiograph unit. The x-ray cassettes are sealed in plastic and placed underneath the stretcher (Photograph courtesy of Mike Walsh, Sea World Orlando, USA).

Figure 22.12. A large, anesthetized sand tiger shark (*Carcharias taurus*) positioned for radiographs with a portable radiograph unit. The x-ray cassettes are sealed in plastic and placed underneath the stretcher (Photograph courtesy of Mike Walsh, Sea World Orlando, USA).

To aid in radiographic interpretation it is always best to acquire two different views whenever possible. This precaution allows the clinician to form a multi-dimensional image and helps interpret potential artifacts. Usually these views include a dorsoventral (DV) view and a lateral view. When working with some dorsally compressed species (skates and rays), a lateral view may not be realistic.

When radiographing elasmobranchs, specimens should be removed from the water as this provides better images and is safer. Whenever electrical equipment is being used in conjunction with salt water, it is critical that appropriate safety precautions be observed.

It is important that the animal remain immobile during radiographic imaging. Manual or chemical restraint can be used depending upon the size and temperament of the animal. The author has commonly used light sedation for most elasmobranch imaging. When working with smaller animals, a fenestrated plastic bag or sheet is commonly used to minimize direct handling. A stretcher is often helpful to aid in lifting and positioning larger specimens (Figures 22.11 and 22.12).

One of the most difficult tasks associated with imaging elasmobranchs is interpreting the images. In many situations radiographs of animals are not taken unless an animal is ill. If these are the only images available in your database, it can be difficult to decipher normal from abnormal findings. In order to help understand normal elasmobranch radiographic anatomy, it is strongly encouraged that the clinician use any opportunity to take radiographic images of sharks and rays (i.e., during routine examinations of healthy animals and necropsies of deceased specimens). These films will become a valuable database for comparison, and will enable the clinician to create a technique chart and radiographic settings for each group of animals.

ULTRASONOGRAPHY

Ultrasonography is an excellent complement to radiography. While radiographs provide outstanding images of skeletal anatomy, they often provide little information about the associated soft tissue structures. Ultrasound imaging provides useful information about organ location, size, and pathological changes. In addition to imaging for diagnostic purposes, the author has found ultrasound to be useful for anesthetic monitoring; accomplished by placing a transducer on the ventral skin surface, just over the heart (Figure 22.13). Once the heart is in view, pulse and contraction strength can be directly

Figure 22.13. Ultrasonography of an anesthetized bonnet head shark (*Sphyrna tiburo*). The ultrasound transducer has been placed over the heart and the heart rate is being measured (Photograph courtesy of Don Neiffer, Disney's Animal Programs, Orlando, USA).

measured. Dramatic decreases in heart rate during anesthesia may indicate that the animal is too deeply anesthetized, while significant increases in heart rate may indicate that the patient is waking up. Ultrasound is also useful for reproductive studies in elasmobranchs. Gravid animals can be identified and, in viviparous species, viability of fetuses can be confirmed. The number of egg cases or fetuses can be counted and the size of each documented (Figure 22.14). In viviparous animals, fetuses can be seen moving within the uterus and, in some cases, fetal heart movement can be identified.

Real time B-mode ultrasound machines are most commonly used in veterinary medicine. The selected transducer will depend on the size of the patient. In general, a 7.5 MHz transducer is used for medium to small animals (small sharks and rays), while a 5.0 MHz or 3.5 MHz probe is better suited for large sharks. For small or dorsally compressed animals, a 10.0 MHz transducer will provide the best image. As with radiographic units, ultrasound machines are available as large stationary units and smaller portable units. In general, the author recommends a smaller portable unit, that can use transducers of various sizes, for work with elasmobranchs. An ultrasound unit of this type will provide excellent images and is flexible enough for use with various species in different locations. When acquiring ultrasound equipment, it is critical to procure some type of recording device. Many newer units have built-in digital storage devices. Older ultrasound machines will need to be equipped with an external recording device. These images are not only an integral part of the animal's medical record, but should become part of a data bank for future reference.

During ultrasound scanning the animal can remain in water and thus acoustic gel is not required. The animal can be maintained in a normal sternal position and the transducer placed in the water on the animal's ventral surface. The animal can be turned, with its ventral surface facing up, for easier access to the ventrum. The animal should be scanned in a routine, systematic manner each time it is evaluated. The author prefers to start by placing the transducer on the ventral midline, between the opercula, providing an initial view of the heart. Starting at the heart provides an easy landmark and allows the clinician to get oriented as well as make appropriate adjustments to the ultrasound unit. After the heart is evaluated, the transducer is moved caudally to image the liver, gall bladder, stomach, spleen, intestines, spiral colon, and reproductive structures. It is important

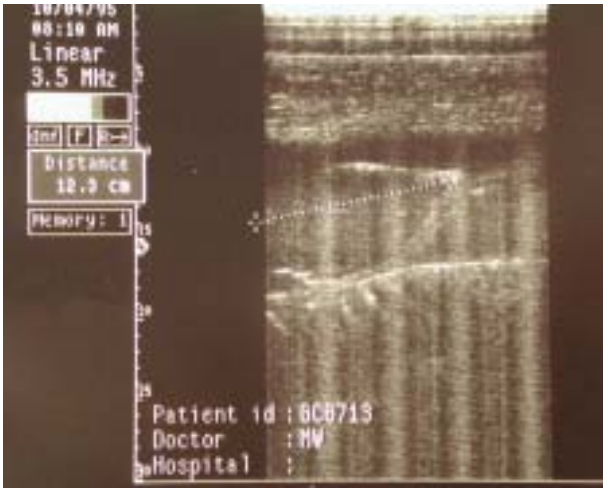


Figure 22.14. Ultrasound image of an egg case within the uterus of a shark. Note how a caliper is used to measure the size of the egg case (Photograph courtesy of Mike Walsh, Sea World Orlando, USA).

to remember that elasmobranchs have a large, lipid-filled liver and that, compared to mammals, it will appear enlarged and hyperechoic. The spiral colon can be easily recognized by its characteristic rotating layers of hyper- and hypoechoic lines (Figure 22.15). On cross-section view, the spiral valve will resemble an onion with many layers. When the transducer is turned 90° to give a longitudinal view, the structure will no longer be round but alternating layers will persist (Figures 22.15 and 22.16). On ultrasound, the gall bladder is round and normally anechoic (black)

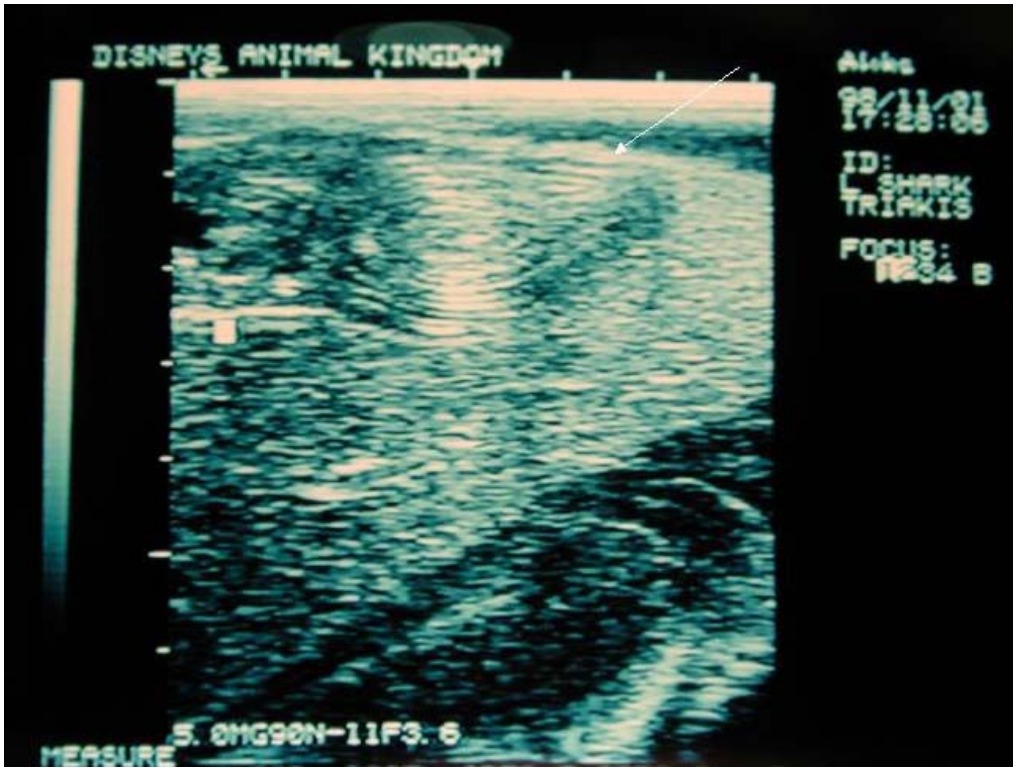


Figure 22.15. Ultrasound image of a leopard shark (*Triakis semifasciata)*. This image shows the spiral colon (arrow) in cross-section, and a portion of the liver.

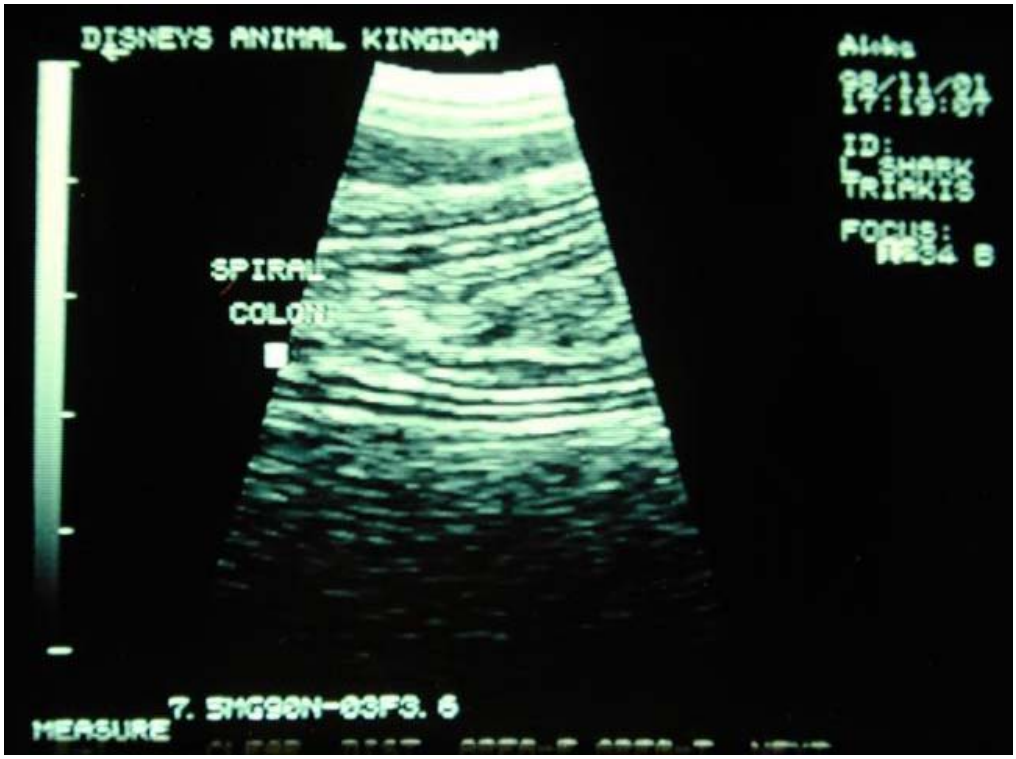


Figure 22.16. Ultrasound image of a leopard shark (*Triakis semifasciata*). This image shows the spiral colon in longitudinal view. Note the characteristic alternating patterns of hyperechoic and anechoic lines.

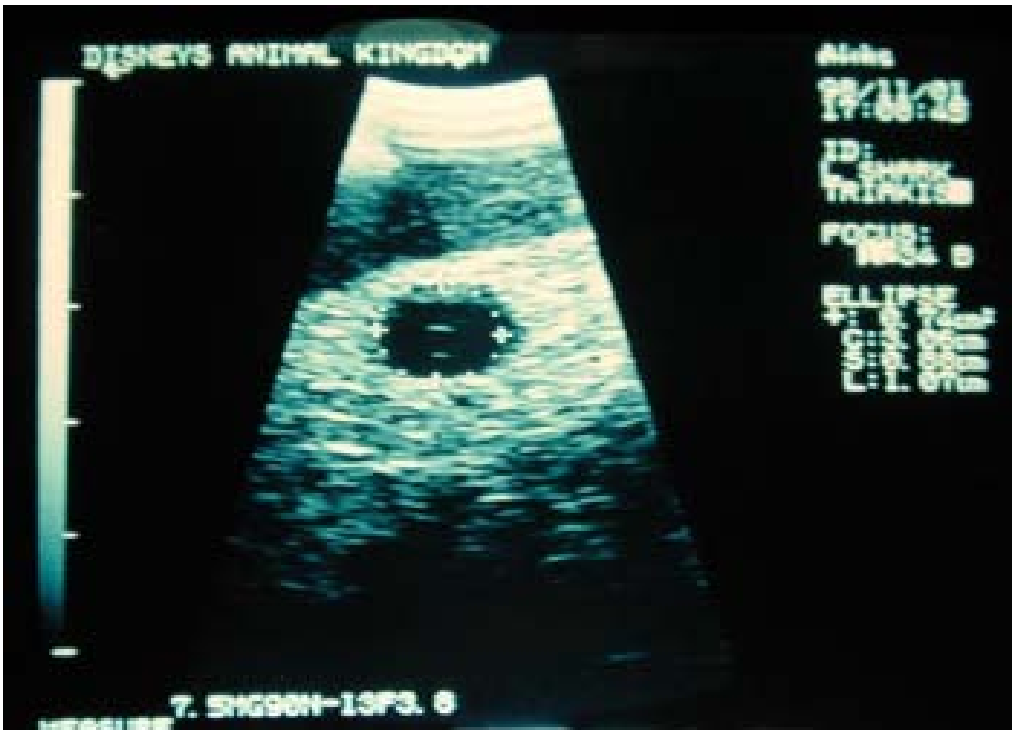


Figure 22.17. Ultrasound image of a leopard shark (*Triakis semifasciata*). The round anechoic (black) area is the gall bladder and is surrounded by normal liver.

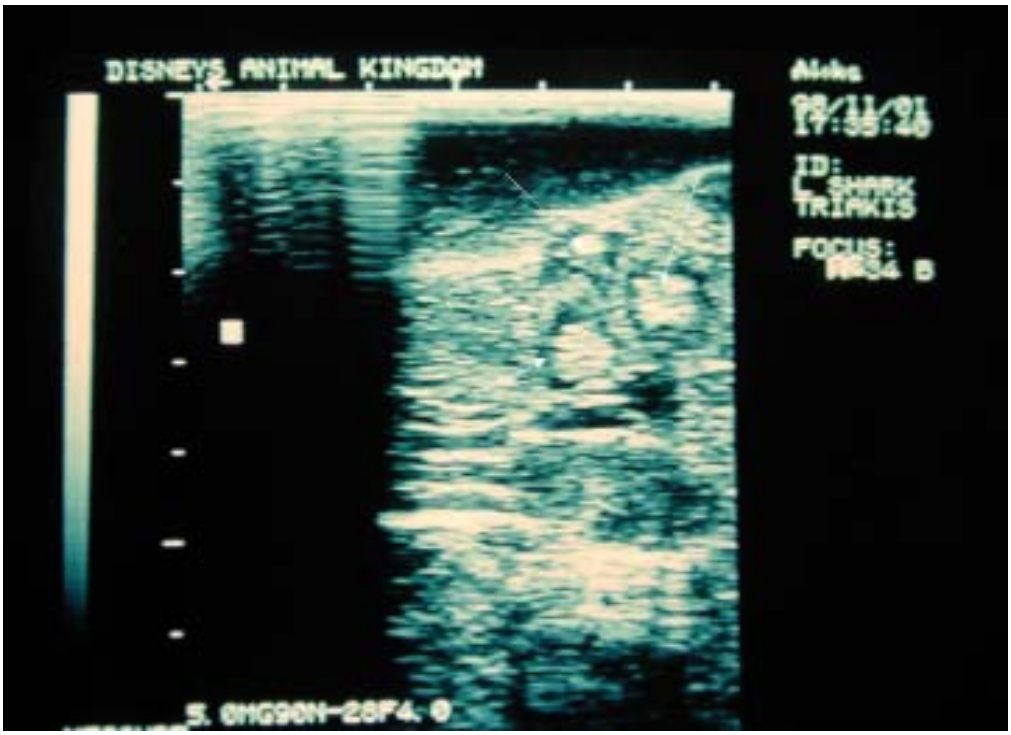


Figure 22.18. Ultrasound image of a leopard shark (*Triakis semifasciata*). The numerous round hypoechoic circles represent cross sections of the intestines (arrows).

(Figure 22.17). Cross-sectional ultrasound of intestines appears as round structures with a hypoechoic rim (Figure 22.18).

Species with thick scales may be difficult to image because ultrasound waves do not penetrate hard objects and image quality can be reduced. In larger sedated sharks, excellent images of internal organs can be acquired by placing the transducer into the mouth and down the esophagus. A mouth gag or PVC pipe should be used to help protect the transducer and clinician. This trans-esophageal image often allows excellent viewing of the heart, liver, gall bladder, and gastrointestinal tract.

Unlike other diagnostic modalities, ultrasound imaging and interpretation are greatly limited by the experience of the operator. For the clinician who is learning how to use ultrasound in aquatic animals, it is important to improve diagnostic skills by correlating surgical and postmortem anatomy with ultrasound findings. Ultrasound scanning of all animals on a routine basis will help the clinician become comfortable with locating different organs and interpreting ultrasound images.

CT AND MR IMAGING

Computerized Tomography (CT) and Magnetic Resonance Imaging (MRI) provide thin anatomical slices (images) of a selected area of interest. These images often provide much more anatomical detail than radiography or ultrasound. Radiographs and ultrasound are more appropriate for routine scanning, while CT and MRI are reserved for times when a more detailed understanding of a specific lesion or anatomical area is required. Historically, CT and MRI were either not available, or too costly, for use in veterinary medicine. However, these units are now readily available at veterinary colleges and referral hospitals.

Both CT and MRI require the patient to remain motionless for a long period of time and thus it is important to have the patient sedated or anesthetized for both procedures. The length of these procedures will depend upon the type of unit and the size of the area being evaluated. In general, CT is much faster than MRI and limiting the number of slices will decrease the amount of time required. Because these techniques require

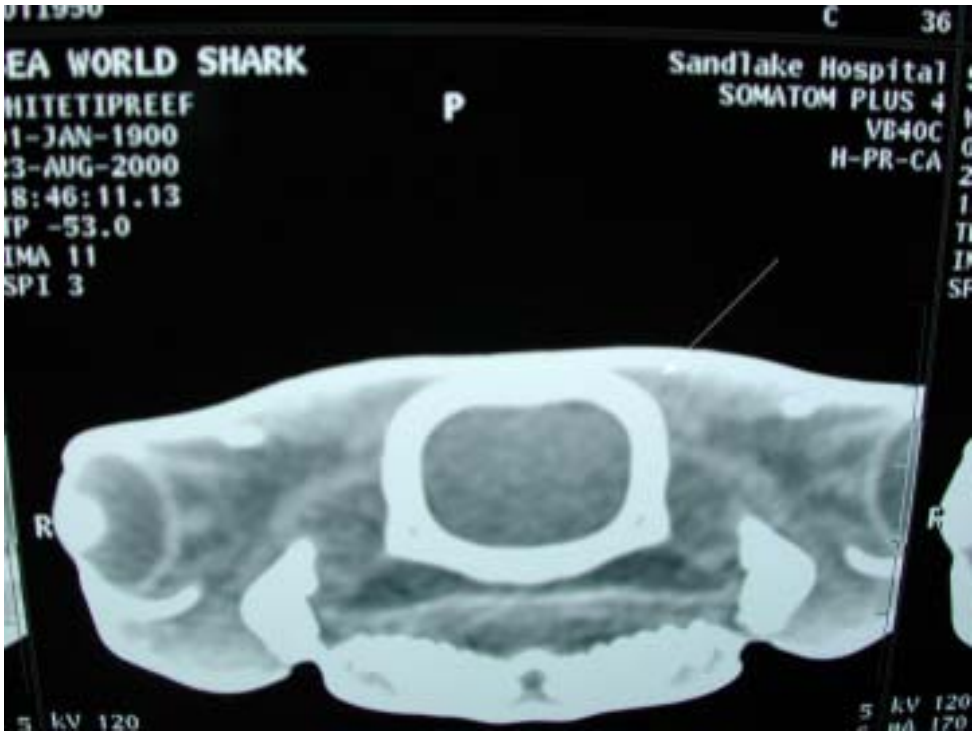


Figure 22.19. An image generated from a CT scan of a whitetip reef shark (*Triaenodon obesus*). This image is a cross-sectional slice at the level of the eye and outlines the skeletal structures of the skull, including the orbit, jaw, and brain encased by cranium (arrow). The right eye can be seen with its internal structures (e.g., lens, scleral ossicles, and vitreous humor) (Photograph courtesy of Mike Walsh, Sea World Orlando, USA).

animals to be sedated and transported for imaging, they will be less commonly used than other methods of imaging. The detail that CT and MRI images provide is outstanding, and thus they provide a useful tool when other diagnostic imaging modalities do not provide adequate information (Figure 22.19).

When performing CT or MRI on elasmobranchs, the sedated animal is placed on a table or gurney that moves through the imaging tunnel. The imaging units can be set to create slices at predetermined distances. For small areas of interest, one-millimeter slices are performed. For larger areas of interest, the range can be increased to one-centimeter slices. Many newer units will create slices in three different planes and can then recreate a computerized 3-D composite of an animal's anatomy.

SUGGESTED READING

Blackband, S. and M. Stoskopf. 1990. In vivo nuclear magnetic resonance imaging and spectroscopy of aquatic organisms. Magnetic Resonance Imaging 8: 191-198.

Goddard, P. 1995. Ultrasonic examination of fish. *In:* Veterinary Ultrasonography, p. 289-302. P. J. Goddard (ed.). CAB International, Wallingford, Oxfordshire, UK.

Love, L. and G. Lewbart. 1997. Pet fish radiography: technique and case history reports. Veterinary Radiology and Ultrasound 38: 24-29.

Sande R. and T. Poppe. 1995. Diagnostic ultrasound examination and echocar-diography in Atlantic salmon (*Salmo salar*). Veterinary Radiology and Ultrasound 36(6): 551-558.

Stetter M. 2001. Diagnostic imaging and endoscopy. *In:* BSAVA Manual of Ornamental Fish, 2nd edition, p. 103-108. W. Wildgoose (ed.). British Small Animal Veterinary Association. Gloucester, UK.

Tyson R., N. Love, G. Lewbart, and R. Bakal. 1999. Techniques in advanced imaging of fish. *In:* Proceedings of the American Association of Zoo Veterinarians, Oct 9-14, 1999, Columbus, Ohio. p. 201-202. Columbus, Ohio, USA.

The Elasmobranch Husbandry Manual: Captive Care of Sharks, Rays and their Relatives, pages 307-323.
© 2004 Ohio Biological Survey

Chapter 23
Elasmobranch Hematology: Identification of Cell Types and Practical Applications

CATHERINE J. WALSH

Marine Immunology Program
Center for Shark Research
Mote Marine Laboratory,
1600 Ken Thompson Parkway,
Sarasota, FL 34236, USA.
E-mail: cjwalsh@mote.org

CARL A. LUER

Marine Biomedical Research Program
Center for Shark Research
Mote Marine Laboratory,
1600 Ken Thompson Parkway,
Sarasota, FL 34236, USA.
E-mail: caluer@mote.org

Abstract: The blood cells that circulate through elasmobranch fishes consist of the same classes of blood cells typically found in other vertebrates, namely erythrocytes, thrombocytes, and leukocytes. Tissue sites of blood cell origin include spleen and thymus as in other vertebrates, but include unique organs associated with the gonads and esophagus. Morphologically, leukocytes resemble those of higher vertebrates and include lymphocytes, granulocytes (heterophils, eosinophils, and basophils), and monocytes. While differential leukocyte counts (relative numbers and varieties of the five basic leukocyte types) will vary with species, leukocytes in the peripheral circulation of healthy elasmobranchs are typically composed of 50-75% lymphocytes, 10-30% heterophils, 0-10% eosinophils, 0-1% basophils, and 0-3% monocytes. The most common method to obtain blood is via caudal venipuncture, after which the sample can be used for preparing smears, for counting cells, for collecting serum or plasma, or for isolating viable leukocytes.

As in other vertebrate animals, blood is the primary circulating fluid in elasmobranch fishes. It is a complex mixture of a variety of cells bathed in a plasma composed of proteins, non-protein nitrogen compounds, carbohydrates, lipids, and organic and inorganic salts and acids. As a transportation medium, blood facilitates numerous vital functions: It carries oxygen and carbon dioxide to and from the tissues and gills; carries metabolic waste to the kidneys; distributes material absorbed by the stomach and intestine to tissues throughout the body; provides for proper water and ion distribution; furnishes a physiologically balanced and properly buffered medium so that reactions in the blood and tissues can be maintained; transports hormones secreted by endocrine tissues to their target sites; contains cells that defend the body against disease-producing microorganisms; and, has the ability to form clots that protect the body from excessive loss of blood volume following injury.

Consequently, blood contains a tremendous amount of information about the condition of the animal and can be used as a valuable diagnostic tool as well as a rich source of research material.

Perhaps the greatest advantage that blood can offer to the husbandry of elasmobranchs is that it can be obtained relatively easily without jeopardizing the life of the animal. Once collected, it can be separated into cellular and non-cellular components for the quantification of a particular constituent or the measurement of a physiological function.

This chapter provides a brief discussion of the tissue sites where blood cells originate, followed by descriptions of the cell types found in the peripheral circulation. The text then focuses on practical aspects of hematology as they apply to elasmobranch fishes, including procedures for collecting and handling blood samples, preparing blood smears, suggesting ways to fix and stain blood cells for visualization, and describing methods for counting cells, isolating leukocytes, and assessing cell viability. Solutions modified for use during elasmobranch hematology procedures have been provided in Table 23.1.

HEMATOPOIETIC TISSUES

The vertebrate animal tissues in which blood cells are produced or stored are referred to as hematopoietic tissues. In higher vertebrates, the primary hematopoietic site is the bone marrow, where both erythrocytes (red blood cells) and leukocytes (white blood cells) are formed. A secondary tissue site for erythropoiesis (red blood cell production) is the spleen, while secondary tissue sites for leukocyte formation include the spleen, thymus, and lymph nodes. Elasmobranch fishes, however, possess neither bone marrow nor lymph nodes and must rely on alternative hematopoietic sites (Zapata et al., 1996). In common with other vertebrates are the thymus and spleen, but unique to the elasmobranch fishes are the epigonal organ and Leydig organ (Zapata, 1980b; Mattisson and Fänge, 1982; Lloyd-Evans, 1993). The anatomical locations of these hematopoietic tissues are shown in Figures 23.1a-c. Isolated

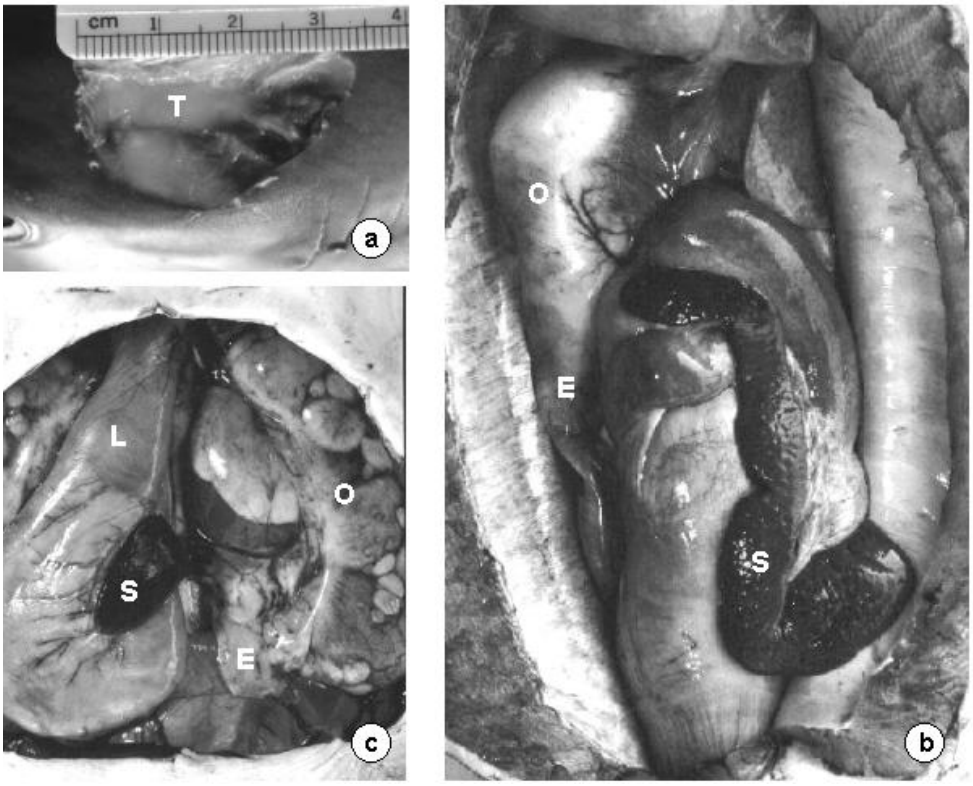


Figure 23.1. Organs. **a**. Dorsal view of dissection showing anatomical location of the thymus (T) in a juvenile blacknose shark, *Carcharhinus acronotus*. The left eye and gill slits are included as reference structures for orientation of the dissected area. **b**. Ventral view of dissection showing peritoneal cavity in a juvenile female nurse shark, *Ginglymostoma cirratum*. Anterior is to the top; posterior is to the bottom. The liver has been removed for easier display of the organs. S, spleen; E, epigonal organ; O, ovary. **c**. Ventral view of dissection showing peritoneal cavity in a mature female clearnose skate, *Raja eglanteria*. Anterior is to the top; posterior is to the bottom. The liver has been removed and the stomach has been reflected from its normal left side orientation for easier display of the organs. S, spleen; E, epigonal organ; L, Leydig organ; O, ovary.

Table 23.1. Solutions modified for use during elasmobranch hematology procedures.

1. Elasmobranch-modified Phosphate Buffered Saline (E-PBS)

	g 100 ml^{-1}	g 500 ml^{-1}	g l^{-1}
NaCl	2.63	13.15	26.3
NaH_2PO_4	0.12	0.6	1.2

Adjust to pH 7.4 with 1N HCl. Filter through 0.2 µm sterile filter and store at 4°C (Final osmolarity ~920 mOsm.).

2. Elasmobranch-modified Heparin-EDTA

Prepare a stock solution of 200 mg EDTA, 2000 units heparin in 10 ml E-PBS. Filter through 0.2 µm sterile filter and use the following volumes for specified amounts of blood: 0.5 ml for 10 ml blood; 0.25 ml for 5 ml blood; 0.15 ml for 3 ml blood. Store at 4°C, or pre-measured aliquots can be frozen and thawed when needed.

3. Elasmobranch-modified ACD Solution "A"

	100 ml	200 ml	500 ml
Citric acid (anhydrous)	0.73 g	1.46 g	3.65 g
or (monohydrate)	0.795	1.59	3.98
Sodium citrate (hydrous)	2.2	4.4	11
Dextrose (hydrous)	2.45	4.9	12.25

For 100 ml, dissolve above ingredients in approximately 67 ml E-PBS and adjust to a final volume of 100 ml with dH_2O. Filter through a sterile 0.2 µm filter and store at 4°C. Use this anticoagulant in amounts equal to the ratio of 7 ml ACD to 40 ml whole blood. For 5 ml samples, add 875 µl per tube.

4. Elasmobranch-modified Natt-Herrick Solution (modified from Natt and Herrick, 1952).

	100 ml
NaCl	2.28 g
Na_2SO_4	0.25 g
NaH_2PO_4	0.29 g
KH_2PO_4	0.025 g
formalin (37% formaldehyde)	750 µl
methyl violet 2B	0.01 g

Stir overnight and filter before use. Store at room temperature.

5. Elasmobranch-modified Trypan Blue (E-trypan blue)

Prepare E-trypan blue (0.2% final concentration) by dissolving 100 mg trypan blue in 50 ml E-PBS. Cover and stir overnight, filter through Whatman No. 1 filter paper to remove undissolved dye particles and store at room temperature in a sterile container.

NOTE: It is not advisable to make large volumes of E-trypan blue at one time because microbial growth occurs readily in solutions containing trypan blue. If contamination is observed, discard solution and prepare fresh E-trypan blue.

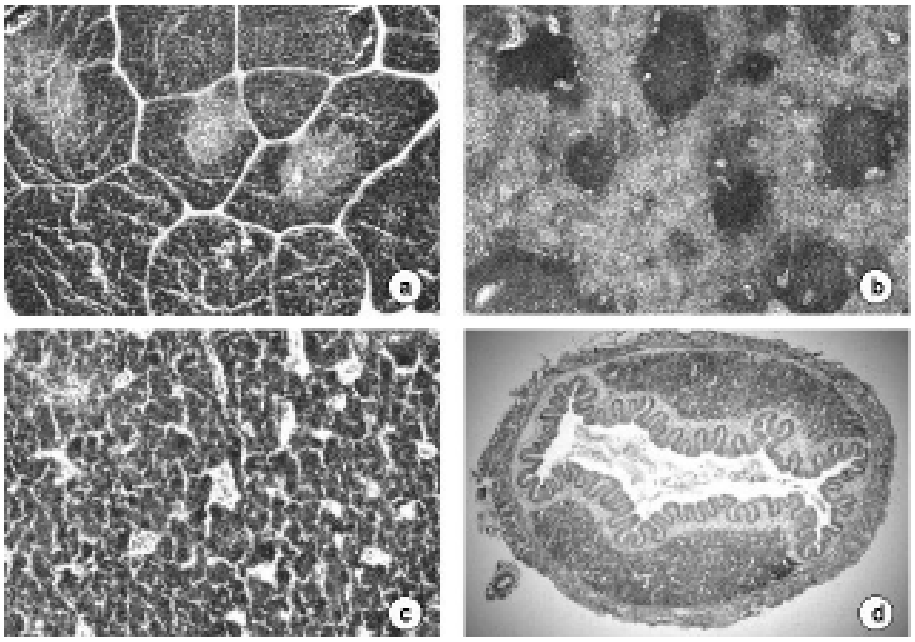


Figure 23.2. Paraffin-embedded 10 μm sections of representative elasmobranch lymphomyeloid tissues stained with hematoxylin and eosin. **a**. Section through thymus from near-term fetal sandbar shark, *Carcharhinus plumbeus*, depicting characteristic lobular architecture with less densely packed, centrally located medullary regions and more densely packed outer cortical areas. (Original magnification, 40x). **b**. Section through spleen from juvenile nurse shark, *Ginglymostoma cirratum*, showing characteristic densely packed white pulp surrounded by less densely packed red pulp. (Original magnification, 40x). **c**. Section through epigonal organ from juvenile nurse shark, showing sinuses filled with granulocytes and lymphocytes. (Original magnification, 100x). **d**. Section through esophagus of mature clearnose skate, *Raja eglanteria*, showing the bi-lobed Leydig organ dorsal and ventral to the esophageal mucosa. (Original magnification, 25x).

patches of lymphoid tissue in the intestine, termed gut-associated lymphoid tissue (GALT), have been described in elasmobranchs (Tomonaga et al., 1986; Hart et al., 1988).

Thymus

The thymus is a paired organ situated dorsomedial to both gill regions (Figure 23.1a) (Luer et al., 1995). Its size and location relative to the surrounding musculature change with somatic growth and sexual maturation of the animal. In fetal and neonatal individuals, the thymus is easily identified, but as the animal grows and matures, the organ gradually involutes and the muscle mass increases, making the thymus extremely difficult to locate in subadult and mature specimens. The thymus is composed of distinct lobules, each lobule consisting of an outer cortex and an inner medulla (Figure 23.2a) (Zapata, 1980a). The cortex and medulla contain lymphocytes, also called thymocytes, at various stages of maturation. Only a small percentage of thymocytes complete their maturation in the thymus prior to release into the peripheral circulation and lymphoid tissues. Because of their thymic origin, they are referred to as thymus-derived lymphocytes, T lymphocytes, or T cells.

Spleen

The spleen is conspicuous among elasmobranch visceral organs by its rich dark red to purplish color. In sharks, the spleen is elongate and positioned along the outer margin of the cardiac and pyloric regions of the stomach (Figure 23.1b). In batoids, however, with their relatively compressed peritoneal cavity, the organ is more compact and situated along the inner margin of the stomach (Figure 23.1c). Histologically, the elasmobranch spleen is typical of other vertebrate spleens in that it is composed of regions of red and white pulp (Figure 23.2b) (Zapata, 1980a). The scattered regions of white pulp are dense accumulations of small lymphocytes with asymmetrically placed central arteries. Areas of white pulp are surrounded by less dense areas of red pulp composed of venous sinuses filled primarily with erythrocytes and, to a lesser extent, with lymphocytes (Andrew and Hickman, 1974).

Epigonal and Leydig Organs

Two tissues that produce cells of both lymphocyte and granulocyte lineages (lymphomyeloid tissues) are unique to the elasmobranch fishes. These include the epigonal and Leydig organs (Zapata,

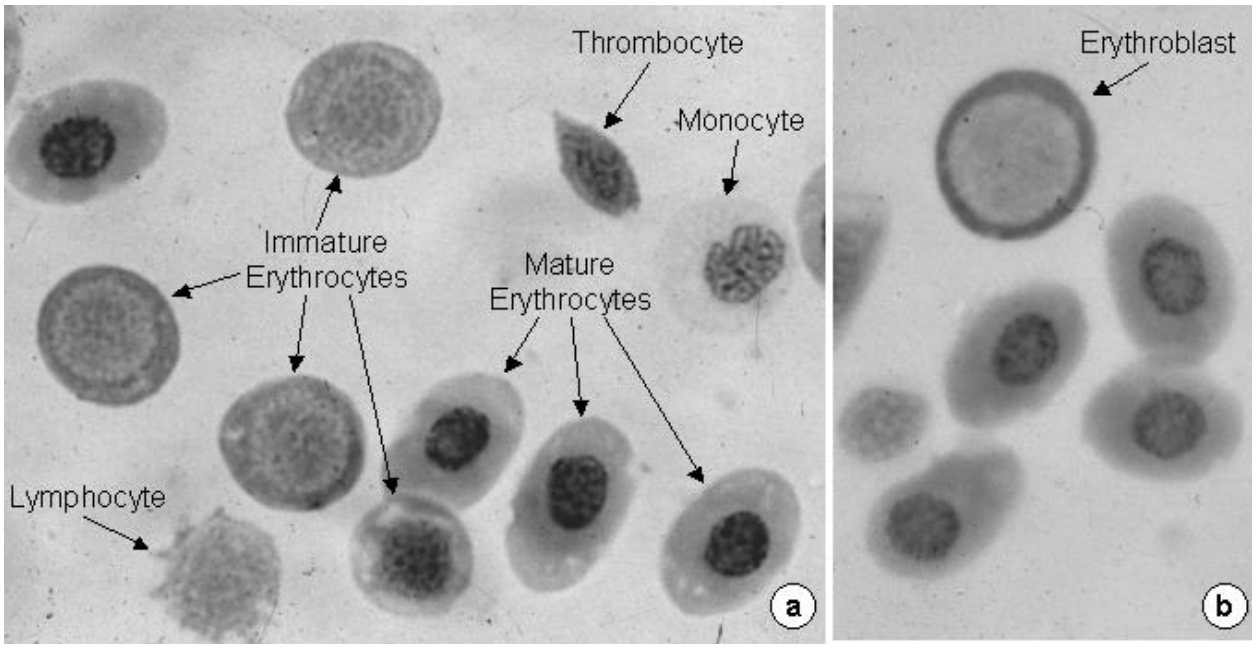


Figure 23.3. Representative peripheral blood cells from a mature bull shark, *Carcharhinus leucas*, showing characteristic morphologies for mature and immature erythrocytes (**a**) and an erythroblast (**b**); original magnification, 1,000x.

1980b; Fänge and Mattisson, 1981; Mattisson and Fänge, 1982; Honma et al., 1984). While many elasmobranchs possess both tissues, some have only the epigonal organ. The epigonal organ continues caudally from the posterior margin of the gonads in all shark and batoid species (Figures 23.1b-c). Histologically, the epigonal is composed of sinuses reminiscent of mammalian bone marrow (Figure 23.2c), except for the absence of adipose cells (fat cells). The sinuses are filled with leukocytes at various stages of maturation. Most of the cells are granule-containing leukocytes (granulocytes), with lymphocytes present to a significant but lesser degree. The Leydig organ, when present, lies beneath the epithelium on both dorsal and ventral sides of the esophagus (Figure 23.2d). Histology of the Leydig organ is virtually identical to that of the epigonal organ.

CELLS OF THE PERIPHERAL BLOOD

The primary cell types characteristic of peripheral blood in higher vertebrates can be found in elasmobranch blood (Hyder et al., 1983; Parish et al., 1986a; Fänge, 1987). These include erythrocytes, leukocytes, and thrombocytes. While it is acknowledged that there is inconsistency in the literature regarding the nomenclature of elasmobranch blood cells (Saunders, 1966; Sherburne, 1974; Fänge and Pulsford, 1983; Hyder et al., 1983; Parish et al., 1986a), the descriptions in this chapter are offered in an attempt to standardize the terminology and minimize the confusion. Where possible, photographs of representative cell types complement the text.

Erythrocytes

The most abundant cell type in elasmobranch blood is the erythrocyte. When blood smears are fixed and visualized with any of the Romanowsky stains, including Wright, Leishmann, May-Grünwald, Giemsa, etc. (see section entitled Practical applications), mature erythrocytes appear as oval or elliptical cells that are some 2½ times larger than their mammalian counterparts. Elasmobranch erythrocytes possess a centrally located nucleus that is round to slightly oval and stains dark blue or purple (Figure 23.3a). The cytoplasm is abundant and stains a pale, orange-red. Vacuoles are frequently visible in the cytoplasm of mature erythrocytes. The nature of these vacuoles is not clear, although it has been suggested that they may represent degenerating mitochondria (Stokes and Firkin, 1971). Immature erythrocytes are commonly observed. They are distinguished by their pale blue or blue-gray cytoplasm and are typically more

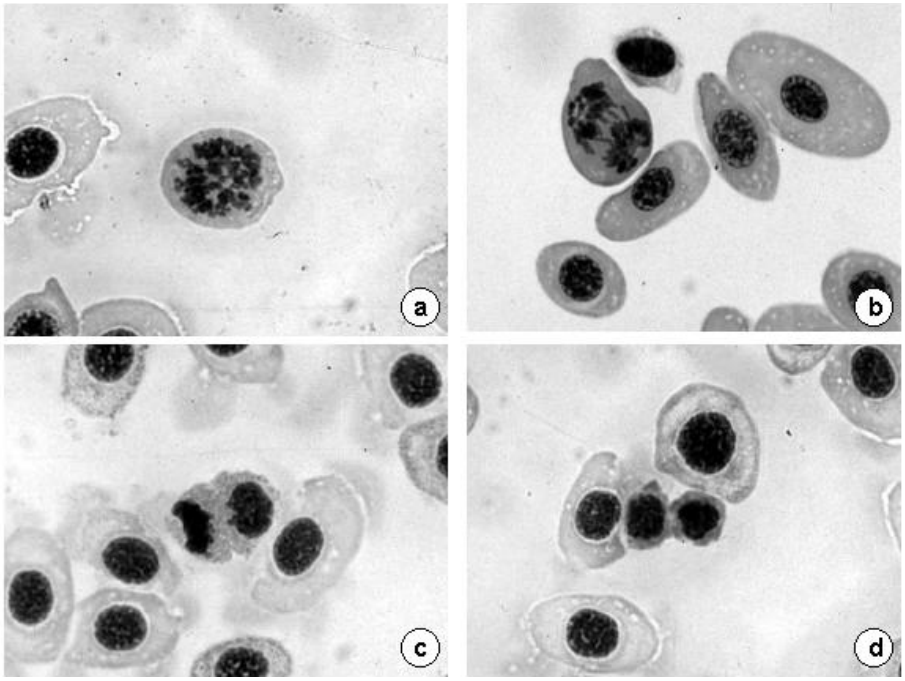


Figure 23.4. Peripheral blood smears from an immature nurse shark, *Ginglymostoma cirratum*, showing erythrocytes in different phases of mitosis: metaphase (**a**); anaphase (**b**); telophase (**c**); daughter cells (**d**). Original magnification, 1,000x.

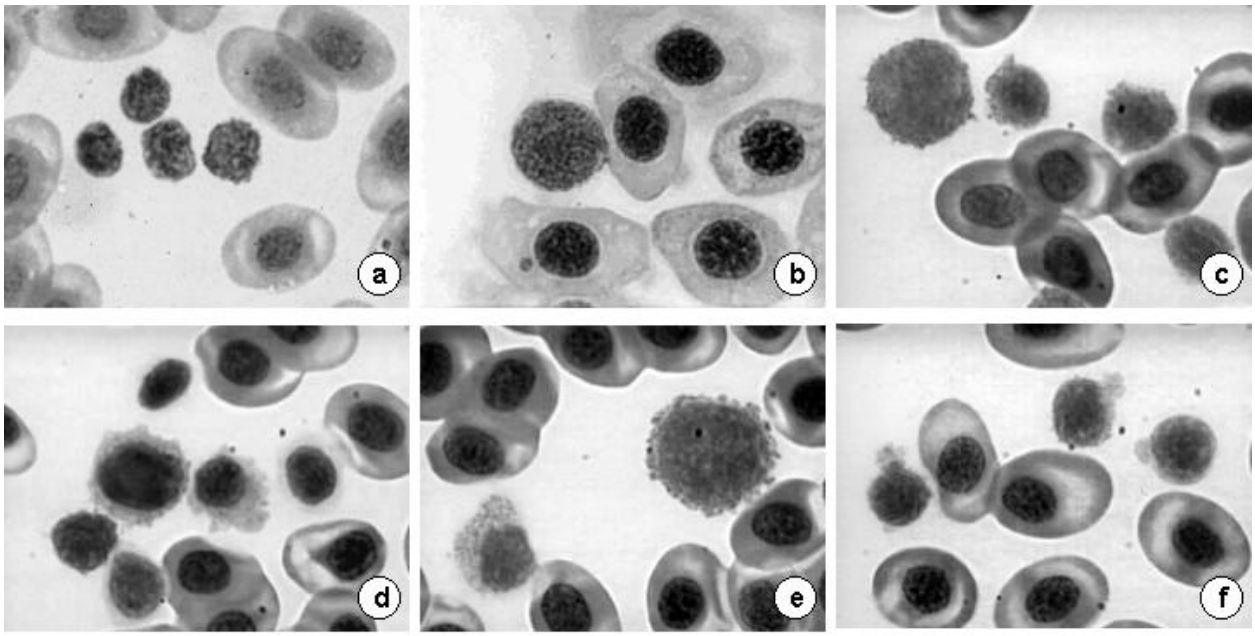


Figure 23.5. Peripheral blood smears showing representative lymphocytes. Small and medium lymphocytes from a nurse shark, *Ginglymostoma cirratum* (**a** and **b**), and blacktip shark, *Carcharhinus limbatus* (**c**), showing no visible cytoplasm. Medium and large lymphocytes from a blacktip shark showing varying degrees of visible cytoplasm (**d** and **e**) and the occasional appearance of cytoplasmic "blebbing" (**f**). Original magnification, 1,000x.

round than mature cells (Figure 23.3a). As erythrocytes mature and their hemoglobin content increases, the cytoplasm becomes less basophilic. Occasionally, erythroblasts and proerythrocytes can be observed in peripheral blood and appear as round cells with a central purple nucleus and a small amount of deeply basophilic cytoplasm (Figure 23.3b). Mitotic activity is frequently seen in elasmobranch peripheral blood (Figure 23.4), supporting the notion that replication as well as maturation of erythrocytes occurs regularly in the peripheral circulation in elasmobranch fishes (Saunders, 1966; Stokes and Firkin, 1971; Sherburne, 1974; Ellis, 1977; Zapata and Carrato, 1981). Cycling of erythrocytes in elasmobranch peripheral blood has been confirmed using cell flow cytometric analyses (Kendall et al., 1992).

Leukocytes

Lymphocytes are the most common leukocyte found in the blood of elasmobranchs, accounting for between 50-75% of total leukocytes in the peripheral circulation. Morphologically, they resemble lymphocytes found in blood smears from other vertebrates and characteristically possess a round- and dark-blue-staining nucleus. The nuclear chromatin is densely clumped and the pattern is generally distinct from that of monocytes, thrombocytes, and blast cells. Lymphocytes are commonly distributed into two to three size categories, reflecting their degree of maturation (Figures 23.5a-e). The majority of circulating lymphocytes are small (mature) or medium (maturing), but large (immature) lymphocytes are not uncommon. The ratio of nucleus to cytoplasm is typically high in lymphocytes, but varies with stage of maturity. As lymphocytes mature, the nucleus occupies an increasingly greater proportion of the cytoplasm (Blaxhall and Daisley, 1973), so that in mature lymphocytes, the cytoplasm is often not clearly visible (Figures 23.5a-c). The amount of cytoplasm in medium to large lymphocytes varies from a narrow rim to a fairly wide and often irregular area (Figures 23.5d and 23.5e). Although lymphocytes are characteristically round in shape, smearing procedures can often result in irregular cytoplasmic projections or “blebbing” (Figure 23.5f).

Granulocytic leukocytes, or granulocytes, have been described in several species of elasmobranchs, but due to the enormous heterogeneity in size, shape, and staining properties of the granules, inconsistency in identification and terminology across species has been prevalent (Saunders, 1966; Sherburne, 1974; Fänge, 1987; Rowley et al., 1988; Campbell and Murru, 1990). The different interpretations revolve around the realization that not all of the granulocytes have a clear mammalian counterpart (Hine and Wain, 1987; Hine, 1992). Attempts to classify these cells using both classical nomenclature, as well as novel terminology, have further complicated the issue. That cells at different stages of maturation are often observed adds to the confusion (Ellis, 1977; Hine and Wain, 1987; Hine, 1992). In addition, there is a tendency to classify thrombocytes containing cytoplasmic granules as a type of granulocyte (Fänge, 1987). As a practical assessment, the morphologies and staining characteristics of elasmobranch blood cells, and of fish blood cells in general, are more easily described using terminology based on avian rather than mammalian hematology (Lucas and Jamroz, 1961; Campbell, 1988). Avian hematology provides the basis for the nomenclature offered here.

The most common granulocyte in elasmobranch blood is a cell type that is referred to in non-mammalian hematology as the heterophilic granulocyte, or heterophil (Figures 23.6a-c). Generally considered to be analogous to the mammalian neutrophilic granulocyte (neutrophil), heterophils are so named because of their variable staining characteristics. Heterophils possess a colorless cytoplasm containing granules that typically range from rod or needle shaped to bead-like or spherical, and stain lightly eosinophilic with Romanowsky stains. Granule shape, size, and staining intensity vary among species as well as with maturity of the cell. The nucleus is often partially obscured by the granules in the cytoplasm. Mature heterophils have an eccentric, multi-lobed nucleus (usually two or three lobes) with a coarse, clumped chromatin that stains blue or purple. Although heterophils in early stages of development (heterophilic granuloblasts) are rarely seen in the peripheral blood of normal elasmobranchs, immature and maturing cells are common and are distinguished by their round, kidney-shaped, or band nuclei. It is not uncommon for the individual granules of heterophils to be difficult to distinguish, resulting in the appearance of a pink, hazy cytoplasm rather than a cytoplasm with distinct eosinophilic granules. While heterophils are the predominant granulocyte, their numbers vary widely among elasmobranch species, ranging from 10 to 30% of the total leukocytes. As in other vertebrates, elasmobranch heterophils play an active role in

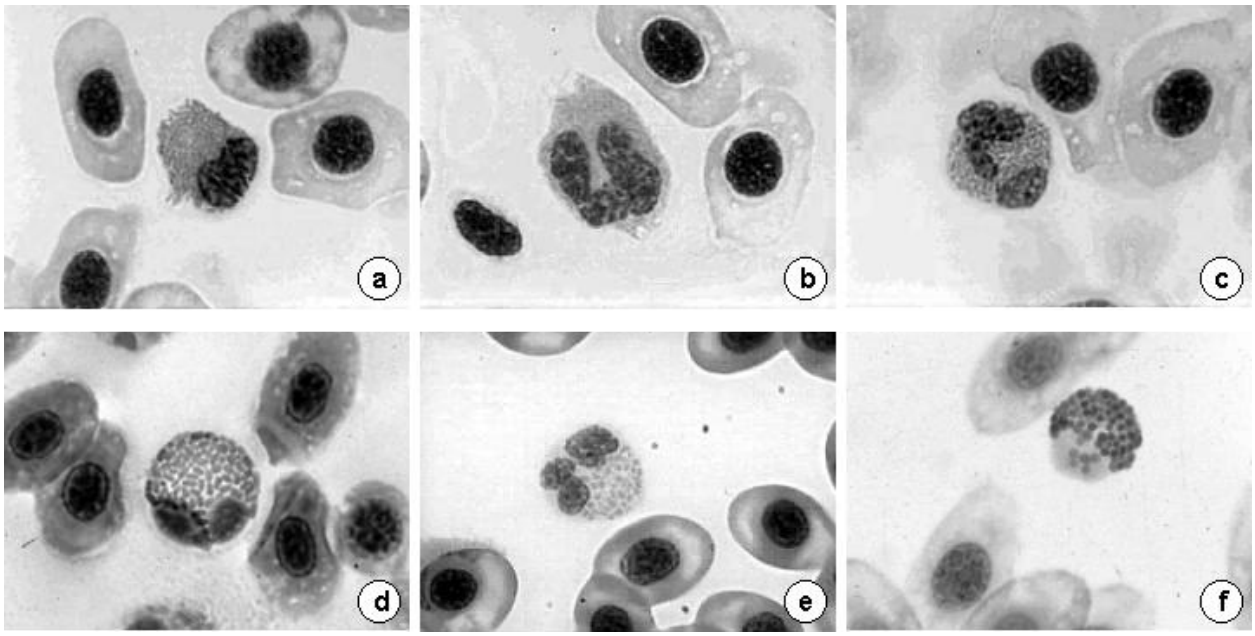


Figure 23.6. Peripheral blood smears showing representative granulocytes. Heterophils from a blacktip shark, *Carcharhinus limbatus*, depicting different nuclear morphologies: kidney-shaped (**a**), band (**b**), and multi-lobed (**c**). Eosinophils showing range of granule shape as a function of species: Atlantic stingray, *Dasyatis sabina* (**d**), blacktip shark (**e**), and clearnose skate , *Raja eglanteria* (**f**). Original magnification, 1,000x.

phagocytosis (Hiemstra, 1993; Parish et al., 1986b; Parish et al., 1986c; Walsh and Luer, 1998). Infection, disease, and stressful conditions will result in even greater numbers of heterophils (Ellsaesser et al., 1985).

A second type of granulocyte with red-staining (eosinophilic) granules is present in elasmobranch peripheral blood, although typically present in much fewer numbers than heterophils. Termed eosinophils, this granulocyte contains granules that stain considerably more intensely eosinophilic with Romanowsky stains, and appear distinctly sharper than granules of heterophils (Figures 23.6d-f). Numbers of eosinophils vary considerably among species and can range from nonexistent to greater than 10% of the total leukocyte count. In most species, eosinophils and heterophils are approximately the same size and can be difficult to distinguish. Nuclei of eosinophils are lobed with coarse, clumped chromatin that stains dark-blue or purple, and are often more noticeable than nuclei of heterophils.

As with heterophilic granulocytes, granule shape in eosinophils varies with species. In the nurse shark (*Ginglymostoma cirratum*), granules of eosinophils are thin rods, while those of cownose ray (*Rhinoptera bonasus*) eosinophils are more cylindrical. Clearnose skate (*Raja eglanteria*) eosinophils have exceptionally large, spherical granules that stain bright red with Wright's stain. In vitro studies have demonstrated that elasmobranch eosinophils can phagocytize bacteria and other foreign substances, but not with the efficiency of heterophils (Parish et al., 1986b; Walsh and Luer, 1998). In other vertebrates, eosinophils respond to parasite infection (Taverne, 1989; Abbas et al., 1991) through the process of degranulation, resulting in the release of cytotoxic factors from their granules. It is likely that this cell type performs a similar function in elasmobranchs.

Purple-staining (basophilic) granulocytes are extremely rare in elasmobranch blood smears and are usually present as less than 1% of the total leukocyte count (Saunders, 1966; Sherburne, 1974; Fänge, 1987). Basophils are round, with an eccentric nucleus that is usually lobed. The nucleus stains a light blue and is often obscured by the large and deeply basophilic cytoplasmic granules. These deep purple or dark blue stained granules distinguish this cell type from other granulocytic cells when viewed with light microscopy. Granules in basophils are round and fewer in number than granules in either heterophils or eosinophils. While the function of basophils in elasmobranchs has yet to be characterized, these cells may participate in hypersensitivity reactions as in higher vertebrates (Brostoff and Hall, 1989; Abbas et al., 1991).

In some species, granulocytes in which the granules do not appear to take up stain have been observed (Saunders, 1966; Sherburne, 1974; Fänge, 1987). The nuclei of these cells are typically not lobed and are usually located off to one side of the cell, although mature multi-lobed nuclei are occasionally seen. It is not known whether these cells represent an additional granulocyte (perhaps equivalent to the mammalian neutrophil) or result from artifacts of the fixing and staining process.

Monocytes are present in relatively low numbers in the peripheral blood of elasmobranchs, ranging from 0 to 3% of total leukocytes. They are typically larger than mature lymphocytes and although they are usually round, they can be irregular in shape. The cell margins may be indistinct or rough because of cytoplasmic protrusions (pseudopodia). In blood smears, the monocytes appear as large leukocytes with an abundant blue to blue-gray cytoplasm that lacks granules and is occasionally vacuolated. The nucleus occupies less than half of the cell volume, is eccentric in location, and has a characteristic kidney-shape, often appearing to be bilobed or indented (Figure 23.7a). The monocyte nuclear chromatin is less densely packed than in lymphocytes, and gives the nucleus a more lace-like and delicate appearance than the typically clumped chromatin in lymphocyte nuclei. The monocyte cytoplasm often contains vacuoles and is frequently described as having the appearance of ground glass or glass beads. Monocytes have a higher cytoplasm-to-nucleus ratio than lymphocytes, and are usually larger with more abundant cytoplasm than large lymphocytes. As in higher vertebrates, circulating monocytes likely migrate to tissue sites where they differentiate into macrophages (Lydyard and Grossi, 1989; Abbas et al., 1991). As macrophages, these cells function in phagocytosis as well as the release of immune regulatory factors termed cytokines, some of which are involved in inflammation.

Thrombocytes

As in other non-mammalian vertebrates, the circulating cell that serves the same role in blood clot formation as mammalian platelets is the thrombocyte. It is not surprising, then, that thrombocytes tend to clump in peripheral blood smears (Figure 23.7b). This process aids in their

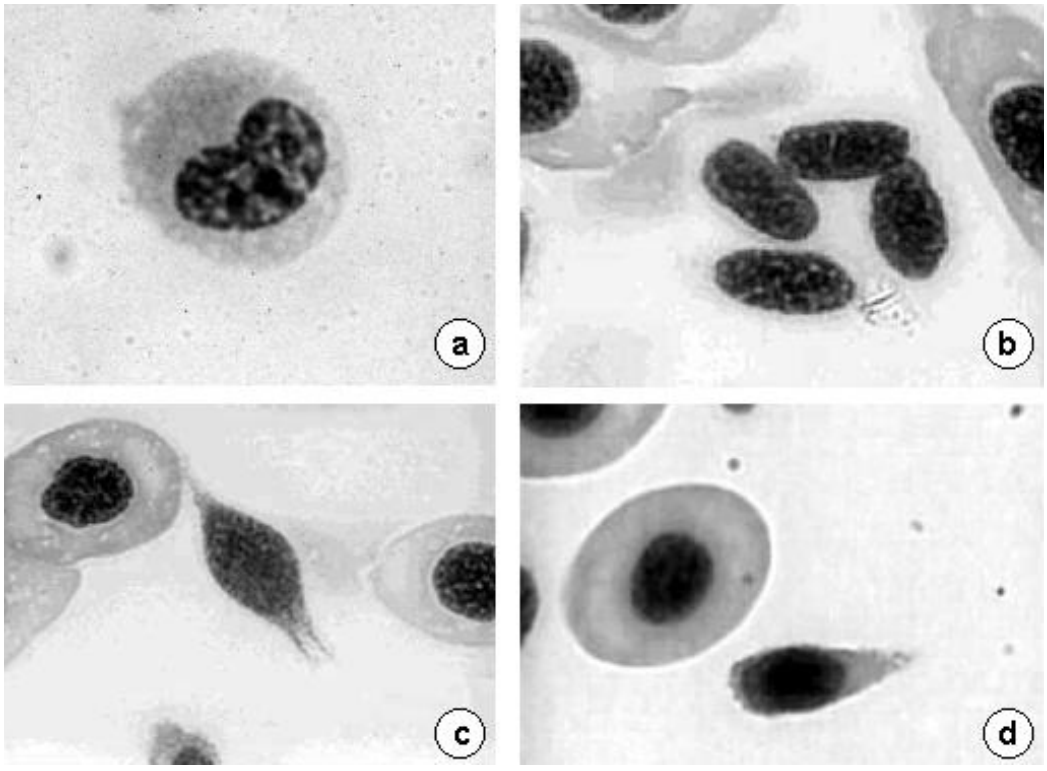


Figure 23.7. Peripheral blood smears showing a representative monocyte from a blacktip shark, *Carcharhinus limbatus* (**a**), clumped thrombocytes from a nurse shark, *Ginglymostoma cirratum* (**b**), and spindle-shaped thrombocytes from a blacktip shark (**c**), and a bull shark, *Carcharhinus leucas* (**d**). Original magnification, 1,000x.

identification, but complicates differential counting procedures. Unlike platelets, thrombocytes are nucleated and can assume a variety of shapes, including spindle-shaped, elliptical, or round (Stokes and Firkin, 1971; Fänge, 1987) (Figures 23.7c and 23.7d). The shape may vary with the stage of maturity or degree of reactivity. Mature thrombocytes are generally elliptical and are smaller than erythrocytes. In general, thrombocyte nuclei are larger in relation to the amount of cytoplasm, and more round, than erythrocyte nuclei. Nuclei stain dark purple with dense and clumped chromatin, while the cytoplasm is clear and colorless to pale blue, and is only visible as a faint rim around the nucleus. Visually, the distinction between small lymphocytes and thrombocytes is often difficult, although thrombocytes are typically smaller and more darkly staining than lymphocytes. In some species, thrombocytes are conspicuous by the presence of numerous small red spherical granules. An easily recognizable and fairly common form of the thrombocyte is an elongated spindle-shaped cell, with long spicules extending from one or both ends of the cell.

BLOOD COLLECTION

If blood is to be used for research or for assessment of health, it is recommended that the sample be taken using a sterile disposable needle and syringe. Depending upon whether a particular test requires serum, plasma, or intact cells, the sample can be collected in the presence or absence of an anticoagulant. If serum is required, blood is collected in the absence of an anticoagulant and a clot is allowed to form. Subsequent removal of the clot by centrifugation will yield a supernatant fluid called serum, which is devoid of all clotting factors.

If plasma or intact cells are required, the collected blood should be mixed with an anticoagulant solution, preventing the formation of a clot. The supernatant fluid remaining after sedimentation of intact blood cells by centrifugation is called plasma. If it is known that the blood of a particular species will coagulate rapidly, or if the clotting dynamics of a particular species are not known, it is advisable to coat the syringe with the anticoagulant prior to collection to prevent clotting of the sample prior to mixing with the remaining anticoagulant. If premature clotting is not a problem, the blood can be drawn into an uncoated syringe before mixing with the anticoagulant. Resuspending or mixing of the blood sample, whether anticoagulant is added or not, should be performed by gentle inversion of the container (i.e., culture tube or centrifuge tube) to avoid disruption of the cells. Any reddish coloration in the resulting serum or plasma is an indication of hemolysis, in which hemoglobin is released from ruptured erythrocytes into the otherwise clear supernatant fluid.

Since hemolysis can result from osmotic shock, it is recommended that anticoagulants be balanced for elasmobranch osmolarity. Recipes for two anticoagulant solutions that prevent clotting of elasmobranch blood without hemolysis are included in Table 23.1. One solution combines two anticoagulant compounds, heparin and EDTA. The combination has proven to be more effective with elasmobranch blood than either compound used individually, especially if leukocytes are the desired product. The second solution, known as ACD solution “A”, is commonly used when collecting human blood for transfusions. This solution combines citric acid, sodium citrate, and dextrose. It must be noted that use of either anticoagulant will increase the final volume of the blood sample, necessitating a correction factor if volume is a critical parameter in a particular measurement.

The most common method of obtaining blood from small to medium sized sharks is through caudal venipuncture (Stoskopf et al., 1984). The caudal vein lies ventral to the caudal artery, both vessels encased in the hemal arch of the caudal vertebrae (Figure 23.8a). The animal should be restrained with the ventral side up, care being taken not to injure the gill regions. While steadying the tail with one hand (Figure 23.8b), the needle should enter the tail at the ventral midline and remain as close to a midline position as possible during penetration of the muscle until the vertebral column is reached. Slight penetration of the caudal vertebrae will allow access to the caudal vein. For most small to medium sharks, a 38 mm, 18-20 gauge needle is adequate.

When sampling large sharks, restraining a conscious animal may be impractical, necessitating light anesthesia with MS-222 (tricaine methanesulfonate) (Gilbert and Wood, 1957) prior to penetrating the tail. Alternatively, blood may be obtained from lightly sedated large sharks through a vascular sinus behind the dorsal fin. Since the precise location and size of this sinus depends on the species, this method meets with varying success and generally requires considerable practice.

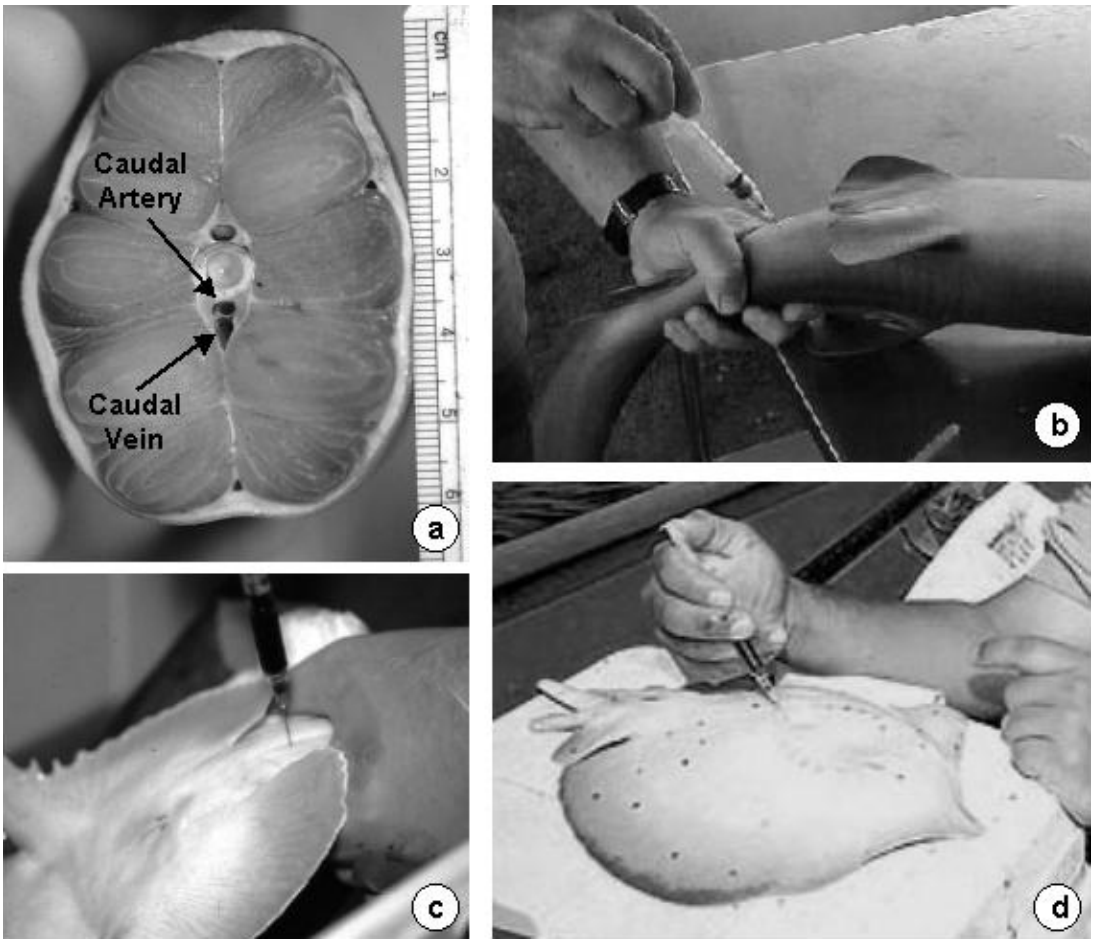


Figure 23.8. Cross section through the tail of a nurse shark, *Ginglymostoma cirratum*, showing location of caudal artery and vein encased within the hemal arch of the caudal vertebrae (**a**). Recommended positioning of syringe to obtain blood from caudal artery/vein from small to medium sized sharks (**b**) and from batoids possessing substantial tail structure (**c**). Batoids with whip-like tails can be bled via cardiac puncture (**d**) penetrating the ventral surface of the body immediately anterior to the pectoral girdle and at a slight angle toward the rostrum.

Blood can be collected from the caudal artery from batoids possessing substantial tail structures (i.e., skates, guitarfish, sawfish, and certain rays), although a 25.5 mm, 23-24 gauge needle is recommended due to the reduced diameters of blood vessels relative to those in sharks. In skates, the caudal artery/vein complex can be accessed through intervertebral spaces visible along the ventral surface when the tail is gently arched (Figure 23.8c). In those batoid species with whip-like tails, the reduction of circulating blood to this area makes the caudal artery/vein complex a poor option. In these species, cardiac puncture can be used (Figure 23.8d), although administration of light anesthesia with MS-222 may be necessary if restraining a conscious specimen proves to be difficult or potentially stressful to the animal. With practice, any of the methods described can be safely performed, although due to the risk of damage to the heart, cardiac puncture is only recommended when other options are impractical.

PRACTICAL APPLICATIONS

Preparation of blood smears

For general cytological staining, thin smears of peripheral blood are preferred. Use of bevel-edged slides to perform the smearing action is recommended. Compared with the sharper edges found on typical microscope slides, bevel-edge slides have rounded edges, reducing cell disruption resulting from mechanical stress. Granulocytes are particularly susceptible to mechanical stress, but “smudging” of other cells can occur. Even when using “pre-cleaned” slides, slides should be wiped with 70% ethanol using a tissue to remove fingerprints, lint, or any other surface contaminants.

A small (<5 mm in diameter) drop of blood should be carefully placed on the slide near the frosted end. Larger drops of blood will result in blood smears that are too thick. Steadying the frosted end of the slide with one hand, use the other hand to place the leading edge of the bevel-edge slide just in front of the blood drop at an angle approximately 45° to the slide. Carefully move the slide backwards until it just makes contact with the drop, allowing the drop to spread evenly across the edge of the bevel. Using a smooth, even motion and while applying light, but steady, pressure, glide the bevel-edge slide towards the opposite end of the slide. This action distributes the blood in a thin film across the surface of the microscope slide. The drop should not remain on the slide too long before smearing since the blood will dry quickly, or possibly clot, interfering with the smearing process and distribution of cells across the slide. A well-prepared smear should occupy approximately three-quarters of the surface of the slide, with the leading edge of the blood film having a "feathered" appearance. The "feathering" indicates that the cells are distributed on the slide as a monolayer, an optimal condition for examining blood cells.

Fixing and staining of blood smears

To preserve the morphology of the cells, blood smears must either be fixed in cold (4°C), 100% methanol for five minutes or stained as soon as possible after they have dried. It is essential that blood smears do not come in contact with water before fixation is complete. Once they are fixed, slides can be stored indefinitely before staining if they are kept free of dust or other contaminants. Methanol is the preferred fixative, although ethanol can be used as well. To prevent the fixative from absorbing water, keep it in a tightly sealed container, or seal the lid of a staining jar with stopcock grease. It may be necessary to replace the methanol frequently in humid climates and if many smears are prepared in one day.

Romanowsky stains, such as Wright's or Giemsa, are typically used for visualizing elasmobranch blood smears, although other staining protocols can be used. The results with Wright's or Giemsa stains are similar. Both are available from chemical supply companies as prepared stains, eliminating such problems as batch-to-batch variability and presence of precipitated or undissolved crystals commonly encountered if these stains are self-prepared using published recipes.

Immediately after blood smears are completely dry, place slides in a staining rack and place the rack in a staining tray containing cold (4°C) 100% methanol for five minutes. Allow slides to air-dry after fixation. Place the slides on a horizontal staining rack and immerse in Wright's stain. Allow stain to remain on the slides for 8 minutes and 30 seconds, then rinse each slide individually with distilled water. Place slides on a paper towel in a position where they can drain vertically. Allow slides to dry completely before viewing or applying a cover slip. Staining slides with Giemsa stain gives good results with elasmobranch blood smears. For this procedure, place dry, unfixed slides in a staining jar containing Giemsa stain for one to two minutes. Rinse slides in gently running tap water for 2 minutes, followed by an additional quick rinse with distilled water. Allow to air-dry vertically, as before, while draining.

After the slides have completely dried, cover slips may be applied. Covering the smears with a cover slip allows examination of the smear with all magnification microscope lenses, including oil immersion. A rectangular cover slip that is wider and longer than the smear works best, allowing cells at the edges of the smear to be properly examined. Cover slips help preserve the smear during storage and multiple viewings. Cover slips can be attached with a thin layer of a toluene based, low viscosity mounting media, applied in a thin line down the center of a completely dry, stained blood smear. Align one edge of a cover slip to the edge of the blood slide at an ~45° angle. Gently lower the cover slip until it comes in contact with the thin line of mounting media, which should spread evenly under the edge of the cover slip. As the mounting media spreads, continue lowering the cover slip until both mounting media and cover slip completely cover the slide. Place the slide on a flat surface, and gently tap on the cover slip using the eraser end of a pencil or other similar object to facilitate the even spreading of mounting media. Small air bubbles can be removed using this method. Too much mounting media will impair the ability to focus through a microscope, while too little mounting media will result in inadequate coverage of the slide. Excess mounting media exuding from under the cover slip can be removed with a razor blade after being allowed to dry.

Obtaining differential cell counts

Microscopic examination of a blood smear can provide information about the relative numbers and varieties of the five basic leukocyte types.

This information is referred to as a differential cell count. In some elasmobranch literature, thrombocytes are considered as a distinct leukocyte type and are included as part of a leukocyte differential (Saunders, 1966; Sherburne, 1974; Fänge, 1994), while other literature recognizes thrombocytes as a specific cell type distinct from erythrocytes and leukocytes (Campbell and Murru, 1990; Stoskopf, 1993). Since thrombocytes in fish respond to physiological situations in much the same manner as platelets in mammals (Stoskopf, 1993), it makes sense to include them in hematological evaluations. Total thrombocyte counts may be more useful than differential thrombocyte counts, however, due to the tendency of thrombocytes to clump in smears and vary greatly in number.

Before beginning a differential cell count, conduct an initial survey of the smear using low power (100x to 200x) to assess stain quality and cell distribution, then use a higher magnification (400x) to evaluate the staining characteristics of the different cell types. During this phase, cells characteristic of the different leukocyte types should be identified and used as standards for eventual decisions regarding ambiguous cells. The differential cell count can be performed under "high-dry" magnification (400x) or oil immersion (1000x). Using oil immersion facilitates analysis of nuclear and granule characteristics, which may aid in leukocyte identification. Several methods exist for canvassing the slide in order to produce a consistent and representative count. A traditional method, known as the battlement method, examines horizontal fields along the edges of the smear (MacGregor et al., 1940). Crossing back and forth across the smear in a zigzag pattern is an accepted procedure. Whatever method is chosen, it is essential to be consistent and to cover a large area of the slide in order to obtain the most representative cell count.

The question of how many cells should be counted to determine a differential cell count has not yet been resolved (Stoskopf, 1993). The differential count is subject to considerable error because only a small number of leukocytes are counted in relation to the total sample, even in the most thorough counts. The distribution of cells on the smear and in the areas counted greatly influences the results obtained. Obviously, counting a greater number of cells allows more representative cell percentages to be obtained, but performing differential cell counts is time-consuming, and counting large numbers of cells per smear limits the total number of samples that can be counted. Stoskopf (1993) suggests that 200 leukocytes should be counted in routine differential counts, except in cases where a low number of leukocytes in the smear would make achieving 200 cells difficult and the count considerably more time-consuming. In this situation, 100 cells are sufficient.

The procedure used by the authors is to count the number of leukocytes present per 1000

Table 23.2. Representative values for hematology parameters from a variety of elasmobranch species. Values are reported as the mean ± SE, with the range given in parentheses. [a] n = 4; [b] n = 8; [c] n = 24.

	Clearnose skate (*Raja eglanteria*) n = 7	Atlantic stingray (*Dasyatis sabina*) n = 10	Blacktip shark (*Carcharhinus limbatus*) n = 15	Nurse shark (*Ginglymostoma cirratum*) n = 13	Bonnethead shark (*Sphyrna tiburo*) n = 7
Total Erythrocytes per ml ($x10^8$)		2.19 ± 0.41[a] (1.15 - 3.05)	4.76 ± 0.27 (3.06 - 6.60)		
Hematocrit (%)	22.4 ± 4.2 (16 - 27)		22.8 ± 1.3[b] (17 - 28)	24.7 ± 3.7[c] (17 - 30)	23.3 ± 3.5 (18 - 27)
Total Leukocytes per ml ($x10^8$)		0.55 ± 0.15 (0.24 - 0.84)	1.95 ± 0.18 (1.03 - 2.85)		
Differential Counts:					
% Granulocytes	21.4 ± 3.6 (15 - 40)	29.3 ± 2.7 (17 - 42)	24.8 ± 2.2 (12 - 46)	24.5 ± 2.2 (11 - 38)	
% Lymphocytes	77.4 ± 3.5 (59 - 83)	69.1 ± 2.6 (56 - 83)	72.5 ± 2.5 (47 - 86)	73.3 ± 2.2 (62 - 87)	
% Monocytes	1.3 ± 0.3 (0 - 3)	1.6 ± 0.4 (0 - 4)	2.8 ± 0.6 (0 - 4)	2.2 ± 0.5 (0 - 4)	

erythrocytes, using oil immersion and a final magnification of 1000x. Using a mechanical tallying device, label individual tally buttons with various cell types to be counted, including erythrocytes. After completing the field in which the erythrocyte count reaches 1000, calculate the relative contribution of each leukocyte type as a percentage of the total number of leukocytes counted. Granulocytes are typically grouped as a single cell type for this evaluation. (Example: total erythrocytes, 1,029; total leukocytes, 61; granulocytes, 20; lymphocytes, 40; and monocytes, 1: resulting in a differential cell count of 32.8% granulocytes, 65.6% lymphocytes, and 1.6% monocytes). Representative values for differential cell counts from several elasmobranch species are shown in Table 23.2.

Cell counts per measured volume

While differential cell counts provide information on relative amounts of leukocyte types, determining cell numbers per volume of blood gives absolute quantities. Elevations (leukocytosis) or reductions (leukopenia) outside the normal ranges in leukocyte numbers for a particular species are indicative of infection or inflammation, while reductions in erythrocytes (anemia) can be symptomatic of various diseases (Blaxhall and Daisley, 1973). Monitoring hematocrits (relative volume of erythrocytes in a given volume of blood) is a simple method to assess the health status of an animal (Blaxhall and Daisley, 1973). Representative values for total leukocyte counts, total erythrocyte counts, and hematocrits from several elasmobranch species are shown in Table 23.2.

The solution recommended for use when determining total cell counts per volume of whole blood is Natt-Herrick solution (Natt and Herrick, 1952), modified for elasmobranch osmolarity (Table 23.1). Using a sterile pipette tip, add 10 µl of elasmobranch whole blood containing anticoagulant to 1,990 µl Natt-Herrick solution. A 1:200 dilution of whole blood to Natt-Herrick solution is often adequate, but may vary with species and should be determined empirically.

When Natt-Herrick solution is used, the staining characteristics of the cell types are different from those described earlier when stained with Romanowsky stains, such as either Wright's or Giemsa stains. When stained with Natt-Herrick solution, erythrocytes appear as oval cells with a dark purple oval nucleus surrounded by a light purple or nearly colorless cytoplasm. Granulocytes stain a dark purple, although different types of granulocytes will not be distinguishable. Thrombocytes stain light purple, but are usually a little more darkly stained than red blood cells. Lymphocytes stain dark purple with no visible cytoplasm. Lymphocytes will be smaller in size than either the granulocytes or monocytes. Small lymphocytes can be difficult to differentiate from round thrombocytes, but will generally stain more darkly than thrombocytes. If there is uncertainty, peripheral blood smears can be viewed to assess the relative presence of small lymphocytes versus thrombocytes in a particular sample. Monocytes stain a pale purple and will be larger than the other cell types.

To obtain total cell counts, a device called a hemacytometer is used. Specially designed cover slips must be used to ensure even distribution and coverage of counting chambers. In order to fill the hemacytometer chamber properly by capillary action, the cover slip, chamber, and pipette used to fill the chamber must be clean. Prior to each use, the chamber and cover slip should be cleaned with distilled water followed by 95% ethanol, then wiped dry with a clean lint-free tissue. It is important that the cell suspension be thoroughly but gently mixed before adding to the hemacytometer, allowing the cells to settle in the chamber for a few minutes before counting the different cell types.

The Improved Neubauer hemacytometer, with its two x 0.1 mm deep counting chambers, is the most frequently used hemacytometer. Each chamber contains an etched grid divided into nine 1.0 mm x 1.0 mm squares (Figure 23.9). The center square millimeter is ruled into twenty-five 0.2 x 0.2 mm squares containing sixteen 0.05 x 0.05 mm squares. The 0.2 mm squares in the four corners and the 0.2 mm square in the center (labeled "E" on diagram in Figure 23.9) are used to count erythrocytes. When the hemacytometer cover slip is placed over the chambers, the total volume over all five of the 0.2 mm squares used to count erythrocytes is 0.02 mm^3, or 2 x 10^{-5} cm^3. Taking into account any dilution used in preparing the blood sample for counting, the total number of erythrocytes per milliliter will be the number of erythrocytes counted in the five smaller (0.2 mm) squares multiplied by 5 x 10^4. (Example erythrocyte count: total erythrocytes in 5 smaller (0.2 mm) squares = 41; if dilution of blood sample is 1/200, then total erythrocytes per ml = 41 x 200 x (5 x 10^4) = 4.1 x 10^8. Total erythrocytes can be obtained by multiplying the cells per milliliter by the volume of original blood sample.)

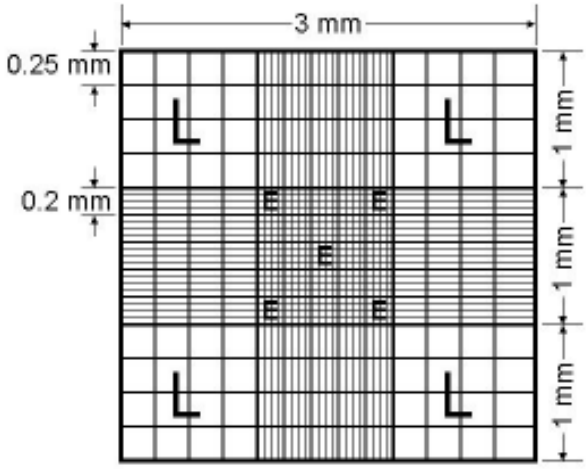


Figure 23.9. Diagrammatic representation of the grid pattern in an Improved Neubauer hemacytometer. The grid is divided into nine 1.0 mm x 1.0 mm squares. The 1.0 mm squares in the four corners of the counting chamber (L) are used to perform leukocyte counts. The 1.0 mm square in the center is divided further into twenty-five 0.2 x 0.2 mm squares containing sixteen 0.05 x 0.05 mm squares. The four 0.2 mm squares in the four corners and the 0.2 mm square in the center (E) are used to count erythrocytes.

The 1.0 mm squares in the four corners of the counting chamber (labeled "L" on diagram in Figure 23.9) are used to count leukocytes. (Note: Depending upon personal preference, as many as all nine of the 1.0 mm squares can be used in the count.) The total volume over each of the 1.0 mm squares is 0.1 mm^3, or 10^{-4} cm^3. Taking into account any dilution used in preparing the sample for counting, the total number of leukocytes per milliliter will be the average count per 1.0 mm square multiplied by 10^4. (Example leukocyte count: average number of leukocytes per 1.0 mm square = 27; if dilution of sample is 1/200, then total leukocytes per ml = 27 x 200 x 10^4 = 5.4 x 10^7. Total leukocytes can be obtained by multiplying the cells per milliliter by the volume of original blood sample.)

Isolation of peripheral blood leukocytes

Whole blood is collected in the presence of anticoagulant, as described in the section entitled "Blood collection," and transferred to sterile centrifuge tubes. Peripheral blood leukocytes (PBL) are isolated through repetitive slow speed centrifugation at room temperature using 5-10 ml volumes of whole blood and centrifuging at ~50x G for 10-15 minutes per spin. Smaller volumes of blood are difficult to separate, but separation can be achieved by shortening the repetitive spins to three minutes in duration. Slow speed centrifugation results in the gradual sedimentation of erythrocytes while PBL remain suspended, but concentrated, above the red cell layer. If leukocyte separation is not adequate, centrifugation can be increased to 100x G, but speeds greater than this will result in the "sticking" of PBL to erythrocytes and inadequate separation of leukocytes from erythrocytes. If erythrocytes are still present in the white cell layer after the first centrifugation step, aspirate the "buffy" layer, transfer to a fresh 15 ml centrifuge tube, and spin at 50-100x G for an additional 10-15 minutes. Once erythrocytes are sufficiently separated from leukocytes, aspirate the suspended PBL, dilute to the original blood volume using E-PBS, and wash at least once by centrifuging at 200x G for seven minutes. Following the wash step, the cell pellet should be carefully resuspended in E-PBS or cell culture medium.

Isolated elasmobranch PBL tend to aggregate readily. Once a cell aggregation forms, it is difficult to disperse. Aggregation can be minimized by resuspending only a small portion of the cell pellet at a time, using a minimal volume of dilution buffer. Repeat with additional small volumes of dilution buffer until the entire cell pellet is resuspended. Cells can be diluted to the desired concentration. Resuspension of cell pellets immediately after removal from the centrifuge, without allowing tubes to sit for any length of time, will reduce the risk of aggregation. Although it is possible to use concentrations greater than 5 x 10^6 cells ml^{-1}, it is recommended that the length of time that cells remain at this concentration, or greater, be kept to a minimum to prevent the irreversible formation of cell aggregates.

The leukocyte population resulting from this procedure is a mixed population and contains all non-erythroid cell types, including thrombocytes. Currently, no procedures have been developed for separating leukocyte subpopulations from elasmobranch blood. Commercially available cell separation media, such as Ficoll or Histopaque, do not achieve separation of elasmobranch blood cell populations, primarily due to differences in cell sizes, cell densities, and osmotic conditions.

Determining viability of elasmobranch PBL

For certain blood tests or assays that rely on functional leukocytes, it is critical to assess the viability (percentage of live cells versus dead cells) of the cells in the sample being tested. To

perform viability assessments, vital stains such as trypan blue are commonly used. When exposed to trypan blue, non-viable cells will take up the dye and appear blue, while live cells will not incorporate the dye and remain colorless. Since viable cells will eventually absorb the dye, it is important to prepare dilutions with trypan blue and perform counts immediately.

To determine cell viability of isolated elasmobranch PBL, dilute 50 µl of isolated PBL using a sterile pipette tip with 450 µl E-trypan blue (Table 23.1). This 1:10 dilution is suitable for most elasmobranch blood samples, although other dilutions can be prepared based on the expected cell density. Mix thoroughly and apply stained cells to one chamber of an Improved Neubauer hemacytometer as described previously. Count all the PBL in the 1.0 mm squares at the four corners of the grid (labeled "L" on diagram in Figure 23.9). The number of blue-stained cells divided by the total number of cells multiplied by 100 will give an estimate of the percentage of dead cells in the sample. To obtain an estimate of the percentage of viable cells, subtract the number of dead cells from 100. (Percent viability = 100 - # viable cells counted ÷ total # of cells counted x 100). If time is limited, a quicker but less accurate method is to stop when 100 leukocytes have been counted and determine how many of this number accumulated the dye (% dead) and how many did not (% viable). If fewer than 100 cells are present in the four squares, repeat with a more suitable dilution factor.

ACKNOWLEDGEMENTS

The authors express their gratitude to the organizers of the 1st International Elasmobranch Husbandry Symposium for the opportunity to prepare this chapter for the Elasmobranch Husbandry Manual. The authors gratefully acknowledge the use of facilities at Mote Marine Laboratory, where the research on which the hematological material presented in this chapter was performed. Special appreciation is extended to David Noyes and Tom Story for their assistance with the maintenance of captive elasmobranchs. Partial support for some of this research was provided to CJW through a grant from the National Science Foundation and to CAL through grants from the Vernal W. and Florence Bates Foundation and the Disney Wildlife Conservation Fund.

REFERENCES

Abbas, A. K., A. H. Lichtman, and J. S. Pober. 1991. Cellular and Molecular Immunology. W. B. Saunders, Company, Philadelphia, Pennsylvania, USA. 576 p.

Andrew, W. and C. P. Hickman. 1974. Histology of the Vertebrates: A Comparative Text. C. V. Mosby Company, St. Louis, Missouri, USA. 439 p.

Blaxhall, P. C. and K. W. Daisley. 1973. Routine hematological methods for use with fish blood. Journal of Fish Biology 5: 771-782.

Brostoff, J. and T. Hall. 1989. Hypersensitivity – Type I. *In:* Immunology, p. 19.1-19.20. I. Roitt, J. Brostoff, and D. Male (eds.). Harper and Row Publishers, New York, USA.

Campbell, T. W. 1988. Avian Hematology and Cytology. The Iowa State University Press, Ames, Iowa, USA. 101 p.

Campbell, T. and F. Murru. 1990. An introduction to fish hematology. The Compendium 12: 525-532.

Ellis, A. E. 1977. The leucocytes of fish: A review. Journal of Fish Biology 11: 453-491.

Ellsaesser, C. F., N. W. Miller, and M. A. Cuchens. 1985. Analysis of channel catfish peripheral blood leukocytes by bright-field microscopy and flow cytometry. Transactions of the American Fisheries Society 114: 279-285.

Fänge, R. 1987. Lymphomyeloid system and blood cell morphology in elasmobranchs. Archives of Biology 98: 187-208.

Fänge, R. 1994. Blood cells, haemopoiesis and lymphomyeloid tissues in fish. Fish and Shellfish Immunology 4: 405-411.

Fänge, R. and A. Mattisson. 1981. The lymphomyeloid (hemopoietic) system of the Atlantic nurse shark, *Ginglymostoma cirratum*. Biological Bulletin 160: 240-249.

Fänge, R. and A. Pulsford. 1983. Structural studies on lymphomyeloid tissues in the dogfish, *Scyliorhinus canicula* L. Cell and Tissue Research 230: 337-351.

Gilbert, P. W. and F. G. Wood, Jr. 1957. Method of anesthetizing large sharks and rays safely and rapidly. Science 126: 212-213.

Hart, S., A. B. Wrathmell, J. E. Harris, and T. H. Grayson. 1988. Gut immunology in fish: A review. Developmental and Comparative Immunology 12: 453-480.

Hiemstra, P. S. 1993. Role of neutrophils and mononuclear phagocytes in host defense and inflammation. Journal of the International Federation of Clinical Chemistry 5: 94-99.

Hine, P. M. 1992. The granulocytes of fish. Fish and Shellfish Immunology 2: 79-98.

Hine, P. M. and J. M. Wain. 1987. The enzyme cytochemistry and composition of elasmobranch granulocytes. Journal of Fish Biology 30: 465-476.

Honma, Y., K. Okabe, and A. Chiba. 1984. Comparative histology of the Leydig and epigonal organs in some elasmobranchs. Japanese Journal of Ichthyology 31: 47-54.

Hyder, S. L., M. L. Cayer, and M. L. Pettey. 1983. Cell types in peripheral blood of the nurse shark: An approach to structure and function. Tissue and Cell 15: 437-455.

Kendall, C., S. Valentino A. B. Bodine, and C. A. Luer, 1992. Flow cytometric DNA analysis of nurse shark, *Ginglymostoma cirratum* (Bonaterre) and clearnose skate, *Raja eglanteria* (Bosc) peripheral red blood cells. Journal of Fish Biology 41: 123-129.

Lloyd-Evans, P. 1993. Development of the lymphomyeloid system in the dogfish, *Scyliorhinus canicula*. Developmental and Comparative Immunology 17: 501-514.

Lucas A. M. and C. Jamroz. 1961. Atlas of Avian Hematology. U. S. Department of Agriculture, Agriculture Monograph 25, Washington, D.C., USA 271 p.

Luer, C. A., C. J. Walsh, A. B. Bodine, J. T. Wyffels, and T. R. Scott. 1995. The elasmobranch thymus: Anatomical, histological, and preliminary functional characterization. Journal of Experimental Zoology 273: 342-354.

Lydyard, P. and C. Grossi. 1989. Cells involved in the immune response. *In:* Immunology, p. 2.1-2.18. I. Roitt, J. Brostoff, and D. Male (eds.). Harper and Row, Publishers, New York, USA.

MacGregor, R. G. S., W. Richards, and G. L. Loh. 1940. The differential leukocyte count. Journal of Pathology and Bacteriology 51: 337-368.

Mattisson, A. and R. Fänge. 1982. The cellular structure of the Leydig organ in the shark, *Etmopterus spinax* (L.). Biological Bulletin 162: 182-194.

Natt, M. P. and C. A. Herrick. 1952. A new blood diluent for counting the erythrocytes and leukocytes of the chicken. Poultry Science 31: 735-738.

Parish, N., A. Wrathmell, S. Hart, and J. E. Harris. 1986a. The leucocytes of the elasmobranch *Scyliorhinus canicula* L. - A morphological study. Journal of Fish Biology 28: 545-561.

Parish, N., A. Wrathmell, S. Hart, and J. E. Harris. 1986b. Phagoyctic cells in the dogfish, *Scyliorhinus canicula* L. I. In vitro studies. Acta Zoologica (Stockholm) 67: 215-224.

Parish, N., A. Wrathmell, S. Hart, and J. E. Harris. 1986c. Phagoyctic cells in the peripheral blood of the dogfish, *Scyliorhinus canicula* L. II. In vivo studies. Acta Zoologica (Stockholm) 67: 225-234.

Rowley, A. F., T. C. Hunt, M. Page, and G. Mainwaring. 1988. Fish. *In:* Vertebrate blood cells, p.19-127. A. F. Rowley and N. A. Ratcliffe (eds.). Cambridge University Press, Cambridge, England.

Saunders, D. C. 1966. Elasmobranch blood cells. Copeia 66: 348-351.

Sherburne, S. W. 1974. Occurrence of both heterophils and neutrophils in the blood of the spiny dogfish, *Squalus acanthias*. Copeia 74: 259-261.

Stokes, E. E. and B. G. Firkin. 1971. Studies of the peripheral blood of the Port Jackson shark (*Heterodontus portusjacksoni*) with particular reference to the thrombocyte. British Journal of Haematology 20: 427-435.

Stoskopf, M. K. 1993. Clinical pathology. *In:* Fish Medicine, p. 113-131. M. K. Stoskopf (ed.). W. B. Saunders Company, Philadelphia, Pennsylvania, USA.

Stoskopf, M., B. Smith, and G. Klay. 1984. Clinical note: Blood sampling of captive sharks. Journal of Zoo Animal Medicine 15: 116-117.

Taverne, J. 1989. Immunity to protozoa and worms. *In:* Immunology, p. 17.1-17.21. I. Roitt, J. Brostoff, and D. Male (eds.). Harper and Row Publishers, New York, USA.

Tomonaga, S., K. Kobayashi, K. Hagiwara, K. Yamaguchi, and K. Awaya. 1986. Gut-associated lymphoid tissue in the elasmobranchs. Zoological Science 3: 453-458.

Walsh C. J. and C. A. Luer. 1998. Comparative phagocytic and pinocytic activities of leukocytes from peripheral blood and lymphomyeloid tissues of the nurse shark (*Ginglymostoma cirratum* Bonaterre) and the clearnose skate (*Raja eglanteria* Bosc). Fish and Shellfish Immunology 8: 197-215.

Zapata, A. 1980a. Ultrastructure of elasmobranch lymphoid tissue. I. Thymus and spleen. Developmental and Comparative Immunology 4: 459-472.

Zapata, A. 1980b. Ultrastructure of elasmobranch lymphoid tissue. II. Leydig's and epigonal organs. Developmental and Comparative Immunology 5: 43-52.

Zapata, A. and A. Carrato. 1981. Ultrastructure of elasmobranch and teleost erythrocytes. Acta Zoologica 62: 129-135.

Zapata, A. G., M. Torroba, R. Sacedon, A. Varas, and A. Vicente. 1996. Structure of the lymphoid organs of elasmobranchs. Journal of Experimental Zoology 275: 125-143.

The Elasmobranch Husbandry Manual: Captive Care of Sharks, Rays and their Relatives, pages 325-416.
© 2004 Ohio Biological Survey

Chapter 24
Metazoan Parasites and Associates of Chondrichthyans with Emphasis on Taxa Harmful to Captive Hosts

GEORGE W. BENZ

Department of Biology,
P.O. Box 60, Middle Tennessee State University,
Murfreesboro, Tennessee, U.S.A.
E-mail: gbenz@mtsu.edu

STEPHEN A. BULLARD

Gulf Coast Research Laboratory,
Department of Coastal Sciences, The University of Southern Mississippi,
703 East Beach Drive,
Ocean Springs, Mississippi, U.S.A.
E-mail: ash.bullard@usm.edu

Abstract: Metazoan symbionts have significantly impacted captive chondrichthyans. However, although disease in these fishes could be caused by numerous parasite taxa under unusual conditions, only a handful of parasites routinely do so. Monogeneans, leeches, and fish lice are problematic taxa because at least some of their representatives cause health problems for elasmobranchs in captive settings. Nematodes, copepods, and isopods are considered emerging problematic taxa because of their suspected potential to cause disease in captive chondrichthyans. The remaining taxa that infect or otherwise associate with wild chondrichthyans, i.e., some species of myxozoans, turbellarians, digeneans, aspidogastreans, cestodes, acanthocephalans, barnacles, ostracods, amphipods, molluscs, and fishes, are unproblematic taxa in captive settings. Identification of symbionts to taxonomic levels with functional significance regarding husbandry practices is a critical first step in the control of infectious disease. Unnecessary manipulation of hosts and life support systems to eradicate unproblematic symbionts wastes operational resources and can endanger hosts and their communities. Understanding the life histories of parasite species as well as the interactions among parasites, hosts, and their environments facilitates sound husbandry decisions.

Sharks, batoids, and chimaeroids, i.e., chondrichthyans,[1] each host a great variety of metazoan parasites in nature (Cheung, 1993), and chondrichthyan species are probably far outnumbered by the metazoan species that exclusively infect them (Benz, 1995). The present authors' field examinations of thousands of elasmobranchs suggest that many, if not most, individuals are infected by at least one metazoan species, and concurrent infections of five or more species on a single shark have been documented (Hewitt, 1979; Benz et al., 2003). As free-ranging chondrichthyans are captured and transported into captivity,[2] they typically ferry their parasites with them.

[1] Nomenclature and taxonomy used throughout this chapter follows Compagno (1999) for elasmobranchs, FishBase (see http//www.fishbase.org) for other fishes, and numerous authoritative sources for symbionts. Those needing definitions of various biological terms used throughout this chapter should consult Lincoln et al. (1998).

[2] The terms "captivity" and "captive setting," and their derivatives, herein refer to the maintenance of animals in man-made enclosures that include but are not limited to: net pens and other confinements with direct or semidirect connection to natural aquatic systems as well as public, private, and research aquariums. By itself, the term "aquarium" refers to a form of confinement rather than a type of institution, and the term "husbandry staff" refers to anyone who provides care for captive animals.

Metazoan parasites can significantly impact the well being of captive chondrichthyans in several ways. Most obvious are instances when parasites possess the capacity to kill or debilitate these fishes. But even when infected fishes are not in immediate mortal danger, some infections can promote emotionally disturbing host behaviors or aesthetic appearances that can negatively affect the exhibition quality of fishes, and some parasites of fishes are capable of biasing research results (Pascoe and Cram, 1977). Additionally, the anticipated, presumed, or actual presence of parasites often triggers a variety of prophylactic or parasiticidal treatments and host manipulations that can be expensive and may ultimately harm the host and its community. Thus, identifying potential pathogens, preventing or restricting pathogen entry into confined environments, and eradicating pathogens or reducing their presence within host populations are all relevant to the health and appearance of captive fishes— and ultimately to the financial success of the institutions that maintain fishes in captivity.

This chapter is primarily an overview of the metazoan, i.e., multi-cellular animal, parasites that infect chondrichthyans, and it emphasizes taxa that can promote disease in captive hosts. The chapter focuses mainly on elasmobranchs (Elasmobranchii) as hosts, but information on chimaeroids (Chimaeroidei, Holocephali) is also included because of the close phylogenetic relationship between these taxa (Lund and Grogan, 1997) and because Chimaeroidei cradles a small (34 spp. according to Didier, 1995) extant assemblage of peculiar deep-water fishes that in the future will likely be more widely exhibited in public aquariums and maintained at research facilities.

The body of knowledge regarding the metazoan parasites of chondrichthyans is staggeringly large and widely scattered in many languages throughout the literature. This chapter was written as a contemporary synthesis, with the intent that it can serve as an initial source of information and portal to the literature for those with husbandry responsibilities. Readers should be aware, however, that many relevant topics were omitted from this work due to space constraints and that new data germane to this chapter are continually published. The chapter was endowed with several short sections on the general nature of parasites and parasitism because the authors believe that too few husbandry staff are well versed in these important matters, and even most veterinarians have little academic training in Parasitology. In addition to this tutorial, readers are encouraged to train themselves more fully by consulting contemporary Parasitology texts such as Noble et al. (1989), Esch and Fernández (1993), Williams and Jones (1994), Woo (1995), Poulin (1998), Roberts and Janovy (2000), Bush et al. (2001), and Combes (2001).

Pragmatic readers may be irritated because this chapter contains at least minor mention of all major metazoan taxa known to infect or similarly associate with chondrichthyans (Table 24.1). The decision to do so was prompted by the authors' beliefs that parasite identification should be a primary husbandry concern and that a general familiarity with all of the major taxa that infect or associate with these fishes facilitates accurate taxonomic identifications. Furthermore, little is known about most of the parasites and associates of chondrichthyans, and hence recognition of unusual or rare infections or associations can lead to valuable scientific advancement. Antithetically, parasite enthusiasts will be disappointed with the chapter's depth of coverage. But rather than apologize to these parasitophiles, the authors direct the attention of those seeking more information to the chapter's necessarily long reference section.[3]

The morphological diversity displayed by the metazoan symbionts of chondrichthyans is astounding. Decisions regarding how much coverage to afford each symbiont taxon were made by categorizing parasite taxa as belonging to one of three functional groups, i.e., problematic taxa, emerging problematic taxa, and unproblematic taxa (Table 24.1). These groups (each defined below) may seem arbitrary to some; however, the authors believe that they have merit and that their use was necessary to affect chapter economy. To assist those lacking well-formed concepts of the major groups to which these organisms belong, the chapter contains a general identification key (Table 24.2) as well as tabled summary information regarding symbiont biology and life history (Tables 24.3, 24.4). Some information regarding parasite eradication and control was cautiously included throughout the chapter. However, because this topic is a primary focus of another chapter in this volume, the

[3] Because of their relevance and for convenience, three unpublished manuscripts co-authored by G.W.B. have been cited in the text as being in preparation. Copies of these manuscripts are available upon request and the journals in which publication is expected are noted for each in the reference section.

Table 24.1. Classification and common names of metazoan taxa that include associates or parasites of chondrichthyans.[a] Bold entries indicate taxa presented in the text; entries in parentheses indicate functional assignments of taxa discussed throughout the text; asterisks indicate taxa that are entirely parasitic.

Classification— common name(s) (functional assignment)

Kingdom Animalia— animals
- **Phylum Myxozoa***— myxozoans, myxosporidians (unproblematic group)
- Phylum Platyhelminthes— flatworms
  - Superclass Turbellaria— turbellarians
    - **Order Tricladida**— triclads, turbellarians (unproblematic group)
  - Superclass Cercomeria*
    - **Class Aspidogastrea***— aspidogastreans, soleworms, aspidobothreans, aspidogastrids (unproblematic group)
    - **Class Digenea***— digeneans, flukes, digenes, digenetic trematodes (unproblematic group)
    - **Class Monogenea***— monogeneans, monogenoideans, monogenes, monogenetic trematodes (problematic group)
    - **Class Cestoda***— cestodes, cestoideans: gyrocotylideans and eucestodes, tapeworms (unproblematic group)
- **Phylum Nematoda**— nematodes, roundworms, threadworms (emerging problematic group)
- **Phylum Acanthocephala***— acanthocephalans, spiny-headed worms (unproblematic group)
- Phylum Annelida— annelids, segmented worms
  - **Order Hirudinida**— true leeches (problematic group)
- Phylum Arthropoda— arthropods
  - Subphylum Uniramia— uniramians
    - **Subclass Acari**— mites (unproblematic group)
  - Subphylum Crustacea— crustaceans
    - Class Maxillopoda— maxillopodans
      - **Subclass Ostracoda**— ostracods, seed shrimp (unproblematic group)
      - **Subclass Copepoda**— copepods (emerging problematic group)
      - **Subclass Branchiura***— branchiurans, fish lice (problematic group)
      - **Subclass Cirripedia**— barnacles (unproblematic group)
    - Class Malacostraca— malacostracans
      - **Order Isopoda**— isopods (emerging problematic group)
      - **Order Amphipoda**— amphipods (unproblematic group)
- Phylum Mollusca— molluscs
  - **Class Gastropoda**— gastropods, snails (unproblematic group)
- Phylum Chordata— chordates
  - **Subphylum Craniata**— craniates
    - Superclass Agnatha— jawless fishes
      - Class Myxini— hagfishes, slime eels (unproblematic group)
      - Class Cephalaspidomorphi— lampreys (unproblematic group)
    - Superclass Gnathostomata— jawed vertebrates
      - Class Chondrichthyes— chondrichthyans (unproblematic group)
      - Class Actinopterygii— ray-finned fishes (unproblematic group)

[a] A universally-accepted classification scheme for taxa within Animalia does not exist. The classification used here is a mixture of several widely accepted schemes.

Table 24.2. Dichotomous identification key to adults[a] of the metazoan parasites and associates of chondrichthyans.[b]

1. Organisms often appearing as cysts containing many symmetric spores that are not visible to the naked eye, cysts sometimes macroscopic (e.g., Figures 24.89-24.94) ..... **Myxozoa**

Organism visible to naked eye (may nonetheless be quite small and larvae may be microscopic), may or may not be aggregated into clusters of individuals 2

2. Organism with endoskeleton made of cartilage or bone, body fish-like (e.g., Figures 24.132-24.135) ..... **Craniata**

Organism lacking endoskeleton made of cartilage or bone, body not fish-like 3

3. Organism possessing snail's shell (e.g., Figure 24.131) ..... **Gastropoda**

Organism lacking snail's shell 4

4. Organism worm-like, lacking an exoskeleton articulated with segmented appendages 5

Organism with exoskeleton articulated with segmented appendages (appendages may be minute and require a microscope to be observed) **14**

5. Organism segmented along main body axis **6**

Organism not segmented along main body axis **7**

6. Organism with anterior sucker surrounding oral opening; digestive tract and blind posterior sucker present (e.g., Figures 24.30-24.33) ... . **Hirudinida**

Organism with anterior holdfast; lacking oral opening, digestive tract and posterior sucker (e.g., Figures 24.115-24.119) ..... **Eucestoda**

7. Organism with spiny proboscis at anterior end of body, lacking digestive tract (e.g., Figures 24.120-24.122) ..... **Acanthocephala**

Organism with digestive tract **8**

8. Organism with more than two suckers or rugae **9**

Organism with two or less sucker-like holdfasts, holdfast sometimes divided into subunits .. **10**

9. Organism with suckers or rugae arranged in single file along longitudinal body axis (e.g., Figures 24.96-24.99) ..... **Aspidogastrea**

Organism with three or four pairs of circular suckers at posterior end of body (e.g., Figures 24.14, 24.15, 24.18) .... **Polyopisthocotylea (Monogenea)**

10. Organism with holdfast at posterior end of body **11**

Organism lacking holdfast at posterior end of body **13**

11. Organism with posterior holdfast in form of sucker with or without claw-like hooks (e.g., Figures 24.7-24.13, 24.16, 24.17) .... **Monopisthocotylea (Monogenea)**

Organism with posterior holdfast lacking hooks and not in form of sucker **12**

12. Organism possessing a complete digestive tract (e.g., Figure 24.95) ..... **Tricladida**

Organism lacking complete digestive tract (e.g., Figures 24.113, 24.114) ..... **Gyrocotylidea**

13. Organism with two suckers,[c] monoecious, capable of extending itself with nonsigmoidal worm-like movements (e.g., Figures 24.100-24.112) ..... **Digenea**

Organism lacking suckers, dioecious, typically elongate and round in cross section, movement typically eel-like, i.e., sigmoidal wriggling and thrashing (e.g., Figures 24.40-24.48, 24.50-24.60) ..... **Nematoda**

14. Organism with five or less appendages, all appendages unbranched, body compact and possibly tick-like (e.g., Figure 24.123) .... **Acari**

Organism with more than five appendages, body not tick-like **15**

15. Organism possessing bivalve, laterally compressed carapace that completely encloses head, body, and most appendages (e.g., Figure 24.124) ..... **Ostracoda**

Organism lacking bivalve carapace as described immediately above **16**

16. Organism sessile on host, attached to host or associate by peduncle that may or may not not be embedded (e.g., Figures 24.125-24.127) ..... **Cirripedia**

Organism not attached to host by peduncle **17**

17. Organism lacking compound eyes (e.g., Figures 24.61-24.76) ..... **Copepoda**

Organism with compound eyes **18**

18. Organism with two conspicuous sucker-like appendages on ventral body surface (e.g., Figures 24.35-24.38) ..... ***Argulus* (Branchiura)**

Organism lacking sucker-like appendages .. **19**

19. Organism with laterally compressed body (e.g., Figures 24.128-24.130) ..... **Amphipoda**

Organism with dorsoventrally compressed body or uncompressed body (e.g., Figures 24.80-24.88) ..... **Isopoda**

[a] The identification of some larvae and juveniles may also be possible using this key.

[b] This identification key was constructed to require a minimum of manipulation and technology to view characteristics of study specimens. The key does not present taxa according to phylogenetic relationships, including views on monophyly vs. polyphyly. Some key characteristics of higher taxa pertain only to species that infect chondrichthyans.

[c] Except for sanguinicolids (Sanguinicolidae; digeneans that infect the vascular system of some chondrichthyans) that all lack a ventral sucker and obvious oral sucker (see Figure 24.101).

authors generally only provided information regarding parasite control that they, as parasitologists, consider to be most efficacious. It is important to realize that the noted treatments may not resolve all infections and some hosts could die or become debilitated by exposure to certain doses of the compounds mentioned. Because of this, the authors recommend that husbandry staff consult with appropriate specialists before commencing chemotherapy. The chapter concludes with a discussion of parasitological research opportunities open to those maintaining captive chondrichthyans. This is an appropriate ending because such opportunities are best appreciated following taxonomic treatments that each raise questions and highlight ignorance.

PARASITES AND PARASITISM [4]

Although parasites are symbionts that by definition injure a host, empirical specifics regarding how or the degree to which parasite species harm their hosts are usually unknown. In fact, other than information stemming from phylogenetic inference and observed symbiotic association, science has no specific data to support the designation of parasite for many commonly accepted parasites. Nevertheless, biologists embrace the notion that certain natural taxa are entirely composed of parasites (regarding the focus of this chapter these include Myxozoa, Aspidogastrea, Digenea, Monogenea, Cestoda, Acanthocephala, Hirudinida, and Branchiura). Other major animal groups include both free-living and parasitic taxa (regarding this chapter these include Tricladida, Nematoda, Acari, Ostracoda, Copepoda, Cirripedia, Isopoda, Amphipoda, Gastropoda, and Crainiata).

Specialists in some taxonomic realms (e.g., those studying the copepods and isopods that fraternize with invertebrates) forgo the designation of parasite and instead use the term associate to denote a relationship between species without any or with little trophic necessity (Huys and Boxshall, 1991; Bunkley-Williams and Williams, 1998). Removed further yet from parasite membership are scavengers such as hagfishes (Myxinidae) that feed on dead or dying organisms or micropredators such as cookie-cutter sharks (*Isistius* spp.) that take small bites from living prey. Obfuscating matters further is the situation that obligatory parasites (e.g., the monogenean *Dermophthirius penneri*) can only feed in association with a host, whereas facultative parasites (e.g., the eel *Simenchelys parasiticus*) occasionally feed as parasites in addition to being capable of feeding as scavengers or predators. However, even obligatory parasites can be found

[4] Unless otherwise supported by citation, information in this and the following two sections stem from the authors' views as well as from information in Noble et al. (1989), Esch and Fernández (1993), Roberts and Janovy (2000), and Bush et al. (2001).

Table 24.3. Biological summary information regarding metazoan symbionts of chondrichthyans.[a]

Functional category Taxon	Number of species infecting chondrichthyans[b]	Chondrichthyan hosts or associates	Length	Locomotion style[c]
Problematic taxa				
Monogenea	many	sharks, batoids, chimaeroids	<2 cm	inchworm-like crawling
Hirudinida	few	sharks, batoids, chimaeroids	<20 cm	inchworm-like crawling
Branchiura	several	sharks, batoids	<1 cm	swimming
Emerging problematic taxa				
Nematoda	few	sharks, batoids, chimaeroids	<10 cm	sigmoidal writhing
Copepoda	many	sharks, batoids, chimaeroids	<20 cm (most <2 cm)	sessile, crawling, swimming
Isopoda	few	sharks, batoids, chimaeroids	<4 cm	sessile, crawling, swimming
Unproblematic taxa				
Myxozoa	few	sharks, batoids, chimaeroids	spores<20 µm	sessile
Tricladida	1	batoids	<1 cm	inchworm-like crawling
Aspidogastrea	4	sharks, batoids, chimaeroids	<70 cm (most <10 cm)	worm-like probing movements
Digenea	few	sharks, batoids, chimaeroids	<3 cm	worm-like probing movements
Cestoda	very many	sharks, batoids, chimaeroids	<1 m (many <5 cm)	strobila writhes

[a] Tabled information pertains to species that infect or associate with chondrichthyans. This table has been included for summary purposes only. Husbandry personnel are encouraged to fully consult the text as well as the pertinent references cited therein when confronted with concerns involving tabled symbionts.

[b] Tabled information concerning species richness regards known species. Nonnumeric assignments (lowest to highest descriptors) as follows: several, few, many, and very many. It should be noted, however, that many chondrichthyan symbionts (and especially species representing Myxozoa, Monogenea, Cestoda, Nematoda, and Copepoda) await discovery.

[c] Refers to movement style of most mature stage found on or in chondrichthyan host.

[d] Regards horizontal transmission or transfer among individual chondrichthyans within populations or communities. Designations of improbable are bestowed to taxa with at least some gut parasites that theoretically might be transferred from one host to another via predation. However, the authors are unaware of a verified report of such involving a chondrichthyan.

Host or associate switching[d]	Habitat on/in chondrichthyans[e]	Level of host or associate specificity[f]	Treatment[g]
possible but unlikely	skin, buccal and branchial chambers, gills, olfactory sacs, cloaca, heart, circulatory system, pericardial chamber, body cavity, oviduct, rectal gland, rectum, body surface of parasitic copepods	high	praziquantel treatments
possible, especially among sedentary benthic hosts	skin, buccal and branchial chambers, gills, olfactory sacs, cloaca, uterus, body surface of embryos	moderately high	organophosphate pesticide treatments, mechanical removal
highly probable	skin, buccal and branchial chambers	low	diflubenzuron or organophosphate pesticide treatments, mechanical removal
improbable	within any tissue or organ	moderate to high	fenbendazole, levamisole, piperazine[h]
only likely for capable swimmers	skin, buccal and branchial chambers, gills, olfactory sacs, olfactory lobes, cloaca, lateral line, uterus, body surface of embryos	moderately high to high	diflubenzuron or organophosphate pesticide treatments, mechanical removal
only likely for capable swimmers	skin, buccal and branchial chambers, gills, olfactory sacs, heart, pericardial chamber	low to high	diflubenzuron or organophosphate pesticide treatments, mechanical removal
improbable	gall bladder, muscle and other tissues	low to high	none known
unlikely but possible among nearby sedentary benthic hosts	skin	high	praziquantel treatments
improbable	bile ducts, gall bladder, rectal gland	moderate to high	praziquantel treatments
improbable	eye, buccal and branchial chambers, pharynx, gills, circulatory system, pericardium, stomach, spiral intestine, liver, body cavity, oviduct, cloaca	low to high	praziquantel treatments
improbable	stomach, intestine, gall bladder	high	praziquantel treatments

[e] Refers only to attachment sites of adult symbionts on or in chondrichthyans. For attachment site information regarding larvae and juvenile symbionts see text.

[f] Designation conventions as follows: high = taxa whose majority of species infect one or several chondrichthyan species, infections by adult parasites always restricted to chondrichthyans; moderately high = taxa whose majority of species infect several or more chondrichthyan species, infections by adult parasites always restricted to chondrichthayns; low = taxa whose majority of species infect or associate with one to many chondrichthyan species and that are also known to infect or associate as adults with teleosts.

[g] While treatment options are presented for most major symbiont taxa, not all infections or associations require treatment (see text for details). Also, refer to text for details regarding treatment applications, information regarding alternative methods of control, and methods of infection avoidance, prophylaxis, and strategies to break parasite life cycles via habitat or trophic manipulation.

[h] Chemotherapeutics listed are only recommended for treatment of life-threatening enteric infections. Simultaneously killing large numbers of histozoic nematodes may pose a health risk to hosts.

Table 24.3 (Continued). Biological summary information regarding metazoan symbionts of chondrichthyans.[a]

Functional category Taxon	Number of species infecting chondrichthyans[b]	Chondrichthyan hosts or associates	Length	Locomotion style[c]
Acanthocephala	several	sharks	<2 cm	sessile
Acaria	1	sharks	<2 mm	walking
Ostracoda	several	sharks, batoids	<3 mm	swimming
Cirripedia	3	sharks	<3 cm	sessile
Amphipoda	several	sharks, batoids	<1 cm	walking, swimming
Gastropoda	1	sharks, batoids	<2 cm	snail-like movements
Craniata	few	sharks	<1 m	fish-like movements

[a] Tabled information pertains to species that infect or associate with chondrichthyans. This table has been included for summary purposes only. Husbandry personnel are encouraged to fully consult the text as well as the pertinent references cited therein when confronted with concerns involving tabled symbionts.

[b] Tabled information concerning species richness regards known species. Nonnumeric assignments (lowest to highest descriptors) as follows: several, few, many, and very many. It should be noted, however, that many chondrichthyan symbionts (and especially species representing Myxozoa, Monogenea, Cestoda, Nematoda, and Copepoda) await discovery.

[c] Refers to movement style of most mature stage found on or in chondrichthyan host.

[d] Regards horizontal transmission or transfer among individual chondrichthyans within populations or communities. Designations of improbable are bestowed to taxa with at least some gut parasites that theoretically might be transferred from one host to another via predation. However, the authors are unaware of a verified report of such involving a chondrichthyan.

away from their hosts at some point in their life cycles as, for example, during the brief dispersal periods that are so important to parasites. Thus, merely finding a parasite living away from a host is not sufficient to identify it as a facultative parasite. It amuses the authors that the complexities and vagaries of the foregoing relationships irritate some biologists. For these same scientists are usually quick to accept, for example, that adult pigs (generally regarded as omnivores) feed as predators, or omnivores, or herbivores, whereas their piglets (not predator, omnivore, or herbivore) exclusively suckle from their mother's teats before assuming the trophic position of their parents. In short, science's general pigeonholing of species into convenient trophic designations is not always exact, and in some instances trophic designations only apply to a particular life-history stage of a species.

Although parasites can be found in many major metazoan taxa, parasite lineages have independently evolved from free-living lineages many times. This often-overlooked biological axiom is important to science's understanding of parasitism as well as to applied dealings with parasites because it signals that parasites more closely share genetic information with their free-living ancestors than with more distantly related parasites. Sometimes these genetic differences between parasite lineages distinguish parasites from one another in practically exploitable ways. For example, parasitic crustaceans possess a chitinous exoskeleton ancestral to all crustaceans whereas platyhelminths do not. Hence chitin-inhibiting compounds can be useful to eradicate parasitic crustaceans, but they are ineffective against parasitic platyhelminths such as monogeneans, digeneans, and cestodes. Ignorance

Host or associate switching[d]	Habitat on/in chondrichthyans[e]	Level of host or associate specificity[f]	Treatment[g]
improbable	stomach, intestine	moderately high	unknown
possible but not probable	heart	unknown	diflubenzuron or organophosphate pesticide treatments for free-living stages
possible	gills, olfactory sacs	low	diflubenzuron or organophosphate pesticide treatments
not possible	skin	low to high	physical or surgical removal
probable	skin, buccal and branchial chambers, gills, olfactory sacs	low	diflubenzuron or organophosphate pesticide treatments
probable, especially among sedentary benthic hosts	skin	probably low	physical removal
possible	skin or burrowed into body	low	physical removal

[e] Refers only to attachment sites of adult symbionts on or in chondrichthyans. For attachment site information regarding larvae and juvenile symbionts see text.

[f] Designation conventions as follows: high = taxa whose majority of species infect one or several chondrichthyan species, infections by adult parasites always restricted to chondrichthyans; moderately high = taxa whose majority of species infect several or more chondrichthyan species, infections by adult parasites always restricted to chondrichthayns; low = taxa whose majority of species infect or associate with one to many chondrichthyan species and that are also known to infect or associate as adults with teleosts.

[g] While treatment options are presented for most major symbiont taxa, not all infections or associations require treatment (see text for details). Also, refer to text for details regarding treatment applications, information regarding alternative methods of control, and methods of infection avoidance, prophylaxis, and strategies to break parasite life cycles via habitat or trophic manipulation.

[h] Chemotherapeutics listed are only recommended for treatment of life-threatening enteric infections. Simultaneously killing large numbers of histozoic nematodes may pose a health risk to hosts.

of the phylogenetic and ecological relatedness of parasitic and free-living taxa can sometimes precipitate calamitous outcomes for husbandry staff as, for example, when an epizootic of parasitic crustaceans in a community tank containing valuable free-living crustaceans is treated with a chitin-inhibiting compound.

Parasites, like all symbionts, are thought to coevolve with their hosts through a process that has been likened to the adaptive scenario of an international arms race. Whereas the factors that regulate the tempo, intensity, and outcome of these interactions are largely unknown for most host-parasite relationships, it is convenient to consider that they stem from both intrinsic (genetic) and extrinsic (ecological) factors. One phenomenon that results from this is that parasites infect particular sets of hosts under specific sets of intrinsic and extrinsic constraints as, for example, globally in nature, or in a particular region within a parasite's natural range, or in an aquarium. This fidelity to certain hosts is referred to as host specificity, and its manifestations range from parasites that only infect one or several host species, i.e., a high level of host specificity, to others that infect a great many host species, i.e., a low level of host specificity (Table 24.3, Figure 24.1).

Host patterns sometimes emerge when considering parasites, or groups of parasites, that infect multiple host species. For example, some parasites seem restricted to hosts that are phylogenetically closely related, i.e., phylogenetically mediated host specificity, whereas others seem restricted to hosts that share a common habitat or ecological niche, i.e., ecologically mediated host specificity (Figure

Table 24.4. Life history summary information regarding metazoan symbionts of chondrichthyans.[a]

Functional category Taxon	Impact on chondrichthyans[b]	Life cycle	Sexuality
Problematic taxa			
Monogenea	gill displasia and dysfunction, blood loss, skin lesions, heavy infections may cause osmotic imbalance and other deleterious metabolic demands, may open lesions facilitating invasion by opportunistic pathogens	direct[e]	protandrous hermaphrodites
Hirudinida	gill and skin lesions, blood loss, heavy infections may cause osmotic imbalance and other deleterious metabolic demands, may open lesions facilitating invasion by opportunistic pathogens, may vector other pathogens	direct	monoecious
Branchiura	skin lesions, heavy infections may cause osmotic imbalance and other deleterious metabolic demands, may open lesions facilitating invasion by opportunistic pathogens, may vector other pathogens	direct	dioecious
Emerging problematic taxa			
Nematoda	lesions associated with attachment, feeding, tissue migration	direct (minority) indirect (majority)	dioecious
Copepoda	gill displasia and dysfunction, blood loss, skin lesions, heavy infections may cause osmotic imbalance and other deleterious metabolic demands, blindness	direct[h]	dioecious
Isopoda	gill and skin lesions, blood loss, internal lesions heavy infections may cause osmotic imbalance and other deleterious metabolic demands, may open lesions facilitating invasion by opportunistic pathogens, may vector other pathogens	Gnathiids— indirect Flabelliferans— direct	dioecious dioecious or protandrous hermaphrodites
Unproblematic taxa			
Myxozoa	unknown	indirect[j]	autogametic
Tricladida	lesions associated with attachment	direct	monoecious
Aspidogastrea	unknown	indirect	monoecious

[a] Tabled information pertains to species that infect or associate with chondrichthyans. This table has been included for summary purposes only. Husbandry personnel are encouraged to fully consult the text as well as the pertinent references cited therein when confronted with concerns involving tabled symbionts.

[b] Tabled impacts are those documented for chondrichthyans or those highly probable for those hosts.

[c] Refers to taxa that the adult symbiont infects or associates with.

[d] Refers to method via which symbionts infect or come to associate with chondrichthyans.

[e] Some *Dionchus* spp. may exhibit more complex life cycles involving paratenic or intermediate hosts (see text for details).

Definitive host or associate[c]	Intermediate or paratenic hosts	Infection or association mode[d]	Stages capable of living free from hosts or associates
chondrichthyans[f]	none[e]	swimming or crawling larva[g]	egg, oncomiracidium
chondrichthyans[f]	none known	crawling or swimming juvenile or adult	egg, juvenile, adult
chondrichthyans[f]	none	swimming larva or adult	egg, larva, adult
chondrichthyans[f]	copepods and other invertebrates, fishes (including chondrichthyans)	intermediate or paratenic host infected when larva is eaten	egg, larva
chondrichthyans[f]	none[h]	swimming larva	nauplius, copepodid, adult
none[i]	fishes (including chondrichthyans)	swimming larva	zupha, praniza, adult
chondrichthyans[f]	none	swimming larva or adult	larva, adult
chondrichthyans[f]	annelids, bryozoans [j]	probably active penetration by swimming or drifting actinospore [j]	actinospore, spore
chondrichthyans	none	crawling juvenile or adult	egg, juvenile, adult
chondrichthyans	molluscs, crustaceans[k]	intermediate or paratenic host infected when	egg, cotylocidium

[f] Some species associate as adults with other taxa in addition to chondrichthyans (see text for details).

[g] The infection of sharks by *Dionchus* sp. larvae and postlarvae may be facilitated by remoras (see text for details). *Udonella* spp. appear to use parasitic copepods as platforms, from which they are thought to feed on the skin of fishes (see text for details).

[h] At least some members of Pennellidae are known to exhibit indirect life cycles requiring intermediate hosts (Benz, 1993).

[i] Gnathiids are only parasites during their larval development (see text for details).

[j] Some evidence exists that at least some myxozoans possess direct life cycles (see text for details).

[k] Use of paratenic hosts may be significant regarding infections of chondrichthyans (see text for details).

Table 24.4 (continued). Life history summary information regarding metazoan symbionts of chondrichthyans.[a]

Functional category Taxon	Impact on chondrichthyans[b]	Life cycle	Sexuality
Unproblematic taxa, continued.			
Digenea	unknown	indirect	monoecious
Cestoda			
Gryocotylidea	unknown	probably direct	monoecious
Eucestoda	lesions associated with attachment	indirect	monoecious
Acanthocephala	lesions associated with attachment	indirect	dioecious
Acaria	unknown	indirect	dioecious
Ostracoda	gill lesions	direct	dioecious
Cirripedia	lesions associated with attachment, infections possibly retard somatic and gonad growth	direct	dioecious
Amphipoda	lesions associated with attachment and feeding	direct	dioecious
Gastropoda	lesions associated with feeding, blood loss	direct	dioecious
Craniata	lesions associated with feeding, blood loss	direct	dioecious

[a] Tabled information pertains to species that infect or associate with chondrichthyans. This table has been included for summary purposes only. Husbandry personnel are encouraged to fully consult the text as well as the pertinent references cited therein when confronted with concerns involving tabled symbionts.

[b] Tabled impacts are those documented for chondrichthyans or those highly probable for those hosts.

[c] Refers to taxa that the adult symbiont infects or associates with.

[d] Refers to method via which symbionts infect or come to associate with chondrichthyans.

[e] Some *Dionchus* spp. may exhibit more complex life cycles involving paratenic or intermediate hosts (see text for details).

24.2). At other times, however, the factors underlying host-parasite relationships are less apparent, as could be the case when hosts appear to be both phylogenetically and ecologically related (Figure 24.2). Of course, understanding the underpinnings of host-parasite relationships has practical meaning for husbandry staff because it can sometimes facilitate risk assessments as, for example, predicting that an infection of a particular parasite will spread to other members of a community tank because they are phylogenetically or ecologically akin (Figure 24.1). Considering the phylogenetic relatedness of potential hosts is a relatively straightforward process; however, consideration of the ecological relatedness of potential hosts requires an understanding of host and parasite physiology, behavior, and ecology, including the dynamics of parasite life cycles.

Parasite life cycles can be direct or indirect (Table 24.4, Figure 24.3). In a direct (simple) life cycle only one host is required for the parasite to reach adulthood. In an indirect (complex) life cycle more than one host is required to reach adulthood. In indirect life cycles, hosts in which parasites reach adulthood and reproduce are called definitive hosts whereas those in which parasites develop without reaching adulthood are considered

Definitive host or associate[c]	Intermediate or paratenic hosts	Infection or association mode[d]	Stages capable of living free from hosts or associates
chondrichthyans	molluscs, teleosts, chondrichthyans	intermediate host infected when larva is eaten	egg, miracidium, cercarium
chimaeroids	probably none	unknown	egg, decacanth
chondrichthyans	copepods and other crustaceans, teleosts	intermediate or paratenic host infected when larva is eaten	egg, coracidium
chondrichthyans[f]	amphipods and other crustaceans	intermediate or paratenic host infected when larva is eaten	egg
possibly chondrichthyans[f]	possibly chondrichthyans	probably crawling larva or adult	unknown
chondrichthyans[f]	none	probably swimming larva or adult	probably all stages
chondrichthyans	none	swimming larva	nauplius, larva
chondrichthyans[f]	none	swimming or crawling larva or adult	all stages
chondrichthyans[f]	none	crawling symbiont bites host	all stages
chondrichthyans[f]	none	swimming symbiont bites host	all stages

[f] Some species associate as adults with other taxa in addition to chondrichthyans (see text for details).

[g] The infection of sharks by *Dionchus* sp. larvae and postlarvae may be facilitated by remoras (see text for details). *Udonella* spp. appear to use parasitic copepods as platforms, from which they are thought to feed on the skin of fishes (see text for details).

[h] At least some members of Pennellidae are known to exhibit indirect life cycles requiring intermediate hosts (Benz, 1993).

[i] Gnathiids are only parasites during their larval development (see text for details).

[j] Some evidence exists that at least some myxozoans possess direct life cycles (see text for details).

[k] Use of paratenic hosts may be significant regarding infections of chondrichthyans (see text for details).

intermediate hosts. Some parasites with indirect life cycles may also infect paratenic hosts, i.e., hosts in which no development occurs. Some paratenic hosts serve as transport hosts that trophically bridge two parasite life-history stages. Other paratenic hosts are merely dead-end hosts, i.e., hosts in which a parasite can persist but will not progress toward adulthood. An understanding of how parasite life cycles involve various hosts lends itself to important husbandry decisions, for parasites that are discovered in dead-end associations with hosts seldom have epizootic significance, and parasites with indirect life cycles usually only raise concern when the prospect of life-cycle completion is good, i.e., when all of the necessary intermediate and definitive hosts are present. Chondrichthyans typically serve as the only host or the definitive host for most representatives of the major taxa of metazoan parasites that infect them (Table 24.4). This phenomenon is partially explained in that chondrichthyans are generally situated in relatively unassailable trophic positions within the free-living portions of the ecosystems in which they reside, either because many of them are voracious apex predators or because their size excludes them from being eaten by most predators. Hence, parasite life histories that require

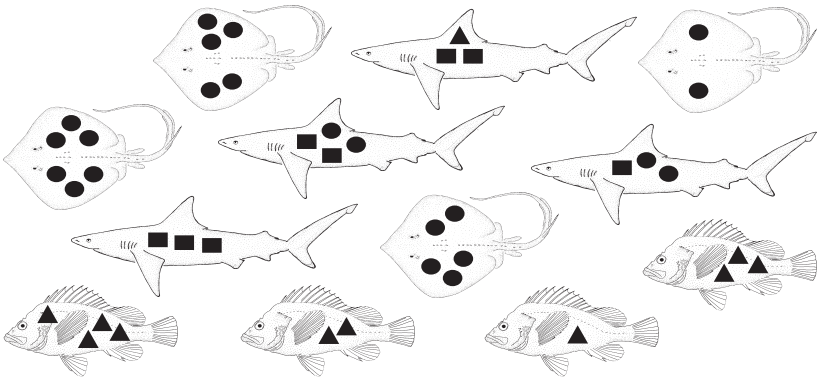
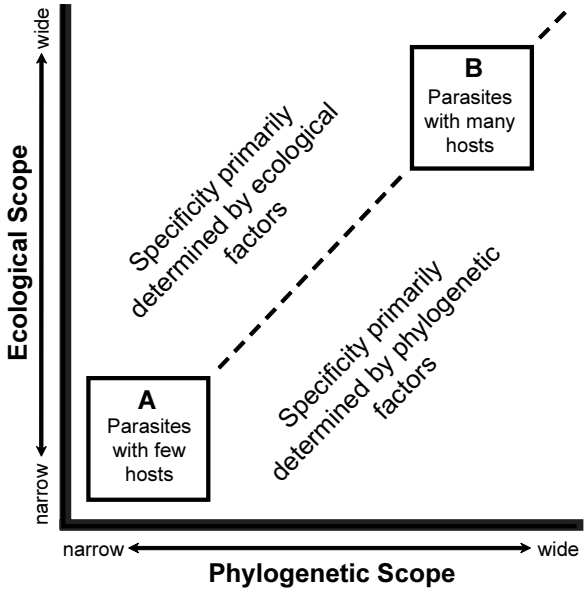


Figure 24.1. The concept of host specificity can be used to predict patterns of infection within various habitats and host communities. This hypothetical example of host specificity considers individuals of three host species (a ray, a shark, and a teleost) and three parasite species (represented by squares, circles, and triangles). Specimens of square only infect the shark, specimens of circle infect both the shark and ray, and specimens of triangle infect the shark (one instance) and teleost (primarily). In this example, the square is considered the most host specific of the three parasite species because it only infects one host species. Regarding the number of host species infected, parasites circle and triangle each are considered to have the same overall level of host specificity in that they each infect two hosts. However, the circle exhibits a higher level of host specificity from a phylogenetic perspective because its hosts are more closely related to one another, i.e., ray and shark, than are the hosts of triangle, i.e., shark and teleost. Without any further data it is impossible to consider how ecological specificity affects these infection patterns. It may be that although parasite triangle can infect sharks, it is restricted to only one shark in this example because the habitat of the teleost, i.e., the primary host of triangle, does not overlap that of the shark. If this were so, then one might expect that if these two hosts were placed together, as might take place at a public aquarium, more sharks might become infected by triangle. Similarly, one might expect that in a large exhibit tank at a public aquarium, parasite circle might have opportunity to infect a greater number of shark and ray species.

Figure 24.2. Phylogenetic and ecological factors influence patterns of infection and host specificity. The phylogenetic scope of parasites ranges from species that can infect only one or several closely related host species (narrow phylogenetic scope) to species that can infect a wide phylogenetic variety of hosts (wide phylogenetic scope). Similarly, the ecological scope of parasites ranges from species that can only infect hosts that intimately share the same habitat or eat the same food (narrow ecological scope) to species that can infect hosts inhabiting a broad variety of habitats or representing a wide trophic diversity (wide ecological scope). Parasites with narrow ecological and phylogenetic scopes (depicted by box A) tend to be restricted to one or several host species in nature. When such parasites are afforded opportunity to infect hosts outside their natural range (as sometimes occurs in captive settings) they often remain faithful to their natural host rather than additionally spreading to other unnatural hosts. An example of such a parasite is *Dermophthirus nigrellii*, a monogenean that is only known to infect the lemon shark, *Negaprion brevirostris*, even in captive settings with multiple shark species present (Cheung and Ruggieri, 1983). Parasites with wide ecological and phylogenetic scopes (depicted by box B) tend to infect a wide variety of species in nature. When such parasites are afforded opportunity to infect hosts outside their natural range they often are successful and can cause epizootics that affect many species. An example of such a parasite is *Neobenedenia melleni*, a monogenean that infects representatives of 14 families of bony fishes in nature (Bullard et al., 2000b) and causes epizootics in aquariums. Considerations of the ecological and phylogenetic scopes of parasites can assist husbandry staff in risk assessments regarding the introduction of parasites into captive settings containing various patterns of ecological and phylogenetic heterogeneity. When using this method, it is important to remember that relative to nature, captive environments do not offer wide ecological variety, especially regarding the physical separation of various niches. Therefore, just as aquarium confinement can afford parasites opportunity to infect a broader phylogenetic array of species than that existing in their natural range, captive settings can also offer parasites better ecological access to hosts than they might have had in nature.



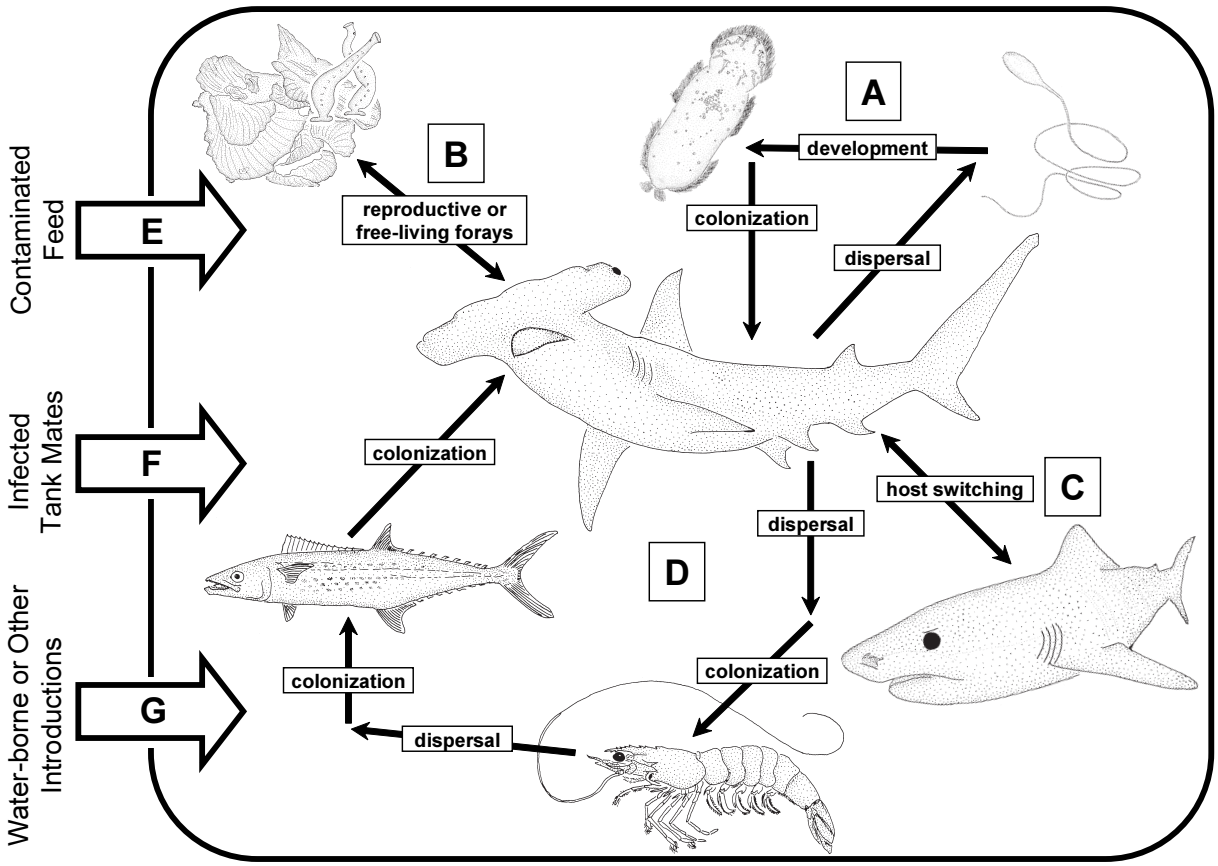


Figure 24.3. Semihypothetical life cycles and routes of infection for some parasites of chondrichthyans in captive settings (captive environment denoted by line framing animals). **A-B.** Parasites with direct life cycles only require one host to produce progeny. **A.** Some obligatory and facultative parasites with direct life cycles mature and reproduce on their hosts, setting forth progeny that disperse in the environment, develop into infective stages, and colonize hosts. **B.** Some obligatory and facultative parasites can move from their hosts to reproduce, and facultative parasites can move from their hosts to feed as free-living organisms. Progeny of these parasites may (all obligatory and some facultative parasites) or may not (some facultative parasites) be parasites during their lives. After a free-living foray, some facultative parasites may take up a host. **C.** Some individual parasites with direct life cycles can switch hosts by actively moving from one host to another. **D.** Parasites with indirect life cycles require two or more hosts to produce a next generation of progeny. Parasites undergo necessary development in each intermediate host, but they only reproduce sexually in the definitive host. In some taxa, asexual reproduction may take place in intermediate hosts. Transfer from one host to the next may involve dispersal and colonization phases carried out by brief free-living stages or colonization phases that are trophically mediated as one host eats another. Some parasites with indirect life cycles must first infect an invertebrate intermediate host before infecting a second or third intermediate or paratenic host such as a fish. **E-G.** Parasites may be introduced into captive settings through several pathways (denoted by hollow arrows). **E.** Some parasites (such as those with indirect life cycles that use trophic pathways for transmission) may be introduced via food that is contaminated with parasites. **F.** Some parasites (both those with direct and indirect life cycles) may gain access through the introduction of infected tank mates that serve as intermediate, paratenic, or definitive hosts. **G.** Some parasites (both those with direct and indirect life cycles) may invade in the form of dispersal or colonization stages of parasites that may be free-living in water or on introduced equipment or props. Some parasites may also be introduced through the entry of infected intermediate hosts (such as small invertebrates) that inadvertently gain access with water, equipment, or props. For more specific information regarding the life cycles of particular metazoan taxa see text and Table 24.3.

chondrichthyans to be eaten as intermediate hosts are generally incompatible with the typical ecological positioning of these fishes.

All parasites follow a general life-history sequence of birth, dispersal, host colonization, and maturation. Obligatory parasites with direct life cycles typically do not stray from this pattern, whereas parasites with indirect life cycles exhibit multiple phases of dispersal, host colonization, and development (Figure 24.3). Facultative parasites can punctuate the aforementioned general pattern with various predatory, scavenging, or reproductive excursions that move them from the host as free-living organisms (Figure 24.3). Parasite dispersal and colonization are critical phases in the lives of all parasites because all individual hosts will die and hence some individual parasites must move to new hosts to ensure that their lineages will persist.

Furthermore, these life-cycle phases provide opportunity for parasites to test new relationships with potential hosts as, for example, in a captive setting when parasites might confront potential hosts that normally reside outside their natural range. For some parasites (e.g., leeches and copepods), dispersal and colonization may exclusively be an active process, with larvae or juveniles swimming or crawling to hosts. For other parasites (e.g., some digeneans), these phases may be completed through various combinations of passive and active mechanisms as, for example, when a first intermediate host eats an embryonated parasite egg and subsequently liberates free-swimming parasite larvae that seek and penetrate a second intermediate host. Some stages of some parasites can only develop into a subsequent life-history stage after being eaten (either by themselves or while they are within an intermediate host) by an appropriate host. Knowing how parasites disperse and colonize their hosts (Tables 24.3, 24.4) is important regarding the health of captive hosts because efficient attempts to avoid or control parasites require knowledge of how hosts are infected.

Parasites do not infect their hosts evenly in nature regarding temporal and spatial perspectives. For example, at certain times of the year host populations and individual hosts can be expected to be more widely or heavily infected (described respectively as prevalence of infection and intensity of infection; Figure 24.4). Likewise, disparities in parasite prevalence and intensity among allopatric hosts are also common. The factors actually mediating temporal and spatial differences in parasite burdens among hosts can be abiotic and obvious (e.g., the effect of water temperature or salinity on parasite survival) or biotic and obscure (e.g., the presence or absence of appropriate intermediate hosts as affected by substrate composition). In addition, because of ontogenetic shifts in diet or immunocompetancy in some hosts, conspecifics of different ages that reside in the same area may be variously susceptible to infection. Generally speaking, in many natural host populations a relative few hosts are infected by many parasites of a particular species whereas most hosts are infected by none or few of these same parasites (Figure 24.4). This pattern is called overdispersion.

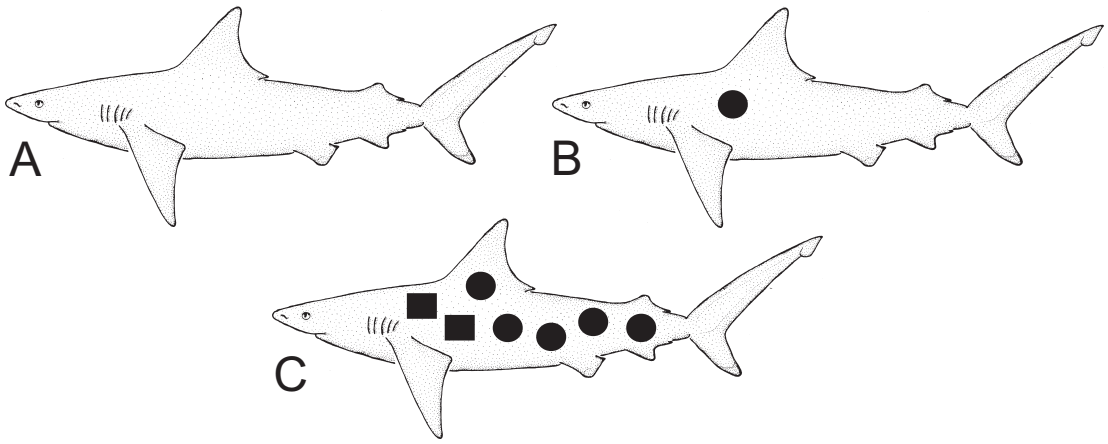
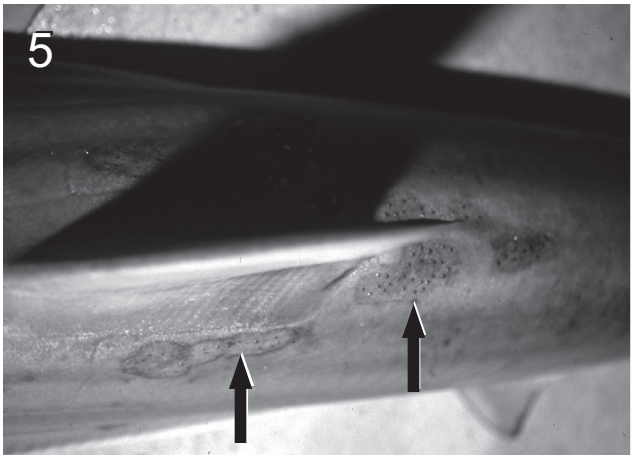
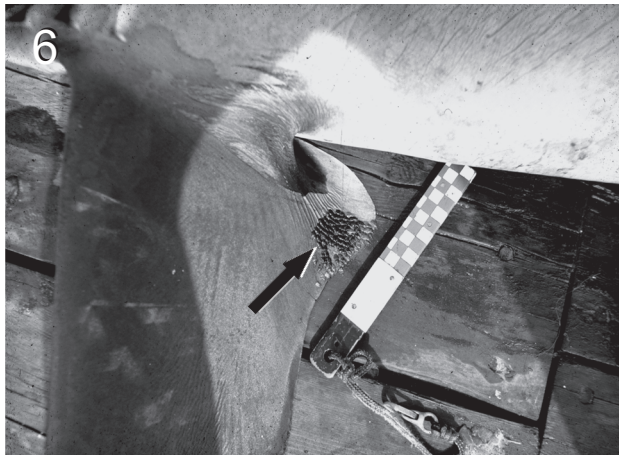


Figure 24.4. Hypothetical example of measures of parasite prevalence, intensity, and abundance using three conspecific hosts that live together as representatives of a population (note that this population can be considered to represent a natural one or one composed of conspecifics held in captivity). Circles and squares represent individuals of two parasite species. Host A is not infected. Host B is infected by one circle. Host C is infected by five circles and two squares. Prevalence is defined as the number of hosts infected by one or more individuals of a specific parasite species divided by the number of hosts examined for the parasite (Bush et al., 1997). This measure of infection presence is usually reported as a percent. In this example, the prevalence of circle is calculated as 2/3 = 0.67 or 67%, and square as 1/3 = 0.33 or 33%. Intensity (often reported as mean intensity) is the term used to denote the number of individuals of a particular parasite species inhabiting an infected host individual (Bush et al., 1997). In this example, the mean intensity of circle is calculated as 6/2 = 3 (±2.83), and square as 2/1 = 2. When intensity is reported as a mean value (as in this example), a statistic of variance (as ± SE) about this mean is usually also reported (note that in this example there is no variance associated with the intensity of square). Abundance (often reported as mean abundance) is the term used to denote the number of individuals of a particular parasite species in or on an organism regardless of whether or not the organism is infected (Bush et al., 1997). In this example, the mean abundance of circle is calculated as 6/3 = 2 (±2.65), and square as 2/3 = 0.67 (±1.16).





Figures 24.5-24.6. Some parasites infect specific sites on or in their hosts. **24.5.** Individuals of the monogenean *Dermophthirius penneri* typically form small groups (arrows) located along the dorsal surface about and posterior to the first dorsal fin on blacktip sharks, *Carcharhinus limbatus*. **24.6.** Individuals of the copepod *Pandarus satyrus* typically cluster (arrow) at a specific region on the pectoral fins of blue sharks, *Prionace glauca*.

Parasites can generally be divided into three ecological groups according to their style of infection. Ectoparasites attach to and feed on superficial host tissues, and on chondrichthyans they can be found infecting the fins, general body surface, exterior of the gills and linings of the branchial (including the spiracles) and buccal chambers, the olfactory sacs, pores of the acousticolateralis system, and the cloaca. Endoparasites infect the interior of the body, and in chondrichthyans they can be found in the gut, body cavity or pericardial chamber, circulatory system, muscle, gall bladder or liver, brain or other nerve tissue, pancreas, spleen or kidney, reproductive system, or rectal gland. Mesoparasites are defined as sedentary parasites that penetrate the host such that a significant portion of their body resides inside the host while a significant portion of the body trails outside the host. While the attachment structures of many parasites penetrate chondrichthyans (Borucinska and Benz, 1999), only one barnacle (Yano and Musick, 2000) and several species of copepods can be considered mesoparasites of these fishes (Benz and Deets, 1986; Deets and Ho, 1988; Diebakate et al., 1997). For example, *Thamnocephalus cerebrinoxius* is a sphyriid (Sphyriidae) whose cephalothorax penetrates the olfactory lobe of the barbeled hound shark, *Leptocharias smithii* (Müller and Henle, 1839), while more posterior portions of its body dangle free within its host's olfactory sac (Diebakate et al., 1997). It is lamentable that the uninitiated often equate the designation of ectoparasite with infections of little consequence; for in considering captive fishes as hosts, ectoparasites are the most problematic of all metazoan parasites (see sections below).

Although parasites as a group can infect any host tissue, almost all parasites tend to infect particular tissues and body regions. Results from studies of tapeworms living in the spiral intestine of elasmobranchs (Cislo and Caira, 1993; Curran and Caira, 1995) as well as monogeneans (Bullard et al., 2000c, 2001) and copepods (Benz, 1986, 1993; Benz and Dupre, 1987) living on the skin and gills of elasmobranchs indicate that gross labels such as spiral intestine, skin, and gills are inadequate to describe exactly where many parasite species reside. For example, adult *Dermophthirius penneri* attach to the placoid scales of blacktip sharks, *Carcharhinus limbatus* (Valenciennes, in Müller and Henle, 1839), at consistent and particular locations on the skin (Figure 24.5). Likewise, many species of copepods are conspicuous in the manner in which they consistently cluster at specific locations on their hosts (Figure 24.6). Knowledge of the attachment specificity of parasites can facilitate efficient host examinations for specific parasites, or it can signal that such examinations are not practical when dealing with live hosts as, for example, when parasites infect difficult-to-access body regions such as the water channels between gill filaments (Benz and Dupre, 1987).

Parasites can affect hosts by stealing nutrients from them, destroying or creating obstructions within host tissues, promoting deleterious physiological conditions within hosts, negatively altering host behavior, serving as vectors for pathogens, and predisposing hosts to secondary infections of opportunistic pathogens (Table 24.4). Many parasites merely steal energy from their hosts by either feeding on the host directly or by competing for nutrients within the host's gut. But,

in addition, lesions and obstructions associated with parasite attachment may be significant in that they can alter the normal function of host tissues and organs (Benz, 1980; Benz and Adamson, 1990; Bullard et al., 2001; Benz et al., 2002b). The impact of infections on host health ranges from negligible to significant, with parasite intensity sometimes having a direct relationship with the level of debilitation. This phenomenon promotes the concept that parasitic infection[5] merely denotes the presence of a parasite on or in a host, whereas infectious disease is the presence of an infection that is significantly debilitating to the host. This distinction is operationally important because it highlights that not all infections are associated with disease, and thus not all infections require treatment to promote the health of hosts. Various tissues, organs, and individual hosts have the ability to accommodate different intensities and mixes of parasites without the expression of disease under specific environmental and physiological conditions. Hence, the process of disease as it relates to infection can only be fully understood from a holistic perspective with knowledge of all contributing factors.

Despite the aforementioned list of parasite-induced afflictions, no parasite individual by itself is known to have caused disease in a chondrichthyan. Furthermore, despite the foregoing, as well as reports of lesions in free-ranging chondrichthyans that were associated with metazoan parasites (Benz, 1980, 1989; Benz and Deets, 1986, 1988; Benz et al., 1987, 1998, 2002a, 2002b; Benz and Adamson, 1990; Honma and Chiba, 1991; Honma et al., 1991; Borucinska and Caira, 1993; Borucinska et al., 1998; Campbell and Callahan, 1998; Borucinska and Benz, 1999; Borucinska and Heger, 1999; Heupel and Bennett, 1999; Borucinska and Dunham, 2000; Bullard et al., 2000c, 2001; Yano and Musick, 2000; Borucinska and Frasca, 2002), the authors are unaware of a chondrichthyan mortality in the wild attributed to a metazoan infection. This certainly does not indicate that parasites play no role in the lives of these or other hosts in nature, as more and more biologists are recognizing the potential and realized influences of parasites on individual hosts, host species, and host communities (Combes, 2001). In short, it is probable that via the coevolution prompted by host-parasite interactions, parasites have subtly co-orchestrated the phylogenetic and ecological trajectory of free-living organisms and vice versa (Combes, 2001).

PARASITES IN CAPTIVE SETTINGS

Unlike the situation in nature, when chondrichthyans are confined and clustered in high densities, some of their metazoan infections can cause health problems (Cheung et al., 1982; Cheung and Nigrelli, 1983; Cheung and Ruggieri, 1983; Bullard et al., 2001). Parasitologists generally assume that captive settings foster some infections by facilitating host colonization, maintaining parasites under stable environmental conditions that deny hosts safe haven or seasonal relief from infection, enhancing parasite development and reproduction, and stressing hosts such that their immunocompetency is lessened.

The overdispersed distributions of parasites within host populations in nature are thought to occur primarily because high numbers of parasites are only successful in colonizing a few hosts. In captive settings, the nearby residency of parasites and potential hosts ensures that parasite larvae and juveniles will be successful colonizers. Additional opportunity for parasites with direct life cycles to increase their infection intensity in captive settings is promoted by autoinfection, i.e., a process through which parasite progeny infect and mature on the same host individual as their parents. As confined infections progress into epizootics, overdispersed distributions of parasites transform into homogeneous distributions with abnormally high levels of infection prevalence and intensity. Exacerbating this situation, confinement places the dispersal and colonization stages of parasites nearby all community members regardless of species' identity or age. This affords parasites opportunities to infect unnatural hosts, i.e., species or age classes that would not be infected in nature due to ecological factors. Knowing what taxa serve as natural vs. unnatural hosts for problematic parasites is important regarding parasite control because unnatural hosts imported from the wild are unlikely to ferry such parasites into captivity.

The tempo of parasite life cycles in nature is thought to be moderated by a variety of diurnal and seasonal cues such as tidal rhythms, light intensity, temperature, and salinity. Thus in

[5] Although some use the term infestation, and its derivatives, to denote hosts inhabited by ectoparasites, the present authors consider that all parasites infect their hosts; i.e., living things are infected while inanimate things or places are infested.

nature, the life cycles of many parasites exhibit distinct reproductive and vegetative phases, each triggered by specific environmental conditions. Few if any chondrichthyans are held in captive settings that mimic nature's rhythms, but rather, these fishes and their parasites are confined under conditions that promote sustained feeding, growth, and reproduction. Under these conditions, some parasites may continually reproduce, and their high reproductive rates, fast maturation rates, and longevity may collude to produce epizootics composed of multiple generations of parasites.

A general relationship between immunocompetancy and stress in fishes is well-established, with immunosuppression and immunostimulation being linked to both environmental and endogenous factors (Iwama and Nakanishi, 1996; Wedemeyer, 1996). Although a thorough understanding of the relationship among stress, immunocompetancy, and metazoan infection in chondrichthyans does not exist, phenomena associated with capture and confinement are known to promote stress in some elasmobranchs (Gruber and Keyes, 1981; Torres et al., 1986; Pike et al., 1993; Stoskopf, 1993a, 1993b). Thus, stress factors associated with the capture and confinement of chondrichthyans possibly play roles in the immunoreceptiveness of these fishes to parasites.

PARASITE CONTROL

The symbionts of chondrichthyans are herein divided into three groups (problematic taxa, emerging problematic taxa, unproblematic taxa) regarding their ability to impact the health of captive hosts. Problematic taxa are metazoan parasites whose infections can cause the death of, or disease in, captive chondrichthyans. Emerging problematic taxa are metazoan parasites with suspected potential to cause the death of, or disease in, captive chondrichthyans. Unproblematic taxa are metazoan parasites and associates whose presence is not associated with health problems, those that can be easily removed from hosts, or those that are seldom encountered with chondrichthyans. Rather than being composed of closely related taxa, each of the aforementioned groups is artificial and composed of organisms with similar functional status regarding animal husbandry.

Unless information supporting the contrary exists, the authors recommend that only problematic taxa be addressed with efforts aimed at routine prophylaxis, and that only problematic taxa and members of one emerging problematic taxon, i.e., Isopoda, be considered for routine eradication. Even though no compelling evidence supports the routine control of any other taxon for health purposes, some of these remaining parasites and associates (e.g., ectoparasitic copepods) are large and can be associated with unsightly lesions that may facilitate disease caused by opportunistic pathogens or might upset the patrons at public aquariums. Given that these parasites are usually easy to mechanically remove or eradicate using various parasiticides, the authors encourage their removal when this poses minimal risk to the host. Under other special circumstances, however, even some parasites listed herein as being unproblematic might require control. The authors consider that these circumstances will be obvious and addressable in light of the information provided below.

The philosophical foundation of parasite control is that infectious disease can be avoided or mitigated by reducing parasite intensity to nonpathogenic levels. For hosts with nonpathogenic parasite intensities, control focuses on blocking the immigration of parasites and on preventing parasites from completing their life cycles such that parasite loads do not become amplified. For hosts with pathogenic parasite intensities, control must not only focus on the former objectives; it must also address the eradication of some parasites to reduce parasite intensity to nonpathogenic levels. Even though the methods used to accomplish each of the aforementioned objectives may sometimes be the same, the distinction among these objectives is important. For example, consider the hypothetical case in which a captive shark hosts a nonpathogenic infection of a parasite that uses an indirect life cycle employing an invertebrate intermediate host that is also a captive community member. In this instance, parasites could be controlled indirectly by removing the intermediate hosts from the system rather than by taking a direct approach by killing the parasites in the shark. Such a control option may pose less risk to the shark than other treatments (e.g., some forms of chemotherapy) that more immediately address its parasite burden. This illustrates the concepts that eradicating an infection is only part of the solution regarding parasite control and that a full consideration of a parasite's life cycle (especially the ability of various life-history stages to live away from hosts) is required to best plan and implement a control program.

The discussion above highlights the importance of understanding how parasites reach problematic levels under specific captive conditions. Regarding such, it is notable that well-documented reports of metazoan parasites of chondrichthyans completing two or more successive generations in a closed life support system only exist for some monogeneans, a branchiuran, and a leech, albeit other members of these taxa and some isopods may do so as well. Additionally, the authors are unaware of a published report of a metazoan parasite using a trophic pathway in captivity to infect a chondrichthyan, i.e., an infection associated with the feeding of infected foodstuffs or the consumption of an infected tank mate. Of course, many parasites use trophic pathways to infect chondrichthyans in nature (see sections below) and thus the possibility exists that such a phenomenon has gone unnoticed because the infection did not cause disease.

The proverb that "an ounce of prevention is worth a pound of cure" is good advice when it comes to importing parasites into captive settings. To follow this advice, parasites can sometimes be avoided when acquiring hosts as, for example, when healthy hosts are bred in captivity or transferred from one captive facility to another. However, when feral hosts must be acquired, precautions can sometimes preclude the importation of problematic parasites. For example, some species of elasmobranchs that are displayed in aquariums are seldom infected with problematic parasites (e.g., the sand tiger, *Carcharias taurus* Rafinesque, 1810, and the sandbar shark, *Carcharhinus plumbeus* (Nardo, 1827)), and thus the risk of problematic infections can be minimized when such species are appropriate for exhibition or research purposes. Risk associated with the acquisition of various host species can be assessed by reviewing host-parasite records contained in the literature as well as through direct communication with experts. In addition, seasonal and geographic selectivity regarding fish acquisition may preclude some parasites from being imported into captive settings.

Establishing an effective quarantine program is critical to maintaining healthy chondrichthyans in captivity, and this is especially so regarding species that serve as hosts for problematic parasites. Concerning metazoan parasites, the two most critical components of a sound quarantine program are the ability to effectively search for and identify parasites during the quarantine period and the ability to quarantine fishes for intervals that are long enough for parasite populations with low intensities to be detected. Vigilance is required throughout the quarantine period, especially because many parasites are inconspicuous until they cause disease. In addition to predicting what infections hosts could ferry into captivity and to whom these infections might spread (as discussed above), knowledge of the site specificity displayed by various parasites can make fish inspections more efficient and may also help to interpret abnormal fish behavior.

The release of fishes from quarantine does not ensure that disease will not subsequently occur. If the low-level infection of a problematic parasite is not detected during quarantine, months may pass before the infection is detected in a postquarantine environment such as a large exhibit at a public aquarium. Furthermore, placing a fish in a community environment may allow parasites with complex life cycles as well as unproblematic parasites to infect new hosts and become problematic. For this reason, entire communities should be monitored closely after animal (fish or invertebrate) introductions.

Although most attempts to control parasites aim to eradicate parasite populations, the immediate objective of control is to prevent parasite populations from becoming problematic or to transform problematic populations into unproblematic ones. Complete eradication of some parasite populations (e.g., some monogenean populations) is difficult, and thus control strategies must sometimes be designed to approach eradication via several to many sequential or concurrent steps, the first of which usually secures the health of the affected hosts. The methods used to control metazoan parasites belong to one of four general categories: environmental control, mechanical control, chemical control, and biological control. Each method of control has its own strengths and weaknesses, and the relevance of specific methods must be evaluated according to the specific mixture of hosts and parasites of concern, the physical and chemical environment, applicable regulations and legislation, ethical considerations, husbandry staff experience, safety considerations, and financial considerations.

Environmental control includes altering the salinity of a life support system to combat parasites, reducing water temperature to prevent or reduce parasite reproduction, and raising water temperature to promote the development of

parasite life-history stages that are vulnerable to other methods of control (e.g., chemical control). Unfortunately, so little is known of the environmental requirements of most parasites that attempts at environmental control often appear rudimentary. Nevertheless, parasite populations in nature are regulated by environmental factors, and thus environmental manipulation holds promise regarding the control of parasites within captive settings where appropriate environmental factors can be altered.

Mechanical control includes a wide variety of practices that can generally be divided into direct and indirect methods. Direct methods include the plucking of parasites from hosts using a pair of forceps. These methods often require manipulation of, and close contact with, the infected host, and thus they involve a degree of risk for hosts and husbandry staff when considering therapies involving some chondrichthyans. Indirect methods of mechanical control include installing physical barriers that prevent the immigration of parasites into life support systems or mechanical filters that remove parasites from recirculating life support water. Although indirect methods of mechanical control theoretically may reduce the size of parasite populations, seldom are the efficacies of such methods known regarding specific parasite challenges. Furthermore, mechanical devices such as filters and barriers must be periodically inspected and maintained so that they perform as intended.

Chemical control of metazoan parasites includes a wide variety of practices that fall into one of three practical categories: water applications, feed additions and other oral applications, and injected applications. The chemicals used to control metazoan parasites of chondrichthyans range from common inorganic molecules and compounds such as ozone and copper sulfate to pharmaceuticals such as praziquantel. None of these chemicals were specifically developed to treat a metazoan infection in a chondrichthyan. Some of these chemicals are insecticides and pesticides (e.g., dichlorvos, diflubenzuron) or parasiticides developed for use with humans or domestic animals (e.g., praziquantel, lufenuron), whereas others are industrial and agricultural chemicals (e.g., copper sulfate). The chemicals commonly used to eradicate metazoan parasites of chondrichthyans include ozone, copper sulfate, formalin, various organophosphate pesticides, diflubenzuron, and praziquantel (see sections below). No chemotherapeutic application will control all of the parasite taxa that afflict chondrichthyans. Anecdotal information based on casual observations and opinions abound regarding the toxicity of various chemicals to different species of chondrichthyans, but little scientific information exists regarding this subject (Stoskopf, 1993c). Some chemicals used to control metazoan parasites are regulated substances, and some are hazardous to humans.

Biological control includes vaccination and employing cleaner fishes to reduce ectoparasite loads. A variety of fishes feed on ectoparasites (Strasburg, 1959; Cressey and Lachner, 1970; Grutter, 1996, 1997); however, the authors are unaware of a practically effective cleaner species or other biological control being used to control parasites of chondrichthyans. Cleaner fishes have been used successfully in conjunction with other forms of control to reduce intensities of sea lice on salmon reared in net pens (Bjordal, 1988; Darwall et al., 1992; Costello, 1993; Treasurer, 1993).

As noted above, controlling metazoan infections is predicated on identifying parasites and assigning them to a functional group based on husbandry significance. However, the authors have witnessed that nonspecialists often misidentify metazoan parasites, and thus aquarists and veterinarians should have their parasite identifications verified by specialists whenever possible. And although the transfer of digital images is becoming more helpful regarding such matters, it is often necessary to ship parasites to specialists so that they can be manipulated for taxonomic purposes. In those instances, it is important to properly fix specimens and to provide accompanying data (see sections below).

COLLECTION OF PARASITES AND BIOPSIES

Examinations of fishes for parasites should be systematic, deliberate, and repeatable. Examinations of live subjects will be incomplete and based on remote sensing techniques (e.g., endoscopy) or biopsy. Those of dead subjects will be more complete and comprised of a necropsy. Necropsy is the ultimate opportunity to search for and collect parasites, especially ectoparasites between the olfactory and branchial filaments and lamellae as well as endoparasites. A complete necropsy comprises microscopic examinations of each tissue, organ cavity, and body cavity. Ideally, and if time were not an issue,

each tissue should be entirely examined. But in any case, at least a significant and unbiased subsample of each tissue should be examined. Intuitively, the proportion of abnormal and normal tissue may indicate the level of tissue dysfunction, and thus noting such proportions may be insightful. Freshly-collected biopsies should be examined under a microscope at various magnifications to search for histozoic (tissue-dwelling) parasites by carefully teasing apart the tissue using a fine pair of needles. Parasites should be properly fixed: individual parasites for taxonomy (see below for fixation methods used for various parasite taxa), and parasites in situ for histology. Parasites for taxonomy should be fixed independently because sectioned parasites are difficult to identify.

Various derivatives of skin scrapings and gill clippings are commonly used by husbandry staff to detect ectoparasites (Blasiola, 1992; Gratzek et al., 1992; Noga, 1996). However, the present authors generally consider these biopsy techniques inadequate regarding the collection of metazoan parasites from large fishes such as chondrichthyans and some teleosts. As noted throughout this chapter, metazoan parasites are overdispersed on their hosts, and thus attempts to sample them by gathering a small, random biopsy will likely provide negative results. Or, in the unlikely event that parasites are collected, the small area sampled may exaggerate intensity. While skin scrapings and gill clippings from small subjects may be appropriate for detecting parasites that are thought to be more evenly distributed (e.g., some prokaryotes, protists, and fungi), such small random samples are inefficient and possibly misleading when dealing with metazoan parasites and chondrichthyans. Furthermore, because of the gill structure of chondrichthyans, gill clippings would need to be restricted to the free distal tips of gill filaments (Benz, 1984a) to avoid causing considerable gill damage. The authors are aware of few metazoan parasites that attach to this small region of the gills and those parasites would be evident via direct inspection. Regarding skin scrapes, these are likely to miss monogenean and copepod larvae that reside beneath or between the placoid scales of some sharks (Kearn, 1965; Benz, 1989; Bullard et al., 2000a). Instead, the authors recommend that specific locations be examined where particular parasite species reportedly attach or where telltale signs such as patches of host mucus, small hemorrhagic lesions, off-colored skin, piping, or flashing, point to possible parasite presence.

Skin examinations should be performed on immobilized and possibly anesthetized fishes using a hand lens under bright illumination. Parasites should be carefully picked from the host using forceps or nudged from the host using the combined effects of a fine artist's brush and small blunt probe. If host immobilization is not possible or advisable, biopsy sites should be chosen based on parasite site records, the presence of lesions, or unusual and pointing behavior.

Gill examinations should be conducted with anesthesia and the gills irrigated such that the buccal and branchial chambers can be easily accessed with the fish's head (at least) above water. The buccal and branchial chambers (and gill arches) should be examined through the mouth and gill slits under a bright, maneuverable light, e.g., fiber-optic. After the efferent (upper) aspect of the gill filaments has been examined, flanking filaments should be reflected to expose lamellae and the water channel (Benz, 1984a). Of course, completely examining the gills is not possible, but these methods at least result in some useful information about portions of the major parasite niches within this body region. As these examinations require the fish to be manipulated out of water, work should be rapid and deliberate. Biopsies of abnormal tissue as well as parasites should be collected and fixed. The use of a thin-edged, long-handled chemical spoon can facilitate superficial biopsy of gill arches, gill filaments, and buccal and branchial chamber walls. Examinations can be terminated before every niche has been examined if intense infections are located and samples have been collected.

All samples should be properly labeled and specimens to be stored or shipped will require special attention. Regarding fixed parasite samples, a label of bonded paper with data written in pencil or indelible ink should be placed in the sample container to provide the following information: host species, host length, host sex, original capture location, parasite attachment location, date of parasite collection, collector's name, method of fixation, and name and concentration of preservative in the specimen container. Observations regarding gross lesions associated with infections, details concerning attempts to control infections, and other additional data should be written in a data log. Small specimens that may be harmed by data labels may be placed in a small vial containing only fixative. This protective vial is then nested in a slightly larger vial filled with the same fixative and

containing the data label. Specimen vials should seal tightly to avoid sample desiccation. For added protection against desiccation during long-term storage, sample vials may be individually wrapped with Parafilm® or they may be nested in a larger, well-sealed container that is filled with the same fixative as that in the sample vials. Specimens should always be shipped with a written specimen inventory, an additional copy of which is sent independently. Care should be taken to properly pad vials to prevent them from breaking during shipment. Separately packing each vial in a small plastic bag can provide some insulation and retain specimens in useable condition along with their data labels should vials leak or break during transit.

The authors have been disappointed on several occasions to find that no parasite specimens were available for examination regarding public aquarium findings that seemed significant or questionable. To prevent this dilemma, husbandry staff should follow the common practice of depositing voucher specimens of properly fixed parasites in appropriate museums so future investigators can examine them. Ideally, specimens should be deposited in well-curated museums with conventional study-loan policies. In some instances, depositing specimens in a "country of origin" museum has become politically fashionable; however, too often such practices place specimens at risk in museums lacking curatorial resources. Further complicating this issue is the fact that not all museums are willing to accept voucher specimens, and thus the authors encourage others to ask a specialist for a recommendation as to where to deposit various types of parasites. These authors highly recommend the following institutions: the United States National Parasite Collection (for myxozoans and helminths), the United States National Museum (Smithsonian Institution) (for all taxa except myxozoans and helminths), and the British Museum of Natural History (for all taxa).

PROBLEMATIC TAXA

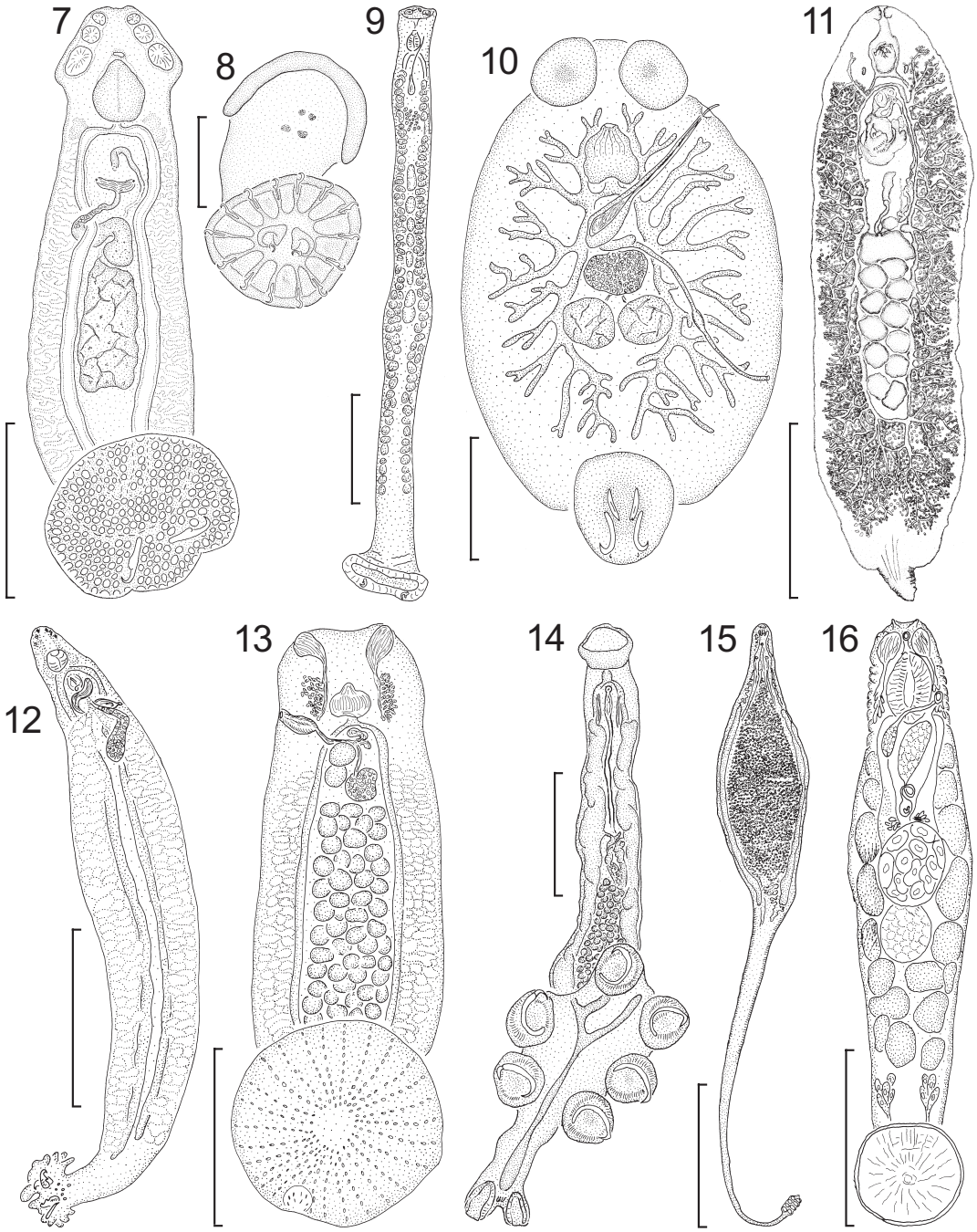
Problematic taxa are herein considered to be metazoan parasites whose infections can cause the death of or disease in captive chondrichthyans. A review of the literature and consideration of unpublished observations only implicates monogeneans, leeches, and fish lice as problematic taxa. Although these taxa are distantly related regarding phylogenetics, they share a handful of life-history characteristics that make them successful pathogens of captive chondrichthyans. All of these taxa are ectoparasites that promote lesions that may serve as infection portals for opportunistic pathogens such as some bacteria and fungi. All exhibit direct life cycles and can develop intense infections on hosts in captive settings due to favorable environmental conditions such as a lack of parasite predators and hyperparasites, easy accessibility to hosts that have no safe haven from parasites, and constant temperatures conducive to parasite reproduction, development, and feeding. All have at least one phase in their life cycle that is not strictly associated with a host, thus necessitating the consideration of the entire environment when attempting to eradicate their infections. In addition, leeches and fish lice may be difficult to control in captive settings because they sometimes infect many host species.

Monogeneans

Monogeneans, formerly known as monogenes and monogenetic trematodes, primarily infect fishes; but they also infect some invertebrates, amphibians, reptiles, and even a mammal (Kearn, 1998). The literature contains many records of monogeneans infecting chondrichthyans (Yamaguti, 1963a; Cheung, 1993). As a group, monogeneans are considered problematic parasites because several species have caused or been closely associated with mortalities of aquarium-held elasmobranchs (Cheung et al., 1982; Cheung and Nigrelli, 1983; Cheung and Ruggieri, 1983; Poynton et al., 1997; Bullard et al., 2001), and probably many more monogeneans are able to harm those fishes.

Systematics, distribution, and taxonomy

Holding all monogeneans, Monogenea is a large class of flatworms (Cercomeromorpha, Platyhelminthes) consisting of about 2,200 known species representing 50 families (Whittington, 1998) and possibly 25,000-30,000 extant species (Kurochkin, 1985). About 200 monogenean species in nine families infect chondrichthyans (Figures 24.7-24.15), and certainly many more species await discovery. Herein, we follow the traditional and most widely accepted classification scheme for monogeneans that identifies the two subclasses Monopisthocotylea and Polyopisthocotylea within the class Monogenea (see Yamaguti, 1963a). However, Bychowsky (1961) and Boeger and Kritsky (2001) refer to these subclasses

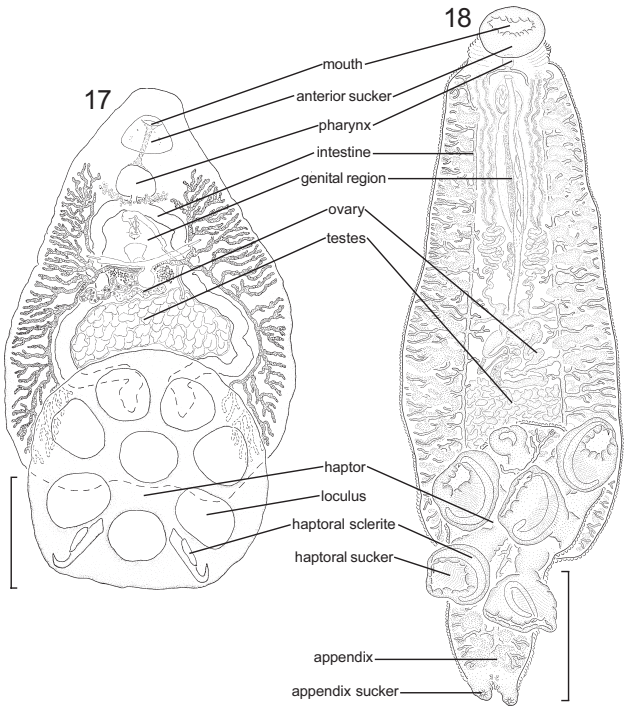


Figures 24.7-24.16. Monogeneans (Monogenea) from (Figures 24.7-24.15) or associated with (Figure 24.16) chondrichthyans, adults unless otherwise noted, ventral views. **24.7.** *Cathariotrema* sp. (Monocotylidae). Original illustration, scale bar = 800 µm. **24.8.** *Dionchus* sp. (Dionchidae), postlarva. Drawn from Bullard et al. (2000a), scale bar = 50 µm. **24.9.** *Loimos scoliodoni* (Loimoidae). Drawn from Hendrix (1994), scale bar = 500 µm. **24.10.** *Benedeniella posterocolpa* (Capsalidae). Drawn from Hendrix (1994), scale bar = 2 mm. **24.11.** *Neodermophthirius harkemai* (Microbothriidae). Original illustration, scale bar = 200 µm. **24.12.** *Amphibdella flavolineata* (Amphibdellidae). Drawn from Hendrix (1994), scale bar = 1 mm. **24.13.** *Pseudacanthocotyla verrilli* (Acanthocotylidae). Drawn from Hendrix (1994), scale bar = 1 mm. **24.14.** *Erpocotyle sphyrnae* (Hexabothriidae). Drawn from Price (1942), scale bar = 1 mm. **24.15.** *Callorhynchicola branchialis* (Chimaericolidae). Drawn from Brinkmann (1952), scale bar = 1 mm. **24.16.** *Udonella caligorum* (Udonellidae). Drawn from Price (1938), scale bar = 500 µm.

respectively as Polyonchoinea and Heteronchoinea within the class Monogenoidea. Representatives of Acanthocotylidae, Amphibdellidae, Capsalidae, Dionchidae, Loimoidae, Microbothriidae, and Monocotylidae are monopisthocotyleans and members of Chimaericolidae and Hexabothriidae are polyopisthocotyleans. Udonellidae species best conform to a monopisthocotylean structure; however, these worms display some characteristics unlike other monogeneans (Boeger and Kritsky, 1993, 2001; Williams and Jones, 1994; Kearn, 1998). Acanthocotylidae (Figure 24.13) is composed of marine species that infect the skin (primarily), olfactory sacs, and gills of batoids.[6] Capsalidae (Figure 24.10) is composed of marine species that infect teleosts and elasmobranchs; those on elasmobranchs infect the skin (primarily) and gills of batoids (primarily) and sharks. Dionchidae (Figure 24.8) appears to contain at least one marine species whose postlarva infects the skin of a shark and whose adult probably infects the gills of a remora (Echeneidae). Loimoidae (Figure 24.9) is composed of several marine species that infect the gills and skin (S. A. Bullard, unpublished observations) of sharks (primarily) and batoids. Microbothriidae (Figure 24.11) is composed of marine species (although *Dermophthirius maccallumi* appears to be euryhaline; Watson and Thorson, 1976) that infect the skin (primarily) and gills of sharks (primarily) and batoids. Monocotylidae (Figure 24.7) is composed of marine (primarily) and freshwater species that infect the gills (primarily), skin, buccal cavity, olfactory sacs, cloaca, rectum, oviduct, and body cavity of batoids (primarily), sharks, and chimaeroids. Amphibdellidae (Figure 24.12) is composed of marine species that exclusively infect the gills and circulatory system of batoids.[7] Hexabothriidae (Figure 24.14) is composed of marine (primarily) and a freshwater species that infect the gills (primarily) and buccal cavity of sharks (primarily), batoids, and chimaeroids. Chimaericolidae (Figure 24.15) is composed of marine species that exclusively infect the gills of chimaeroids. In addition to the aforementioned nine families, Udonellidae (Figure 24.16) is

[6] The authors consider reference to an "unidentified shark" as host to an acanthocotylid (see Cheung, 1993) as a dubious record.

[7] The record of *Amphibdelloides maccallumi* from *Squalus acanthias* (see Price, 1937) is dubious because it is the only record of an amphibdellid from a shark and the specimens of *A. maccallumi* that were deposited by Price in the United States National Parasite Collection were designated as being collected from the skin of the head of the shark, despite this site not being reported in the publication of Price (1937) and the fact that no other record of an amphibdellid from the skin of a host exists.



Figures 24.17- 24.18. Anatomical features of adult monogeneans (Monogenea) from elasmobranchs. **24.17.** Ventral view of the monopisthocotylean *Calicotyle urobati* (Monocotylidae). Modified from Bullard and Overstreet (2000), scale bar = 500 µm. **24.18.** Ventral view of the polyopisthocotylean *Branchotenthes robinoverstreeti* (Hexabothriidae). Modified from Bullard and Dippenaar (2003), scale bar = 1 mm.

treated here because some of its representatives attach to parasitic copepods that infect marine elasmobranchs, and these worms may be parasites of elasmobranchs (see below).

There is no contemporary species identification key to all of the aforementioned monogeneans and, unfortunately, a comprehensive list of taxonomic references for these important parasites is beyond the scope of this chapter. Nevertheless, Bychowsky (1961), Yamaguti (1963a), Schell (1985), Boeger and Kritsky (1989, 1993, 2001), Hendrix (1994), Chisholm et al. (1995, 1997a, 1997b), Whittington et al. (2001), and Chisholm and Whittington (2004) provided information on taxonomic features useful for identifying monogeneans belonging to the various families noted above as well as information on host species and attachment sites.

Morphology

Monogeneans are monoecious, thin, flat or cylindrical, soft-bodied, bilaterally symmetrical, and semitransparent or opaque worms that are typically 1-20 mm long when relaxed (Bychowsky, 1961; Yamaguti, 1963a; Schell, 1985; Hendrix, 1994; Kearn, 1998). They are flexible and many species can stretch to over twice their relaxed length. Monogeneans possess distinct anterior and posterior ends (Figures 24.17, 24.18). The mouth is located at the anterior end of the body, and the posterior end of the body is modified into an attachment structure known as the haptor (opisthaptor or opisthohaptor in the older literature).

Monopisthocotyleans (Figure 24.17) possess an anterior feeding apparatus consisting of a ventrally protrusible pharynx and sometimes an

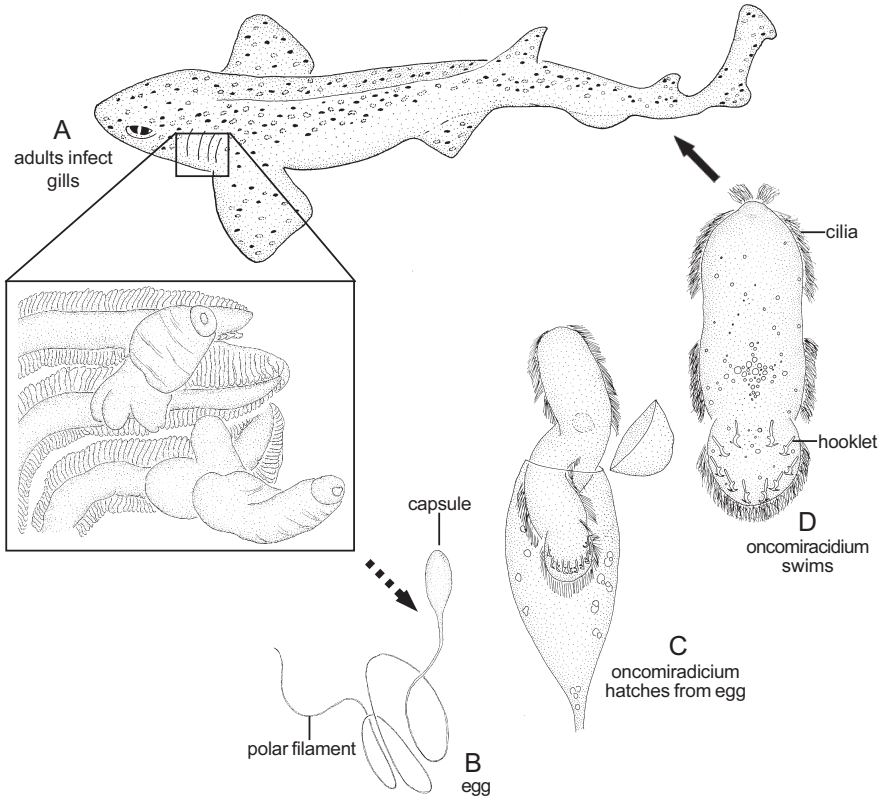


Figure 24.19. Life cycle of *Hexabothrium appendiculatum* (Hexabothriidae), a gill parasite of the small-spotted cat shark, *Scyliorhinus canicula*; based on information and illustrations in Whittington (1987a, 1987b, 1987c). Dashed arrow indicates movement from host (dispersal); solid arrow denotes movement onto host (colonization). **A.** Adults infect gill filaments of shark where they copulate and subsequently produce eggs. **B.** Eggs sink at a rate of approximately 1.26 cm min^{-1}. In 13-14 C water, most 40-d old eggs contain an oncomiracidium. The egg is composed of a spheroidal capsule about 200-µm long and a polar filament about 3-mm long. The anterior end of the oncomiracidium typically resides within the egg near to where an operculum will open at hatching. Drawn from Whittington (1987a). **C.** When the egg is exposed to seawater containing shark secretions, the embryo beats its cilia, and after 20-30 s the oncomiracidium hatches from the egg. Modified from Whittington (1987a). **D.** Oncomiracidia, each about 180-µm long, spiral as they swim at roughly 4 mm s^{-1}. Oncomiracidia swim forward making frequent and erratic changes in speed and course or in circular patterns making no forward progress. Some swim in a relatively confined area for 1-2 min intervals whereas others immediately attach to the substrate and may not swim again. If an oncomiracidium attaches to a small-spotted cat shark, it loses its cilia to transform into a juvenile worm that will feed, mature, copulate, and produce eggs while living on the gills.

attachment organ composed of paired suckers (Figure 24.10) or adhesive pads (Figure 24.13). The haptor of these worms consists of a single cup-like or lappet-like sucker (Figure 24.17). The haptor may be subdivided by tegumental ridges (septa) into loculi that are typically symmetrical (Figure 24.17), or it may possess an array of papillae that aid in attachment and are taxonomically important (Bullard et al., in press). Adults of most species possess a haptor armed with large sclerotized hooks (hamuli) or small peripheral hooks (marginal haptoral hooklets or larval hooklets) that grasp the host (Kearn, 1998). Exceptions to this scheme are species that cement themselves to hard surfaces, such as members of Microbothriidae that attach to the placoid scales of sharks or *Udonella* spp. that attach to the exoskeleton or egg sacs of copepods (Kearn and Gowing, 1990).

Polyopisthocotyleans (Figure 24.18) possess an anterior feeding apparatus consisting of a terminal or subterminal mouth leading to an oral cavity that is enclosed within a bulbous anterior sucker or accompanied by a pair of internal buccal suckers (Kearn, 1994). The haptor is differentiated into three or four pairs of suckers, each associated with one large hooked sclerite (Figure 24.18). Hexabothriids have a haptor with a muscular appendix (Figure 24.18) whose distal region is usually armed with a pair of small hooks and always with a pair of thimble-shaped suckers. In Chimaericolidae the foregoing region of the haptor possesses a small terminal lappet.

Life History

Monogeneans that infect chondrichthyans are protandrous hermaphrodites, and although few of their life cycles are known with certainty, it is presumed that most are direct. Adults copulate with themselves or each other on the host (Figure 24.19). Eggs are generally microscopic, and although they may vary in size and shape between species, they display little intraspecific variation (Kearn, 1986). The eggs of oviparous species are typically voided into the surrounding water singly, in tangled masses, or as chains of connected eggs (Kearn, 1986). The eggs of some monogeneans possess tendrils (filaments) that prevent them from sinking (Figure 24.19; Kearn, 1986). The authors have observed (G. W. Benz and S. A. Bullard, unpublished observations) the tripolar eggs of *Dermophthirius penneri* on the skin of blacktip sharks and the eggs of *Dendromonocotyle octodiscus* on the skin of bluntnose stingrays, *Dasyatis say* (Lesueur, 1817). However, the eggs of some monogeneans that infect elasmobranchs drift to the substrate or are held externally by adult worms before hatching (Kearn, 1967, 1986; Whittington, 1987a). The tendrils or embryo capsule of benthic eggs are sometimes adhesive and can cement eggs to various substrates (Kearn, 1986). Chimaericolids (e.g., *Callorhynchicola multitesticulatus*) are distinguished from other monogeneans that infect chondrichthyans in that they are ovoviviparous, i.e., the egg hatches *in utero* (Manter, 1955; Llewellyn, 1963). Although the eggs of monogeneans are produced one at a time, reproductive output can be high (Kearn, 1986). The authors have observed specimens of *D. penneri* as they produced about one egg min^{-1} *in vitro* (G. W. Benz and S. A. Bullard, unpublished observations). Egg production, embryonic development, and hatching can have seasonal or daily rhythms, or both (Kearn, 1986; Kearn et al., 1992; Yoshinaga et al., 2000). Eggs can hatch in one to over 100 d, with eggs incubated at higher temperatures generally hatching sooner (Kearn, 1986). Whittington (1987a) reported that at 13-14 C the eggs of *Hexabothrium appendiculatum* and *Leptocotyle minor*, both parasites of the small-spotted cat shark, *Scyliorhinus canicula* (Linnaeus, 1758), are fully developed in 16-18 d and about 40 d, respectively. Hatching of monogenean eggs may or may not be prompted by stimuli such as physical perturbations, varying light regimes, host secretions, or varying salinity (Euzet and Raibaut, 1960; Macdonald, 1974; Macdonald and Llewellyn, 1980; Kearn, 1986; Whittington and Kearn, 1986, 1988; Whittington, 1987a; Mueller et al., 1992; Ellis and Watanabe, 1993; Chisholm and Whittington, 2000; Yoshinaga et al., 2000). Whittington (1987a) demonstrated that egg hatching of *H. appendiculatum* and *L. minor* can be stimulated by urea, and Whittington and Kearn (1990) demonstrated that rapid hatching of *Acanthocotyle lobianchi* was stimulated by urea from the spotted skate, *Raja montagui* Fowler, 1910. Long prehatching periods exist for some species. For example, Macdonald (1974) observed that some unstimulated but fully developed eggs of *A. lobianchi* survived without hatching for up to 83 d, and Whittington (1987a) demonstrated that at 13-14 C and without a hatching stimulus, fully developed eggs of *H. appendiculatum* and *L. minor* did not hatch even after 88 d. The ability to postpone hatching probably ensures that emerging monogenean larvae will confront a host; however, specific mechanisms coordinating such delays have not been identified for most species. Regardless, this

scenario is particularly relevant to husbandry staff because it denotes that the eggs of at least some monogeneans may remain viable for almost 3 months and possibly longer. The larva emerging from the egg is called an oncomiracidium (Figure 24.19), and it may be ciliated and capable of swimming (Llewellyn, 1963; Kearn, 1986; Whittington and Kearn, 1986; Whittington, 1987c) or nonciliated and capable of crawling (Euzet and Raibaut, 1960; Llewellyn, 1963; Kearn, 1967, 1986; Macdonald, 1974; Macdonald and Llewellyn, 1980; Bullard et al., 2000a). Once the oncomiracidium attaches to a host it sheds its cilia (if it is a form that has them and has not already done so; see Whittington, 1987c) and is referred to as a postlarva. These juveniles feed and develop directly into adults, and sometimes this process coincides with a migration from a postlarval to an adult niche (Llewellyn, 1960; Kearn, 1965, 1978, 1987). Postlarvae and adults of almost all monogeneans are considered unable to swim, and thus they cannot switch hosts unless the latter are in close proximity to one another. However, in the only report of its kind, a postlarval subadult *Entobdella* sp. collected from a feathertail stingray, *Pastinachus sephen* (Forsskael, 1775), swam vigorously for a short distance in a dish of seawater (Kearn and Whittington, 1991).

Unlike the typical life history presented above, at least some members of Dionchidae apparently use, and may even require, two hosts (Figure 24.20; Bullard et al., 2000a). For example, Bullard et al. (2000a), prompted by the work of Ktari (1977) and Whittington (1990), proposed a life cycle for some *Dionchus* spp. wherein adult worms reside on, copulate on, and attach egg

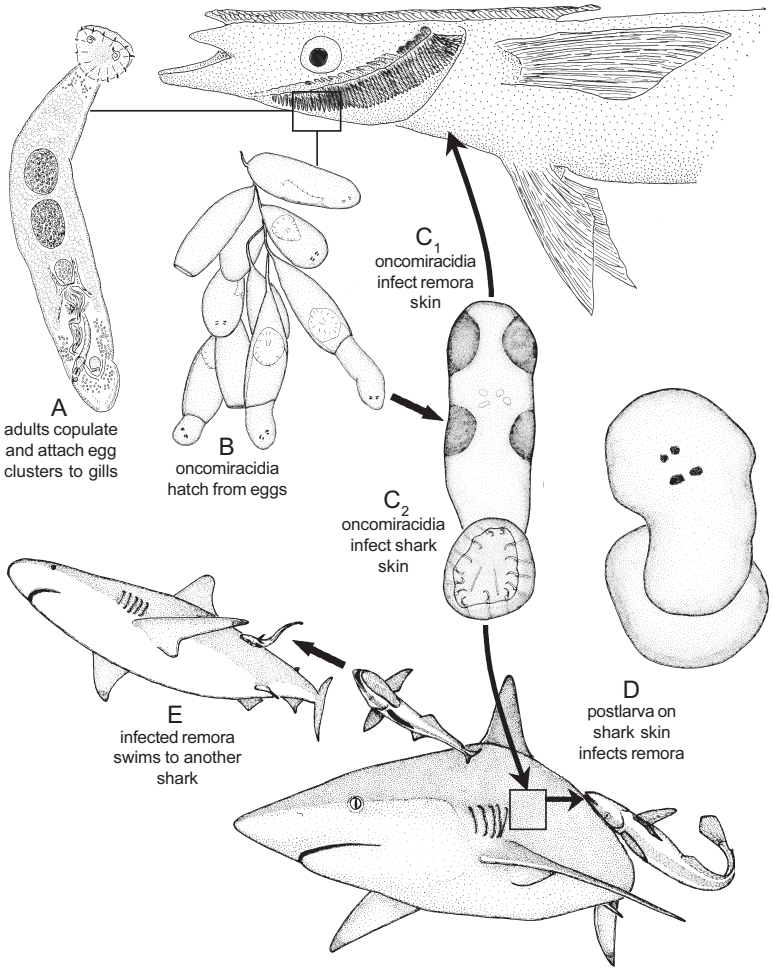


Figure 24.20. Hypothetical life cycle of *Dionchus remorae* (Dionchidae), a gill parasite of remoras (Echeneidae) and skin parasite of the blacktip shark, *Carcharhinus limbatus*; based on information and illustrations in Bullard et al. (2000a). **A.** Adults infect the gill filaments of the remora where they copulate and produce eggs that are attached in grape-like bundles to the distal portion of the gill filaments. **B.** Nonciliated and highly active oncomiracidia hatch from eggs. **C_{1-2}.** Oncomiracidia crawl from the remora's branchial chamber to infect its skin (C_1) or they crawl from the remora onto a shark (C_2). **D.** Postoncomiracidia use the shark as a platform from which they colonize visiting remoras and move to the gills to mature. **E.** Infections of dionchid larvae spread throughout populations of sharks when infected remoras periodically switch sharks.

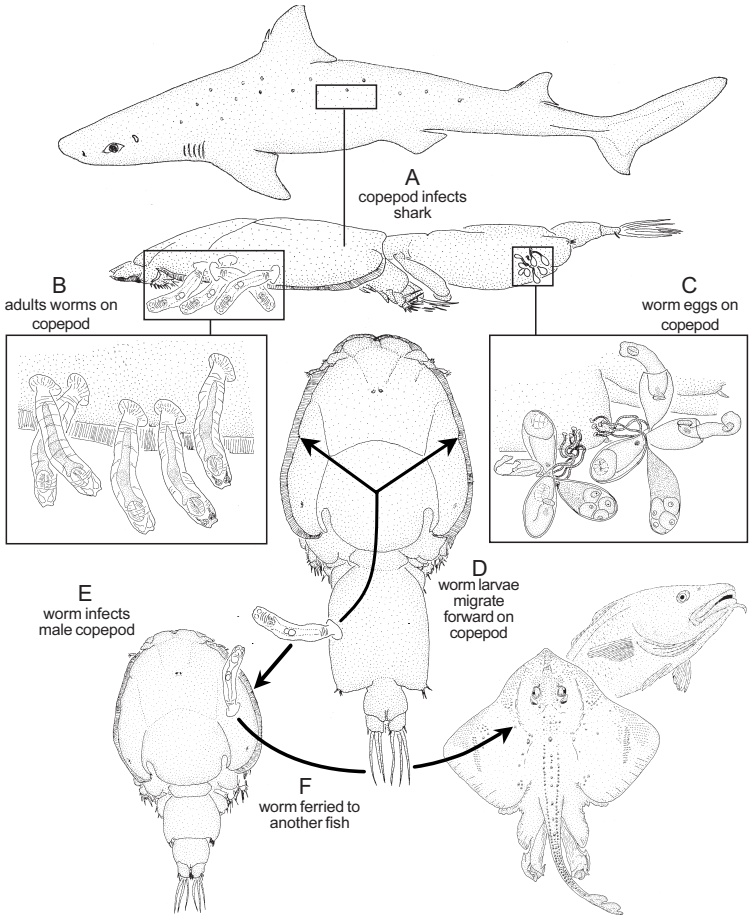


Figure 24.21. Hypothetical life cycle of *Udonella caligorum* (Udonellidae), an associate of copepods that infect fishes; based on information in Kabata (1973) and Aken'Ova and Lester (1996). **A.** Copepods that infect fishes serve as platforms for udonellids. **B.** Adult udonellids typically attach to the lateral regions of the cephalothorax of adult female copepods, from where they can stretch to feed on the copepod's host. **C.** Udonellid eggs are typically attached to the posterior body region of copepods. **D.** After hatching, larvae migrate anteriorly on the copepod where they mature. **E.** Udonellids presumably spread throughout copepod populations by transferring between copulating copepods. **F.** Udonellids presumably spread to new fish hosts when copepods switch hosts.

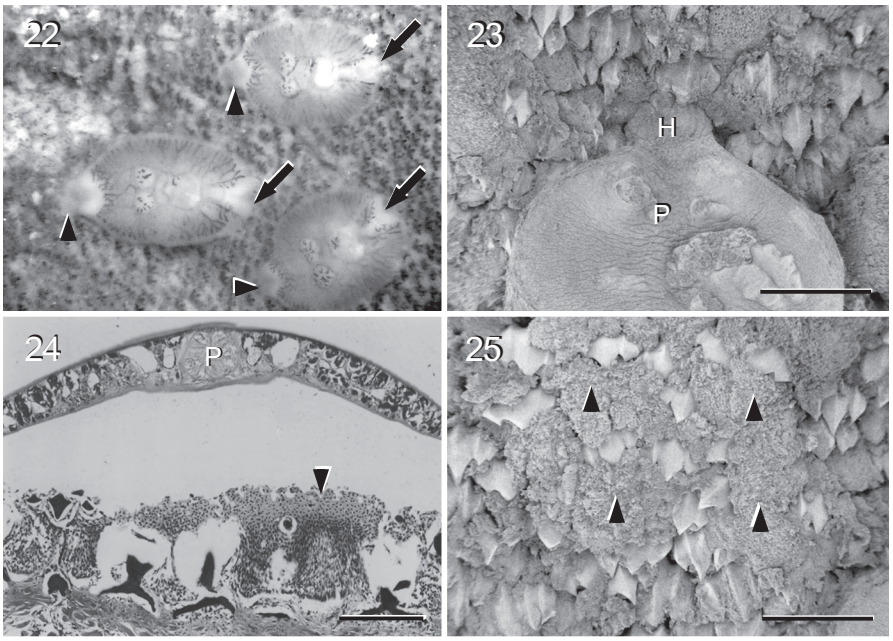
clusters to the gills of remoras (Echeneidae). As an infected remora closely associates with a shark, the eggs hatch and nonciliated oncomiracidia crawl to colonize the shark. Subsequent association between remoras and the infected shark facilitates the infection of remoras and the subsequent completion of the life cycle. Thus, in this scenario, the shark plays an important role as a colonization platform from which juvenile worms may infect new definitive hosts.

Members of Udonellidae also deviate from the general monogenean life history. Although these worms may attach temporarily to fishes (Nichols, 1975),[8] they typically attach to the exoskeleton and egg sacs of copepods that infect teleosts and elasmobranchs (Figure 24.21; Kabata, 1973). Gut contents and attachment location records suggest that udonellids use parasitic copepods as platforms from which they feed on host fishes (Ivanov, 1952; Kabata, 1973; Aken'Ova and Lester, 1996). Adults typically attach their eggs in bundles on the posterior half of copepods and hatching liberates nonciliated juveniles (Kabata, 1973; Minchin and Jackson, 1993). Copepod mating may allow the colonization of copepods, and host switching by vagile copepods may facilitate horizontal transmission within fish populations (Kabata, 1973; Aken'Ova and Lester, 1996).

Host and site specificity

Monogeneans generally display high levels of host specificity, and many species seem restricted to a single or several closely related host species (Yamaguti, 1963a; Cheung, 1993). Although little

[8] Although they are known to associate with parasitic copepods that infect chondrichthyans, no udonellid has been reported directly from a chondrichthyan, and it is unknown if these worms are parasites of these fishes.

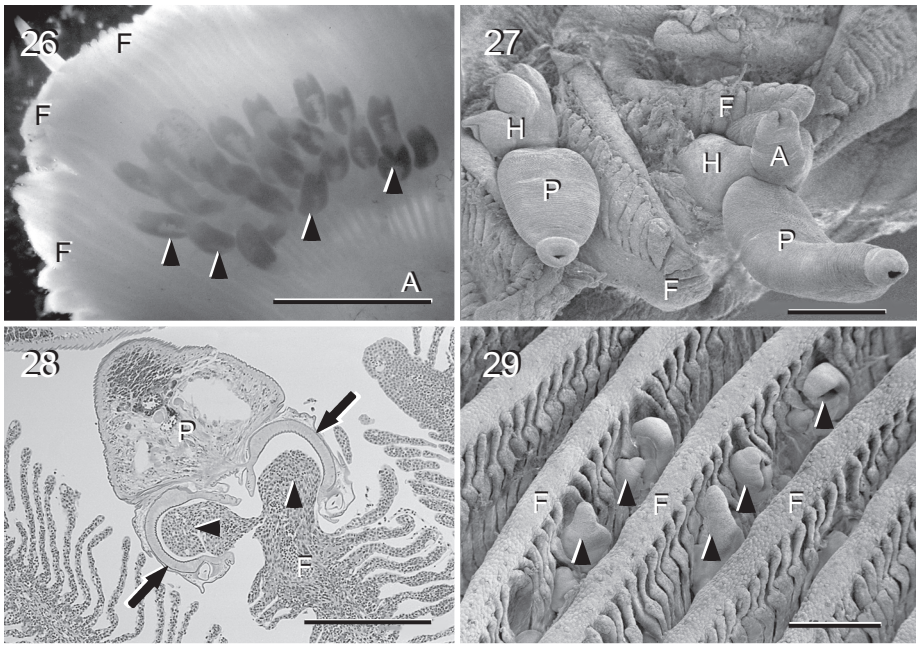


Figures 24.22-24.25. *Dermophthirius penneri* (Microbothriidae) on the skin of wild-caught blacktip sharks, *Carcharhinus limbatus*, from the northern Gulf of Mexico. Figures 24.23 and 24.25 modified from Bullard et al. (2000c). **24.22.** Light micrograph of adult worms, each about 3.5-mm long, attached to skin of shark. Note that the haptor of each worm is attached to the host (arrowhead) in an upstream position relative to the direction of water flowing locally over the host, and that the mouth of each worm (arrows) faces downstream relative to same. **24.23.** Scanning electron micrograph (SEM) of posterior portion of *D. penneri* (P) attached to skin of shark. The haptor (H) is presumably attached to the crown of an underlying placoid scale. Scale bar = 500 µm. **24.24.** Light micrograph of histological section of shark skin beneath transverse section of *D. penneri* (P). The regularity of the array of placoid scales is disrupted and some scales are apparently missing. Epithelial hyperplasia (arrowhead) forms a wide column or mound of cells that fills a space without scales. Scale bar = 200 µm. **24.25.** SEM of the skin of a shark nearby *D. penneri* (parasite not shown). Some placoid scales appear missing, rotated, or tilted relative to normal condition. Trailing edges of some scale crowns appear relatively blunt and valleys between crown ridges on some scales seem shallow. Mounds of hyperplastic epithelium are evident (arrowheads). Scale bar = 500 µm.

is known about the factors that mediate monogenean host specificity (Whittington et al., 2000), host records can be used to predict which species are at risk of infection in captive settings. In turn, these hosts can be closely monitored or initially isolated for prophylactic treatment.

Monogeneans exhibit high levels of attachment site specificity on their hosts in the wild, and this information can be used to great advantage in diagnosing infections. Most monogeneans are ectoparasites (Figures 24.22-24.29; also, see above for general niches of members of the monogenean families that infect or associate with chondrichthyans). However, adults of several species are unusual in that they are endoparasites. *Calicotyle kröyeri* and some other *Calicotyle* spp. infect the rectal gland and oviduct (Kearn, 1987; Chisholm et al., 1997a) and possibly the body and pericardial cavities (Bullard and Overstreet, 2000) of elasmobranchs. *Dictyocotyle coeliaca* infects the coelom and body cavity wall of *Raja* spp. (Lawler, 1981). *Amphibdella* spp. (Amphibdellidae) infect the gills (wherein they release their eggs) and vascular system (including the heart) of torpediniforms (Torpediniformes). The closely-related *Amphibdelloides* spp. (Amphibdellidae) are ectoparasitic only and infect the gill epithelium, thereby exhibiting the more typical mode of infection for a monogenean (Llewellyn, 1960; Euzet and Combes, 1998).

The principal attachment sites of monogeneans remain the same in captivity as in nature, and these worms may infect unusual sites if their infrapopulations become large. For example, infections by *Dermophthirius* spp. in nature appear as small opaque patches at seemingly specific locations on the skin (Figures 24.5, 24.22-24.25), but lesions associated with intense infections on captive hosts appear to have expanded to adjacent areas (G. W. Benz and S. A. Bullard, unpublished observations). Adults of *Neodermophthirius harkemai* typically attach to the small placoid scales on the gill arches of lemon sharks, *Negaprion brevirostris* (Poey, 1868), in the wild (Price, 1963; Euzet and Maillard, 1967); however, they have been collected from the skin of heavily infected captive lemon sharks (Poynton et al., 1997). Adults of *Erpocotyle tiburonis* typically attach to the gill filaments of



Figures 24.26-24.29. Gills of sharks infected with *Erpocotyle* spp. (Hexabothriidae). Figures 24.27-24.29 modified from Bullard et al. (2001). **24.26.** Light micrograph of *Erpocotyle* sp. attached to gill filaments of an aquarium-held leopard shark, *Triakis semifasciata*. Adult monogeneans (arrowheads) are oriented downstream relative to direction of water flowing locally over the filaments, i.e., the mouth of each worm is directed toward the distal tip of a filament (F) and the haptor of each worm is attached closer to the gill arch (A). Scale bar = 1 cm. **24.27-24.29.** Gill of an aquarium-held bonnethead shark, *Sphyrna tiburo*, with an intense infection of *Erpocotyle tiburonis*. **24.27.** Scanning electron micrograph (SEM) of two adult *E. tiburonis* (P) attached along the distal thirds of two gill filaments (F). Note that worms occupy the space between filaments. The haptor (H) of each adult worm grasps a filament, and the appendix (A) of one worm is visible. Scale bar = 500 µm. **24.28.** Light micrograph of tissue section at attachment site of adult *E. tiburonis* (P) in the distal third of a gill filament (F). Note haptoral suckers (arrows) filled with gill epithelium (arrowheads). Nearby lamellae appear normal. Scale bar = 400 µm. **29.** SEM showing juvenile *E. tiburonis* (arrowheads) attached between and oriented parallel to lamellae along the proximal two-thirds of two gill filaments (F). Scale bar = 200 µm.

bonnethead sharks, *Sphyrna tiburo* (Linnaeus, 1758), in the wild, but they have also been collected from the buccal cavity of heavily infected captive hosts (Bullard et al., 2001). Some monogeneans that are life threatening to captive chondrichthyans are only known from intense captive infections (e.g., *Dermophthirius melanopteri* on the blacktip reef shark, *Carcharhinus melanopterus* (Quoy and Gaimard, 1824), (Cheung et al., 1988)), and thus the exact attachment location on wild hosts remains unknown. Furthermore, few biologists have accurately recorded the attachment locations of monogeneans on the gills of chondrichthyans. This is unfortunate because the gills represent a dissimilar array of niches that are not always homogeneously colonized by monogeneans (Bullard et al., 2001).

Locating juvenile monogeneans on hosts that have been in captivity several weeks or more is important because their presence suggests ongoing parasite reproduction that could ultimately overwhelm hosts. Juveniles of some monogeneans may be spatially segregated from their corresponding adults (Kearn, 1965; Euzet and Combes, 1998; Bullard et al., 2001), and because of this, and due to their small size, they may be difficult to detect. For example, adults of *Leptocotyle minor* attach to the upper surface of the crowns of placoid scales of the small-spotted cat shark, whereas postlarvae attach to the lower surface of the crowns (Kearn, 1965). On gills of heavily infected captive bonnethead sharks, juvenile *Erpocotyle tiburonis* attach between gill lamellae along the proximal two-thirds of the gill filaments (Figure 24.29), whereas adults attach to the capping tissue covering the efferent arteriole along the distal third of gill filaments (Figure 24.27) (Bullard et al., 2001).

Problems associated with infection

Although monogenean infections can cause health problems for captive chondrichthyans, details on the effects of these infections are largely unknown. Low intensity monogenean infections apparently do not debilitate hosts (Bullard et al., 2000c, 2001). However, the literature detailing

life threatening infections in captivity typically depicts situations when the gills or skin were overwhelmed by enormous parasite populations (Cheung and Nigrelli, 1983; Poynton et al., 1997; Bullard et al., 2001). Monogeneans that infect the gills of chondrichthyans (e.g., hexabothriids and chimaericolids) may debilitate hosts through the loss of blood associated with parasite feeding, the disruption of normal water flow over the gill lamellae as caused by lesions or by the parasites themselves, or by osmotic imbalance or other metabolic impacts associated with extensive lesions (Figures 24.27-24.29; Bullard et al., 2001). Microbothriids and some capsalids may potentially debilitate hosts by causing osmotic imbalance associated with extensive lesions or, the authors suspect, by metabolic demands imposed by the increased cellular activity associated with extensive lesions, i.e., epithelial cell proliferation and lymphocytic infiltration (Figures 24.22-24.25). Furthermore, because intense monogenean infections are typically associated with extensive lesions, opportunity probably exists for these infections to open significant portals for secondary pathogens such as some bacteria and fungi. Cheung et al. (1982) isolated a species of *Vibrio* (or possibly *Aeromonas*, see Grimes et al., 1985b) from lemon sharks infected with the microbothriid *Dermophthirius nigrellii*, and Grimes et al. (1985b) isolated *V. carchariae* from lemon sharks infected with *D. nigrellii*. It is unknown whether these bacterial infections represented opportunistic colonizations facilitated by lesions associated with monogenean infections or if the monogeneans actually vectored the bacteria, a possibility originally raised by Gruber (1980). The present authors doubt that monogenean infection is a prerequisite for bacterial infection, and some evidence indicates that apparently healthy sharks are commonly infected with potentially pathogenic bacteria that might only result in death or disease under physiologically stressful situations (Grimes et al., 1985a). However, the present authors suspect that under some circumstances monogeneans and bacteria may become powerful partners that cause disease in elasmobranchs. Because of this, husbandry staff should be aware of the potential for complications caused by secondary pathogens associated with monogenean infections.

Diagnosis, prevention, and treatment of infections

Monogenean infections are diagnosed by locating worms on fish, but finding these worms can be difficult because the intensity of wild infections is generally low (Bullard et al., 2001) and infections are not necessarily associated with telltale lesions. In addition, individual monogeneans can be overlooked because some species infect sites that are difficult to examine critically (e.g., interlamellar water channels of the gills, between gill filaments, within the buccal cavity, and within the olfactory sacs). Furthermore, many worms are small and semitransparent or well camouflaged in these sites (Chisholm and Whittington, 1995; S. A. Bullard, unpublished observations). In fact, a thoroughly exhaustive host examination for these worms requires extensive dissection that can only be carried out during necropsy. Considering these observations, and as demonstrated by the common occurrence of monogenean infections on fishes at public aquariums, not locating worms during preliminary examinations of live fishes does not guarantee these subjects to be uninfected; but rather, it indicates that infections, if present, are not intense enough to be detected. Because this concept is seldom appreciated, husbandry staff are sometimes surprised when intense monogenean infections are diagnosed weeks or months later on fishes that were previously thought to be uninfected. Microbothriid and capsalid infections on the skin of elasmobranchs often become noticeable once hemorrhagic or otherwise discolored lesions form (Figures 24.5, 24.22-24.25), or once abnormal behaviors signal irritating levels of infection (Cheung et al., 1982; Poynton et al., 1997; Bullard et al., 2000c). Hexabothriid infections on the gills may induce changes in host behavior such as erratic swimming, flashing, rubbing against hard substrates, rapid respiration, and piping (Bullard et al., 2001). This illustrates a mainstay in quarantine philosophy, i.e., the quarantine period must be long enough to allow low-level infections of potential pathogens to reach detectable levels.

Seeing monogeneans on a submerged fish is difficult. However, closely observing water that is slowly poured over the skin of a fish while it is out of or partially above water can reveal monogeneans and other small raised structures on the skin. If monogeneans are discovered on the skin, the present authors advocate mechanically removing them with forceps or locally treating the infected area with a parasiticide (e.g., a high dose of formalin) directly applied on the parasites. For example, if the host can be temporarily subdued with the infected region of its body above the water, a dam composed of a waterproof substance such as petroleum jelly can

allow the localized application of high doses of parasiticides on the skin for several minutes. Although such efforts only represent the first stage of an effective eradication strategy, they may rapidly reduce the number of egg-producing adult worms, thereby delaying the onset of intense infections.

Although it is unlikely that every monogenean infection of a captive chondrichthyan results in disease, husbandry staff should consider all monogeneans being capable of producing pathogenic populations unless specific information to the contrary exists. Based on host affiliations, husbandry practices, and the authors' personal observations, the following monogeneans are of special husbandry concern and their hosts should undergo routine prophylaxis during quarantine: *Erpocotyle* sp. (Hexabothriidae), found on gills of the leopard shark, *Triakis semifasciata* Girard, 1854, (see Boeger and Kritsky, 1989); *E. tiburonis,* found on gills of the bonnethead shark (see Brooks, 1934; Price, 1942; Yamaguti, 1963a; Boeger and Kritsky, 1989; Bullard et al., 2001); *Dendromonocotyle centrourae* (Monocotylidae), found on the skin of roughtail stingrays, *Dasyatis centroura* (Mitchill, 1815), (see Cheung and Whitaker, 1993); *D. californica,* found on the skin of bat eagle rays, *Myliobatis californicus* Gill, 1865, (see Olson and Jeffries, 1983); *Benedeniella posterocolpa* (Capsalidae), found on the ventral body surface of cow-nosed rays, *Rhinoptera bonasus* (Mitchill, 1815), (see Hargis, 1955; Thoney, 1990); *Dermophthirius maccallumi* (Microbothriidae), found on the skin of bull sharks, *Carcharhinus leucas* (Valenciennes in Müller and Henle, 1839), (see Watson and Thorson, 1976; Bullard et al., 2004); *D. penneri,* found on the skin of blacktip sharks and spinner sharks, *C. brevipinna* (Müller and Henle, 1839), (see Benz, 1987; Bullard et al., 2000c); *D. carcharhini,* found on the skin of dusky sharks, *C. obscurus* (Lesueur, 1818), bignose sharks, *C. altimus* (Springer, 1950), and Galapagos sharks, *C. galapagensis* (Snodgrass and Heller, 1905), (see Mac Callum, 1926; Rand et al., 1986; Benz, 1987); *D. melanopteri,* found on the skin of blacktip reef sharks (see Cheung et al., 1988); *D. nigrellii,* found on the skin of lemon sharks (see Cheung et al., 1982; Cheung and Ruggieri, 1983; Grimes et al., 1985b); *Neodermophthirius harkemai* (Microbothriidae), found on the gills and skin of lemon sharks (see Price, 1963; Euzet and Maillard, 1967; Poynton et al., 1997); and *Dermophthirioides pristidis,* (Microbothriidae), found on the skin of smalltooth sawfish, *Pristis pectinata* Latham, 1794, (see Cheung and Nigrelli, 1983). Ideally, treatments to eradicate infections should begin in transit to the quarantine facility, and transport water should not be mixed with quarantine or other life support water because it may become contaminated with monogenean eggs.

Institutions that do not capture their own fishes should learn how long and under what conditions captive fishes have been maintained by collectors because these parameters can affect the likelihood that newly acquired fishes will be infected. For example, some collectors confine pregnant sharks until they give birth, with the neonates being offered as exhibit or research animals. This practice saves time for the collector, and staff at many public aquariums prefer this practice because the pups are easy and inexpensive to transport and acclimate to captive conditions. Also, there is often a misconception that these pups are not infected by parasites. However, if the confined maternal shark is infected with monogeneans, rapid infection of her neonates is likely. Quarantine personnel that do not suspect the presence of these infections and forego appropriate prophylactic measures oftentimes are disappointed when these young fish subsequently are diagnosed with debilitating monogenean infections (Bullard et al., 2001). For the same reasons, institutions that obtain animals (fishes or invertebrates) from others should learn the containment and medical history of these animals and their tank mates.

A dizzying array of chemicals has been used with poor to excellent success to eliminate or control monogenean infections. Praziquantel is usually the most effective chemical control for these and other platyhelminths. However, limited study (Schmahl and Mehlhorn, 1985) has indicated that the efficacy of praziquantel is not consistent for all monogeneans. The reason for this is unclear, and unfortunately no comparative analysis has focused on this subject as it relates to various monogenean species that infect chondrichthyans. Because of this lack of information, husbandry staff should document and share details of their treatment successes and failures using praziquantel (and other parasiticides), including treatment particulars such as the host and monogenean being treated, the treatment dose and regime, and the pH, salinity, temperature, and physical configuration of the life support system involved.

Praziquantel kills platyhelminths by creating vacuoles in their syncytial tegument (Schmahl and Mehlhorn, 1985) and thus treatments aimed at eradicating monogeneans are usually administered

as short-duration or long-duration water applications (Thoney, 1990; Stoskopf, 1993d; Noga, 1996; Chisholm and Whittington, 2002). Some monogenean eggs are relatively resistant to some chemicals, including praziquantel (Thoney, 1990), and this phenomenon can result in short-duration chemical dips that kill adult worms but not unhatched larvae. On hatching, the resistant eggs produce oncomiracidia that recolonize the original hosts. Thus when treating infections of oviparous monogeneans it is important to consider how the treatment will address egg resistance. One method is to maintain therapeutic levels of parasiticides until all eggs have hatched and all oncomiracidia have been killed. A second method is to keep life support systems uninhabited by susceptible hosts until unhatched eggs become nonviable and hatched larvae have perished for lack of a host. In lieu of continuous therapies, a series of treatments is sometimes employed such that the first treatment kills the adult worms, and two successive treatments kill hatched larvae and juvenile worms before they mature and produce eggs. Unfortunately, without specific knowledge regarding the tempo of parasite development under particular environmental conditions, husbandry staff are often forced to proceed by carrying out successive treatments according to somewhat arbitrary schedules.

In circumstances when hosts are heavily infected with monogeneans, host mucus or thickened epithelium may reduce the efficiency of short-duration chemical baths. Under such a scenario it would be important for treatments to occur before a host response inadvertently shields some parasites from a waterborne parasiticide. In addition, some evidence exists that juvenile monogeneans living in relatively secluded niches on the gills and in the olfactory sacs may be harder to eradicate than their adult counterparts (Chisholm and Whittington, 2002). For example, in studying the efficacy of praziquantel water treatments in treating monogenean infections on the giant shovelnose ray, *Rhinobatos typus* Bennett, 1830, Chisholm and Whittington (2002) found that longer duration baths were necessary to eliminate juvenile worms even though juvenile and adult worms were similarly killed *in vitro*. Oral or injected praziquantel therapy or irrigation of the lumen of the rectal gland or coelom with praziquantel (followed by rinsing) may eradicate debilitating infections of endoparasitic monogeneans in those sites; however, the authors are unaware of anyone having tried the aforementioned irrigation approach. Nevertheless, and in all instances, water treatments should still be employed to prevent recruitment via vertical transmission.

Even at dosing levels of a few parts per million, praziquantel treatment of some life support systems may be too expensive for some institutions. To reduce cost, praziquantel therapy is sometimes carried out as dips or baths in smaller systems, a process that may require the transfer of fish from large life support systems for various lengths of time. Often these treatments provide only temporary relief from infections, as hosts become recolonized by larvae hatched from eggs that remained in the original life support system or from eggs attached to the host that were not killed by the short-duration treatment.

In instances when praziquantel treatment is not practical, other treatments may be attempted to control monogeneans. Freshwater dips and manual removal were used (Cheung and Whitaker, 1993) to eradicate infections of *Dendromonocotyle centrourae* on the skin of roughtail stingrays. Osmotic shock is usually carried out in temporary treatment tanks so that the progeny of detached worms will not colonize hosts. Although osmotic shock may eradicate or control some monogenean infections on elasmobranchs, several reports exist of the ineffectiveness of this treatment (Cheung et al., 1982; Thoney and Hargis, 1991; Poynton et al., 1997). Furthermore, some species of monogeneans that infect elasmobranchs appear to be euryhaline. For example, *Dermophthirius maccallumi* and *Heteronchocotyle leucas* have been collected from bull sharks over a wide range of salinities in Central America (Watson and Thorson, 1976), and *D. penneri* has been collected from blacktip sharks in nearshore waters with salinities of 20-35 ppt (Bullard et al., 2004; S. A. Bullard, unpublished observations).

Copper sulfate, an inexpensive compound that is readily available and easy to measure in solution, has unfortunately had limited efficacy regarding the control of monogenean infections of elasmobranchs (Thoney, 1990; Poynton et al., 1997). Furthermore, the toxicity of parasiticidal concentrations of copper to invertebrates and some fishes, as well as several other factors related to copper toxicity as influenced by water chemistry, prohibit the use of copper sulfate treatments in some instances (Noga, 1996). The organophosphate pesticide trichlorfon is inexpensive and readily available, and it has been used with varying levels of success to eradicate monogenean infections in captive elasmobranchs (Cheung et al., 1982; Thoney, 1990; Poynton et

al., 1997). However, in addition to trichlorfon being highly toxic to invertebrates and some fishes, it is also dangerous to humans if mishandled. In addition to the compounds mentioned above, anthelmintics other than praziquantel may prove useful in controlling monogeneans. For example, mebendazole was reported to be efficacious in treating monogenean infections on the gills of European eels (*Anguilla anguilla*; see Székely and Molnár, 1987), albeit the present authors are unaware of any specific use of this parasiticide in cases involving chondrichthyan hosts. If the efficacy of such treatments approaches that of praziquantel, some of these anthelmintics may be more desirable due to their lower cost.

General dosing guidelines for the application of all of the aforementioned chemotherapeutics can be found in Stoskopf (1993d) and Noga (1996). However, the authors advocate soliciting current information on chemotherapeutics (including information regarding dosing, human safety, and specifics concerning combinations of therapeutics, parasites, hosts, and life support systems) from experienced users before any treatments are initiated. It is notable that monogeneans have apparently demonstrated the ability to become resistant to chemical treatments (Goven et al., 1980). Thus, applying sublethal doses of chemotherapeutics could inadvertently promote resistance and reoccurring infections that are increasingly more difficult to control. Lastly, because monogenean infections may open portals for secondary infections (Grimes et al., 1985b), other health complications concomitant with monogenean infections may need to be addressed.

Fixation and taxonomic study

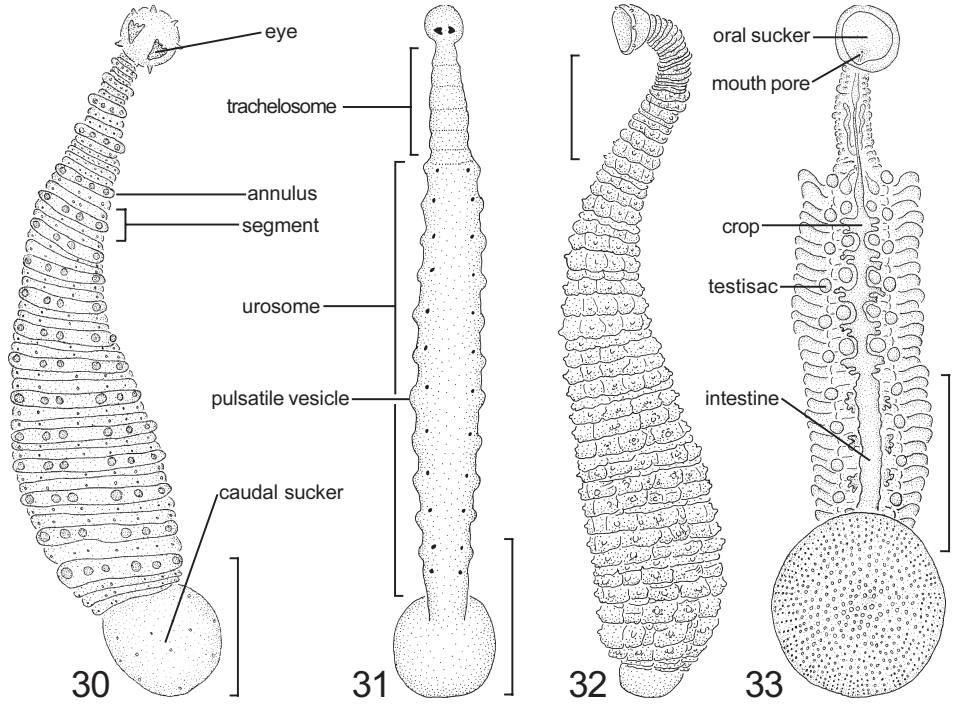
Monogeneans should be similarly relaxed and fixed to facilitate accurate identifications and valid morphological comparisons. Large worms that infect the skin can be carefully collected using forceps or a fine artist's brush to gently lift the base of the haptor free from the host or by cutting the host tissue surrounding the parasite such that the latter is removed still grasping a small piece of host tissue. Particular care is required when removing monogeneans that reside in enclosed sites such as the olfactory sacs and cloaca because these worms are typically small and extremely delicate, i.e., they lack a highly muscular body or thick tegument. In addition, the haptor of some species has taxonomically critical features that are easily damaged by the careless removal of worms. When delicate worms are torn, they relax, fix, and stain poorly. For these reasons, it is often best to fix worms still attached to host tissue or to remove them with the aid of a fine artist's brush while viewing them with a dissection microscope. For ideal fixation, live specimens should be placed in a water droplet on a glass microscope slide, covered with a glass cover slip typically without applying excessive coverslip pressure, killed by passing the microscope slide over the flame of an alcohol lamp for 2-3 sec, and fixed by careful transfer into a vial containing 5% neutral buffered formalin (n.b.f.). An alternative method requires live specimens to be placed in a dish with enough ambient temperature water (relative to the environment from which the samples were collected) to cover the worms. Pour near-boiling water (e.g., coffeepot temperature) into the dish and then immediately flood the dish with 10% n.b.f. After a moment, use a fine artist's brush or a pipette to transfer the specimens into a vial filled with 5% n.b.f.

The species identity of monogeneans is established using morphological characteristics of adult worms that are best observed using a light microscope equipped with Normarski interference optics. Specimens prepared for light microscopy are typically stained, cleared, and permanently mounted on glass microscope slides using standard parasitological techniques (Pritchard and Kruse, 1982). Description of the internal anatomy of some worms requires the use of multiple stains and serial sectioning in various planes using standard histological techniques (Presnell and Schreibman, 1997).

Leeches

Leeches are well-known parasites of elasmobranchs (Sawyer, 1986), and they are infrequently collected from chimaeroids (Love and Moser, 1983; Fernández et al., 1986; Sawyer, 1986; Cheung, 1993). Leeches can pose a threat to captive chondrichthyans because their life cycles are direct and their numbers can increase to pathogenic levels in captive settings; they have been implicated in mortalities of elasmobranchs in semi-open and closed life support systems;[9]

[9] The phrases "semi-open life support system," "semi-open environment," and "semi-open setting" refer to a captive setting in which small organisms such as metazoan parasites may move from the captive setting to natural waters or vice versa. The phrase "closed life support system" herein refers to a captive setting in which small organisms such as metazoan parasites may not move from the captive setting to natural waters or vice versa.



Figures 24.30-24.33. Leeches (Piscicolidae, Hirudinida) from elasmobranchs, adults; dorsal (Figures 24.30, 24.31), twisted (Figure 24.32), and ventral (Figure 24.33) views. **24.30.** *Stibarobdella bimaculata*. Modified from unpublished sketch by S. S. Curran, scale bar = 200 µm. **24.31.** *Calliobdella vivida*. Modified from Sawyer (1986), scale bar = 2 mm. **24.32.** *Pontobdella muricata*. Drawn from Sawyer (1986), scale bar = 10 mm. **24.33.** *Branchellion lobata*. Modified from unpublished sketch by S. S. Curran, scale bar = 500 µm.

they are known to vector pathogens and open portals for opportunistic pathogens; and, they are often difficult to eradicate from captive settings.

Systematics, distribution, and taxonomy

All leeches that have been reported from elasmobranchs are members of Piscicolidae (Rhynchobdellida, Hirudinida, Annelida). Piscicolids (Figures 24.30-24.33) inhabit freshwater, estuarine, and marine environments throughout the world; however, a leech infection from a freshwater chondrichthyan has not been reported. New species of piscicolids are routinely described, and it is not unusual for these to be discovered on common nearshore elasmobranchs (e.g., see Burreson and Kearn, 2000; Curran et al., in preparation). Those wishing to begin the process of identifying piscicolids should refer to Mann (1962), Soós (1965), Llewellyn (1966), Sanjeeva Raj (1974), Sawyer et al. (1975), and Sawyer (1986).

Morphology

Piscicolids are monoecious, subcylindrical to cylindrical segmented worms (Figures 24.30-24.33). The adults of various species range from a few millimeters long to over 10-cm long when relaxed, and these highly muscular worms can usually stretch to several times their relaxed length. Piscicolids use a disk-like or cup-like caudal sucker (Figures 24.30-24.33) as the principal attachment organ; however, a smaller (typically) oral sucker can also be used for attachment to facilitate crawling movements via a looping inchworm-like motion (Figure 24.34; Sawyer, 1986). A highly inflatable crop (Figure 24.33) allows leeches to gorge themselves on blood and lymph, and the level of engorgement can drastically influence the general shape of leeches. Feeding is accomplished by extending a proboscis through a mouth pore located in the oral sucker (Figure 24.33), and the secretion of anticoagulants assists in blood feeding (Sawyer, 1986). Piscicolids may vary in color, from small, thin, and relatively unpigmented species to those with highly noticeable eyes and greenish coloration (e.g., *Stibarobdella macrothela*). For more information on the morphology of piscicolids see Sawyer (1986).

Life history

Few specifics are known regarding the life cycles of most piscicolid species that infect

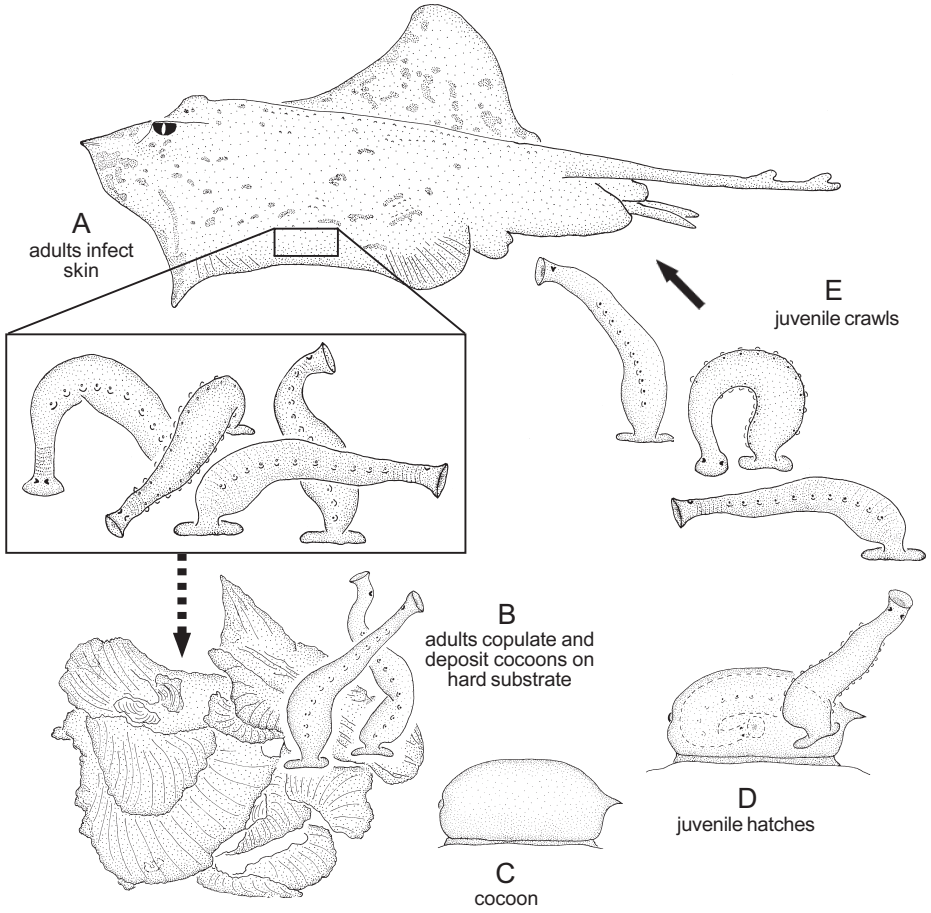


Figure 24.34. Life cycle of *Calliobdella vivida* (Piscicolidae), a skin parasite of the clearnose skate, *Raja eglanteria*; based on information in Sawyer and Hammond (1973). Dashed arrow indicates movement from host (dispersal); solid arrow denotes movement onto host (colonization). **A.** Adults infect skin of skate. **B.** Adults leave skate and copulate with each other while attached to hard substrate; as for example, an oyster bed or rubble pile. **C.** After copulation, adults adhere cocoons to shells or other hard substrates, including aquarium walls, equipment, and props. The cocoon of *C. vivida* is approximately 1.6-mm long and contains one larva. Drawn from Sawyer and Hammond (1973). **D.** A juvenile leech that looks like a small adult emerges from the cocoon after about 6 months. **E.** Juveniles crawl or sporadically swim to infect a host. Sawyer and Hammond (1973) speculated that juveniles of *C. vivida* emerged in winter when low water temperatures facilitated the infection of lethargic hosts living in the benthos.

chondrichthyans. Nevertheless, these life cycles are probably similar to those of better-known species such as *Calliobdella vivida*. The life cycle of *C. vivida* (Figure 24.34), a piscicolid that sometimes infects elasmobranchs, is direct and correlated to water temperature. Sawyer (1986) reported that in South Carolina estuaries, neonate *C. vivida* make their first appearance on fish in mid-December and become most abundant during the winter months when water temperatures are coldest, i.e., 9-10 C. In spring, when water temperatures rise to 14-17 C, the mature leeches detach from their hosts, lay cocoons, and die. From May until early December almost no leeches are found, and the hatching of cocoons in nature takes place in about 6 months, i.e., after the water has cooled (Burreson and Zwerner, 1982). However, at a constant 17 C, hatching can take place in 4 months, and at 5 C with subsequent warming to 17 C, hatching can take place in 1 year (Sawyer, 1986). An individual *C. vivida* may lay about 50 helmet-shaped cocoons (Figure 24.34; Sawyer, 1986), and the colonization of hosts by juveniles is probably facilitated by the lethargic winter behavior of fishes. The cocoons of piscicolids are sometimes attached to the shells of invertebrates (Figure 24.34; Sawyer, 1986), and *Stibarobdella macrothela* adults have been collected from the shells of crabs and bivalves (Sawyer et al., 1975). How these parasites colonize their chondrichthyan hosts is unknown, but it seems probable that they crawl, swim, or are transported by paratenic hosts (Figure 24.34; Sawyer et al., 1975).

Host and site specificity

No detailed information exists regarding the host and attachment site specificity of the leeches that

infect chondrichthyans. Sawyer (1986) categorically stated that within Pontobdellinae, *Pontobdella* and *Oxytonostoma* contain species that feed primarily on batoids and *Stibarobdella* contains species that feed primarily on sharks. However, records of platybdellines (Platybdellinae) and piscicolines (Piscicolinae) infecting elasmobranchs also exist (Cheung, 1993; Burreson and Kearn, 2000). Like most metazoan parasites of chondrichthyans, piscicolids display at least some degree of host specificity. For example, Goldstein and Wells (1966) found 26 specimens of *Branchellion ravenelii* on 31 smooth butterfly rays, *Gymnura micrura* (Bloch and Schneider, 1801), but not on 190 individuals representing 17 other fish species in the Gulf of Mexico off Florida. Although some leeches seem to be restricted to one or several closely related hosts (e.g., *Oxytonostoma typica* on the winter skate, *Leucoraja ocellata* (Mitchill, 1815)), others can be found on a variety of elasmobranchs (e.g., *Stibarobdella macrothela* was reported by Sawyer et al. (1975) from at least eight species of sharks). In addition, some piscicolids that normally associate with elasmobranchs have also been found attached to teleosts (e.g., *S. macrothela* on *Paralichthys dentatus*) and vice versa (e.g., *Calliobdella vivida* on the spiny dogfish, *Squalus acanthias* Linnaeus, 1758), (Sawyer et al., 1975). However, a leech attached to a fish may not feed, and some reports suggest phoretic rather than parasitic associations (Sawyer, 1986). Piscicolids with wide-ranging hosts (e.g., *S. macrothela*, a species that commonly attaches to members of Carcharhiniformes) typically have extensive global distributions (Llewellyn, 1966; Sawyer et al., 1975; Sawyer, 1986). In addition, salinity and temperature can influence the seasonal distribution of at least some piscicolids (Sawyer et al., 1975; Sawyer, 1986).

Piscicolids attach to the skin, cloaca, or within the olfactory, buccal, or branchial cavities of chondrichthyans. In addition, one species (*Branchellion lobata*) has been reported (Moser and Anderson, 1977) from the skin of embryos of the Pacific angel shark, *Squatina californica* Ayres, 1859. Future studies of piscicolids will likely reveal that particular species exhibit a moderate degree of feeding site specificity, as demonstrated by several piscicolids that infect teleosts (Sawyer, 1986).

Problems associated with infection

The intensity of piscicolid infections in nature seems highly variable and is possibly species dependent. For example, Rudloe (1971) reported as many as 50 *Branchellion ravenelii* from a single smooth butterfly ray, and one of the present authors (G. W. Benz, unpublished observations) has often collected only a single *Stibarobdella macrothela* from various individual carcharhiniforms (Carcharhiniformes). Regarding chondrichthyans, the present authors are only aware of casual observations of captive ray mortalities associated with piscicolid infections (e.g., see Curran et al., in preparation). Nevertheless, the literature contains numerous reports of leeches killing or otherwise harming teleosts (Burreson, 1995). The feeding activities of leeches can cause localized hemorrhaging on fishes, and intense leech infections can cause anemia (Burreson, 1995). Furthermore, the extensive epidermal erosion caused by intense piscicolid infections may establish debilitating osmotic imbalances in fishes and may open portals for infection by opportunistic species of bacteria and fungi. Some piscicolids vector viruses and haematozoic protozoa (Sawyer, 1986; Burreson, 1995). For example, the Atlantic starry skate, *Raja asterias* Delaroche, 1809, can be infected by a blood parasite, *Trypanosoma raiae*, that is vectored by *Pontobdella muricata* (see Sawyer, 1986). Some haematozoans vectored by piscicolids can cause the death of or disease in teleosts (Burreson, 1995); however, the health significance of piscicolid-vectored protozoan infections of chondrichthyans has not been studied.

Diagnosis, prevention, and treatment of infections

Leech infections are diagnosed by finding leeches on fishes. However, as these parasites can live away from their hosts for considerable periods, finding leeches elsewhere within life support systems or finding their cocoons should raise concern because it indicates the likelihood of future infections.

Leech infections may be difficult to control in semi-open settings. If leeches are entering these systems from the immediate surroundings, then repeated invasions might be expected. In such cases, mechanical barriers such as fine mesh screening or supply water filters might prevent leech invasions. Of course, quarantine provides opportunity to eliminate leech infections and prevent the introduction of exotic leeches into semi-open environments from which they might escape into nature.

In closed life support systems, quarantine is the first line of defense against leech infections. Fish and life support systems should be examined

periodically for the presence of leeches, and this process should begin immediately after capture to prevent leeches from retreating into difficult to examine body cavities and preclude gravid leeches from detaching and infesting captive settings. Removal of leeches from hosts using forceps can be an effective method of eradication if worms are easily accessible and if adult leeches have yet to leave their hosts to colonize the life support system. Although osmotic shock via freshwater or saltwater dips as well as formalin treatments have been reported to eradicate some leech infections (Stoskopf, 1993d; Burreson, 1995; Noga, 1996; Cruz-Lacierda et al., 2000), the present authors consider such treatments to be ineffective against the large leeches that commonly infect chondrichthyans. Organophosphate pesticides such as trichlorfon or dichlorvos applied as water treatments seem to be the most effective parasiticides to eradicate large leeches. However, because leeches can live away from their hosts for significant periods and because piscicolids deposit their cocoons away from the host, treating infected fishes without treating their entire environment may only provide temporary relief from infections. Furthermore, the potential exists for aquarium props, nets, and other equipment to inadvertently facilitate the transfer of leeches or their cocoons into or between life support systems. And lastly, because leeches may vector pathogens and open portals for secondary bacterial and fungal infections, concomitant health complications may need to be addressed when leeches are present.

Fixation and taxonomic study

Leeches, particularly thick-bodied specimens, must be properly fixed or they will be useless for identification purposes. Fixation for traditional taxonomic study ideally involves relaxation using menthol crystals followed by fixation in 8-10% n.b.f. or alcohol-formalin-acetic acid (AFA) (Pritchard and Kruse, 1982). Alternatively, and if menthol crystals are not available, small to medium size leeches can be placed between glass microscope slides and killed by passing the slides over a flame before fixation in AFA. Larger leeches can be doused with near-boiling water immediately before fixation. Because the gut contents of leeches can obscure critical morphological features, engorged leeches should be starved before fixation. Live leeches are easily maintained away from their hosts for long periods, and specialists sometimes prefer to be sent living leeches so that they may observe and properly fix them.

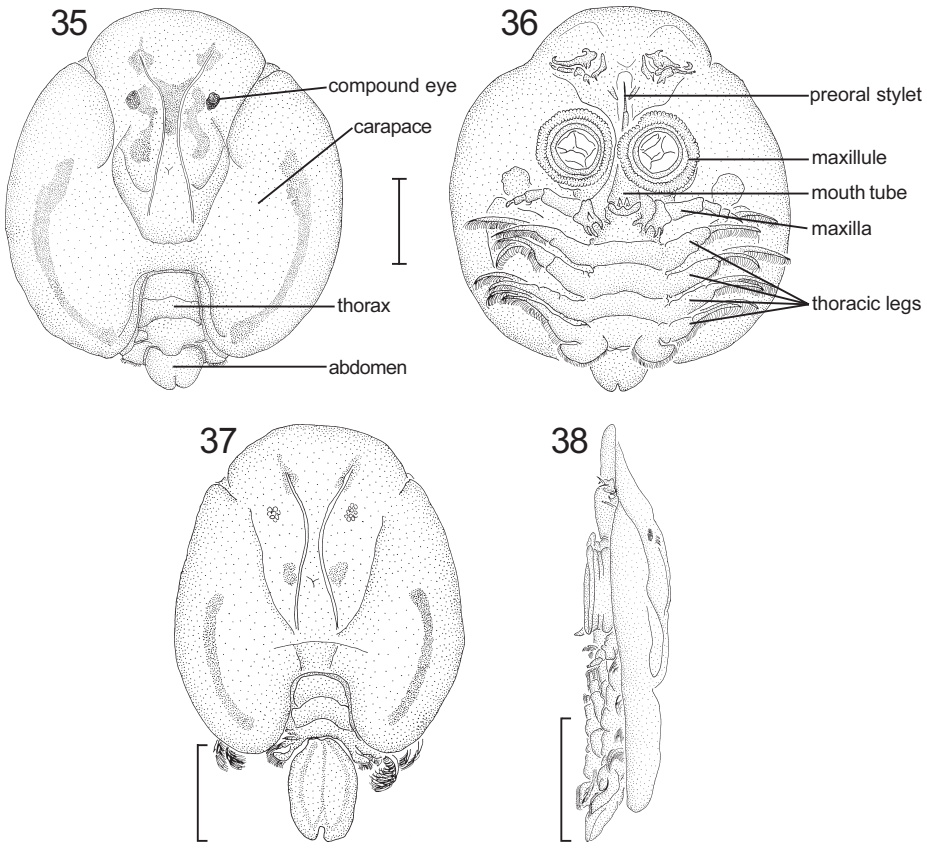
Leech taxonomy is based on a variety of morphological characteristics of mature worms (Sawyer et al., 1975; Sawyer, 1986) that are typically observed using a light microscope. Small specimens are usually stained, cleared, and permanently mounted on glass microscope slides (Pritchard and Kruse, 1982). Large specimens are not permanently mounted as whole specimens. Understanding the internal anatomy of some leeches requires specimens to be serially sectioned in various planes using standard histological techniques (Presnell and Schreibman, 1997).

Fish lice

Argulus spp. (Argulidae, Branchiura, Crustacea, Arthropoda) are commonly referred to as fish lice because they are primarily ectoparasites of fishes. Although there is no report of a fish louse infecting a chimaeroid and only a few reports of them infecting sharks and batoids (e.g., Wilson, 1902, 1904; Monod, 1928; Bere, 1936; Cressey, 1976, 1978; Ross, 1999), fish lice pose a threat to captive chondrichthyans because their life cycles are direct and their numbers can rapidly increase in captivity to pathogenic levels. Fish lice have been implicated in mortalities of wild fishes (Allum and Hugghins, 1959; Kolipinski, 1969) as well as those in captivity (Wilson, 1902; Poulin and FitzGerald, 1987), and it is commonly held that they exhibit low levels of host specificity.

Systematics, distribution, and taxonomy

One of four branchiuran genera, *Argulus* consists of about 120 nominal species that together have a cosmopolitan distribution and can be found in freshwater, estuarine, or marine habitats (Benz and Otting, 1996). There is no contemporary identification key to all *Argulus* spp., and the literature can sometimes thwart attempts to identify these parasites because many *Argulus* spp. have been inadequately described. A complete list of taxonomic references for *Argulus* is beyond the scope of this chapter; however, those wishing to begin the process of identifying fish lice should refer to Wilson (1902), Meehean (1940), Cressey (1976, 1978), Kabata (1988), Thatcher (1991), and Rushton-Mellor (1994). Because some *Argulus* spp. have been widely introduced (Rushton-Mellor, 1992), fish lice should be identified in light of global rather than local records.



Figures 24.35-24.38. *Argulus* sp. (Argulidae, Branchiura) adults from a short-tailed river stingray, *Potamotrygon* sp. Figures 24.35 and 24.36 modified from and Figures 24.37 and 24.38 drawn from unpublished sketches by T. A. Tarpley, scale bars = 1 mm. **24.35.** Female, dorsal view. **24.36.** Female, ventral view. **24.37.** Male, dorsal view. **24.38.** Male, lateral view.

Morphology

Argulus spp. range from several millimeters long to 2-cm long and can be shaded in various patterns of yellow, green, purple, brown, and black that camouflage them on their hosts. The general habitus of the adult fish louse (Figures 24.35-24.38) is composed of a dorsoventrally-flattened discoid carapace, a narrower thorax, and a bilobed abdomen. Dorsally the carapace has two anteriorly located compound eyes (Figures 24.35, 24.37). Ventrally the carapace bears five pairs of true appendages as well as accessory spines, a preoral stylet, and a mouth tube (Figure 24.36). The most obvious of these structures are the maxillules (Figure 24.36), portions of which form two large and powerful suction cups that secure the parasite to its host. Laterally the thorax bears four pairs of biramous cirriform legs (Figure 24.36) that are used for swimming and drawing water over the respiratory areas located ventrally on the carapace. Fish lice are dioecious. Mature males typically are smaller than their corresponding mates, and the basal portions of their second, third, and fourth thoracic legs are modified to clasp the female during copulation. The abdomen of the male contains two large ovoid testes; not to be confused with the smaller more spherical seminal receptacles of the female. Eggs can often be seen through the thoracic cuticle of gravid females. For more information on the morphology of fish lice see Martin (1932), Sutherland and Wittrock (1986), Overstreet et al. (1992), Swanepoel and Avenant-Oldewage (1992), Avenant-Oldewage and Swanepoel (1993), Gresty et al. (1993), and Benz and Otting (1996).

Life history

Fish lice exhibit direct life cycles (Figure 24.39). Mating takes place on or off the host, and gravid females attach their eggs to hard substrates such as stones, sticks, aquarium props, and aquarium glass or acrylic. Eggs are spherical to ellipsoid, approximately 0.4-mm long, sometimes tanned, and typically clustered together in a variety of characteristic patterns (Figure 24.39) that are best appreciated upon close inspection (Shafir and van As, 1986). Egg laying appears to be seasonal in

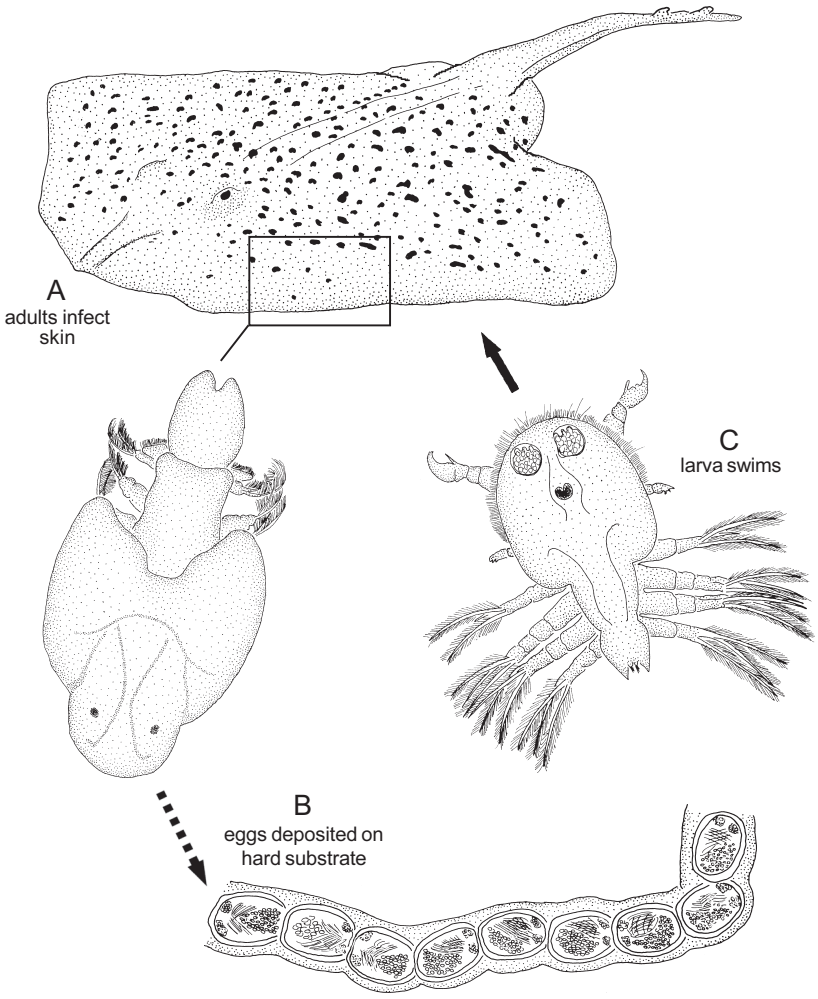
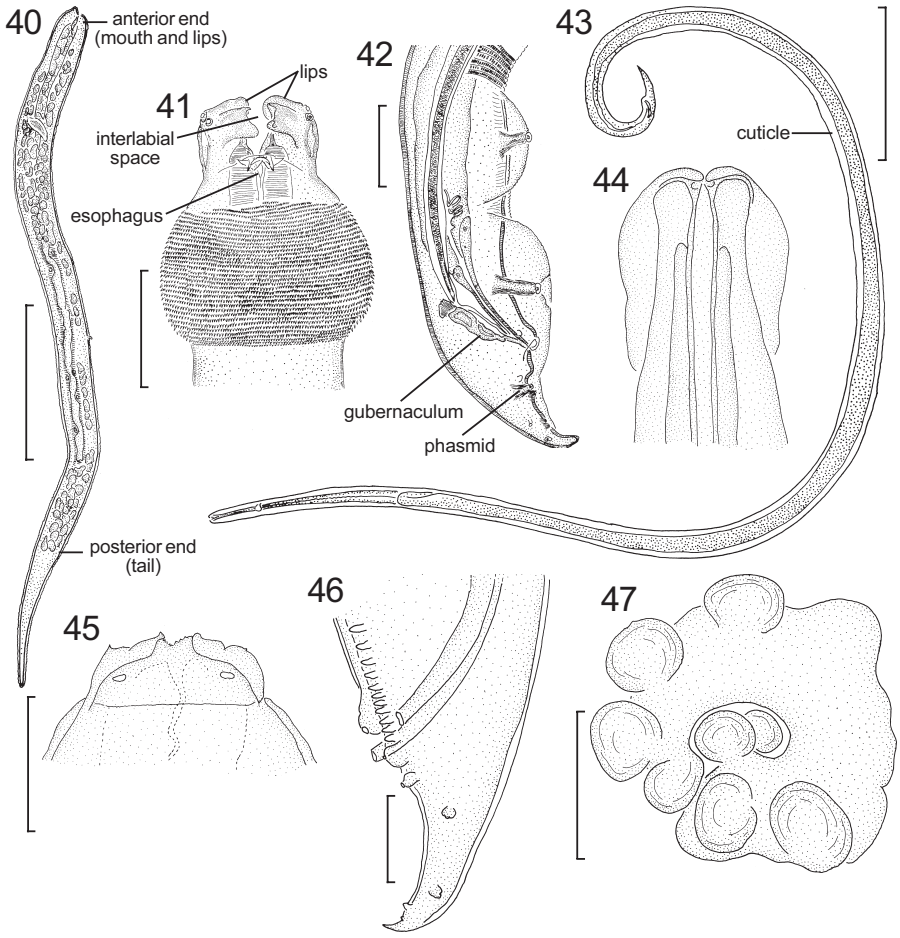


Figure 24.39. Life cycle of *Argulus megalops* (Argulidae), a skin parasite of the little skate, *Leucoraja erinacea*; based on information in Wilson (1904). Dashed arrow indicates movement from host (dispersal); solid arrow denotes movement onto host (colonization). **A.** Mature males and females are ectoparasites that copulate on or off skate. Scale bar = 500 µm. Parasite drawn from Kabata (1988). **B.** Gravid females swim from host and attach eggs to hard inanimate objects, such as sticks, stones, and aquarium walls, equipment, and props. The eggs, each about 170-µm long, are tanned and clustered in characteristic patterns. Time to hatching is inversely related to water temperature and usually takes from weeks to months. Drawn from Wilson (1904). **C.** The infective larva, about 3.8-mm long, swims until it attaches to a host. Juveniles molt several times before maturing, and adults continue to molt throughout life. Drawn from Wilson (1904).

nature (Shafir and van As, 1986), but it can be continuous in thermally stable environments such as aquariums (G. W. Benz, unpublished observations). Development time to hatching is inversely correlated to water temperature. For example, the eggs of *A. japonicus* hatched in 61 d when incubated at 15 C and in 10 d at 35 C (Shafir and van As, 1986). Larval fish lice can infect hosts immediately after hatching (Shimura, 1981). Although the juveniles of some *Argulus* spp. are morphologically similar to their respective adults, those of other species must undergo considerable development to reach their adult form (Shimura, 1981). Development from hatching to adulthood can involve up to nine molts (Shimura, 1981) and maturation can be rapid. For example, Shimura and Asai (1984) reported that at 15-16 C, females of *A. americanus* became gravid several weeks after hatching. Fish lice continue to molt throughout life (Lester and Roubal, 1995).

Host and site specificity

Fish lice reportedly do not exhibit a high degree of host specificity (Shafir and van As, 1985; LaMarre and Cochran, 1992). For example, in addition to being parasites of fintail stingrays (Dasyatidae), *A. chesapeakensis* and *A. flavescens* each infect many species of teleosts (Cressey, 1976, 1978). The potential for some *Argulus* spp. to infect a wide array of captive hosts is foreshadowed by the ability of these same species to infect a wide array of hosts in nature. It is commonly observed that some individual



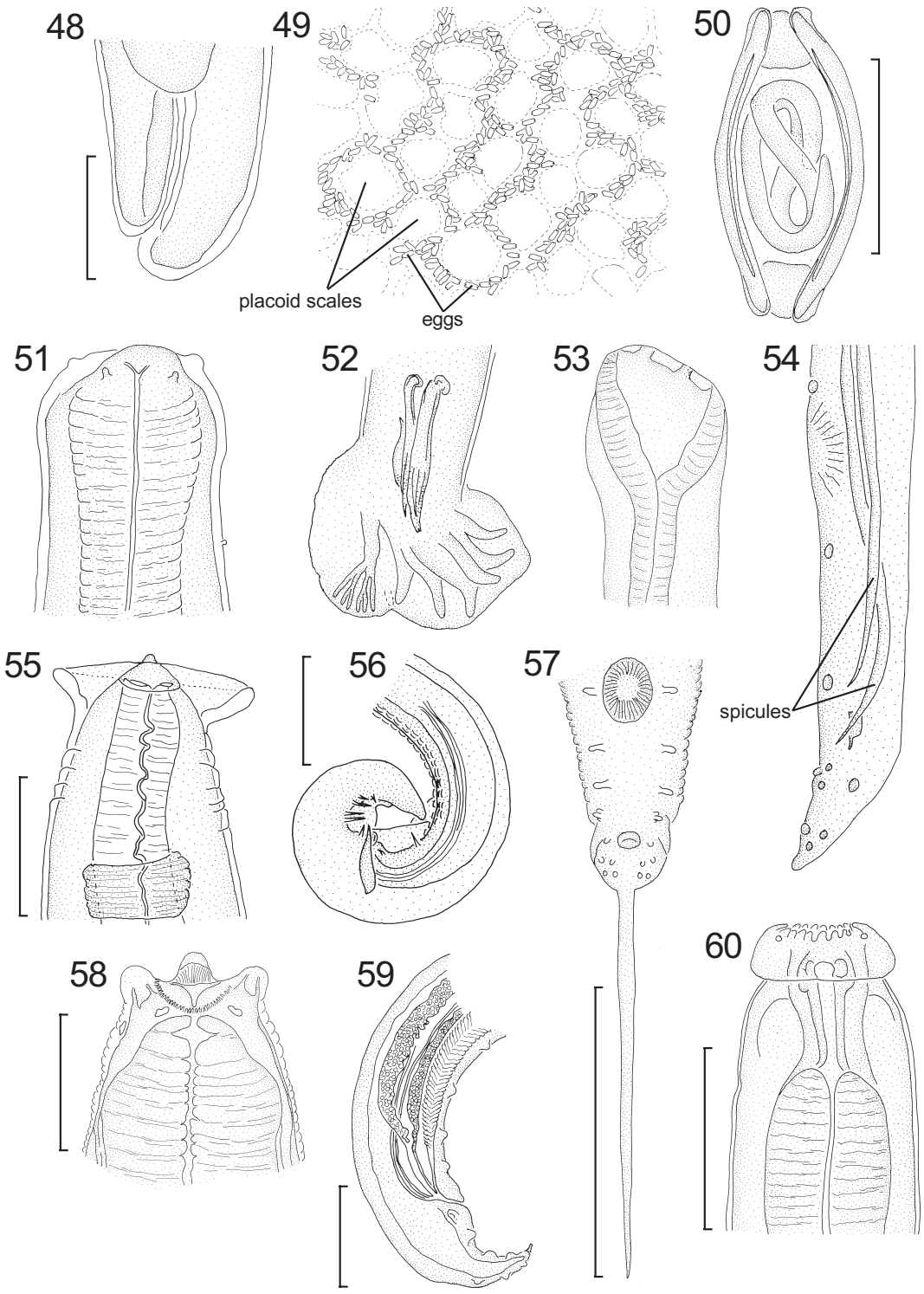
Figures 24.40-24.47. Nematodes (Nematoda) from elasmobranchs. **24.40.** *Phlyctainophora squali* (Dracunculoidea *incertae sedis* according to Adamson et al., 1987), female, intrauterine larva, lateral view. Modified from Adamson et al. (1987), scale bar = 60 µm. **24.41-24.42.** *Echinocephalus janzeni* (Gnathostomatidae). Modified from Hoberg et al. (1998). **24.41.** Anterior end showing cephalic bulb with rows of spines; dorsal view. Scale bar = 200 µm. **24.42.** Male, posterior end, lateral view. Scale bar = 150 µm. **24.43.** *Phlyctainophora squali*, male, lateral view. Modified from Adamson et al. (1987), scale bar = 300 µm. **24.44.** *Parascarophis sphyrnae* (Cystidicolidae), anterior end, lateral view. Drawn from Yamaguti (1961). **24.45-24.46.** *Terranova ginglymostomae* (Anisakidae). Drawn from Olsen (1952). **24.45.** Anterior end, dorsal view. Scale bar = 100 µm. **24.46.** Male, posterior end, lateral view. Scale bar = 100 µm. **24.47.** *Phlyctainophora lamnae* (Philometridae), female, lateral view. Drawn from Yorke and Maplestone (1962), scale bar = 8 mm.

hosts are infected by significantly more fish lice than others, and a variety of physical, ecological, and behavioral factors have been suggested to mediate this phenomenon (Poulin and FitzGerald, 1988, 1989a, 1989b, 1989c, 1989d).

Fish lice attach to the skin, eyes, fins, and within the buccal and branchial chambers of their hosts; however, close observations of aquarium-held hosts suggest that at least some *Argulus* spp. infect particular sites (G. W. Benz, unpublished observations). Fish lice are strong swimmers and can journey free from or scuttle over the surface of their hosts. Their vision seems acute and possibly used to avoid predators, as evidenced by fish lice that repeatedly retreated into the buccal or branchial chambers of their hosts when approached by husbandry staff (G. W. Benz, unpublished observations). This tendency to hide can make the mechanical removal of fish lice with forceps difficult, frustrating, and almost like sport.

Problems associated with infection

Although fish lice are well-known pathogens of captive teleosts (Kabata, 1970; Lester and Roubal, 1995), the present authors are only aware of one report of these parasites infecting a chondrichthyan in captivity. Ross (1999) reported *Argulus* sp. infections of aquarium-held short-tailed river stingrays (*Potamotrygon* sp.). These parasites were apparently reproducing vigorously in captivity before the infection was noted, and they were eliminated via diflubenzuron water treatments (Ross, 1999; R. Ross and G. W. Benz, unpublished observations). Although the ramifications of a branchiuran infection on a



Figures 24.48-24.60. Nematodes (Nematoda) from chondrichthyans. **24.48.** *Piscicapillaria hathawayi* (Trichuridae), female, posterior end, lateral view. Drawn from Read (1948), scale bar = 25 µm. **24.49.** Eggs of *Huffmanela carcharhini* (Trichuridae) embedded in epithelium between basal plates of a shark's placoid scales. Modified from MacCallum (1925). **24.50.** *H. carcharhini*, larva inside egg. Drawn from MacCallum (1925), scale bar = 50 µm. **24.51-24.52.** *Icthyostrongylus clelandi* (taxonomic status uncertain). Drawn from Yamaguti (1961). **24.51.** Anterior end. **24.52.** Male, posterior end. **24.53-24.54.** *Truttaedacnitis heterodonti* (Cucullanidae). **24.53.** Anterior end, lateral view. Drawn from Caira (1990). **24.54.** Male, posterior end, lateral view. Modified from Caira (1990). **24.55-24.56.** *Proleptus obtusus* (Physalopteridae). Drawn from Yorke and Maplestone (1962). **24.55.** Anterior end, lateral view. Scale bar = 150 µm. **24.56.** Male, posterior end, lateral view. Scale bar = 50 µm. **24.57-24.58.** *Pseudanisakis rotundata* (Acanthocheilidae). Drawn from Williams and Richards (1968). **24.57.** Male, posterior end, lateral view. Scale bar = 500 µm. **24.58.** Anterior end, ventral view. Scale bar = 200 µm. **24.59-24.60.** *Kathlania chiloscyllii* (Kathlaniidae). Drawn from Thwaite (1927). **24.59.** Male, posterior end, ventrolateral view. Scale bar = 100 µm. **24.60.** Anterior end, dorsal view. Scale bar = 200 µm.

chondrichthyan has not been studied, an untreated infection presumably could result in death of the host by any of the mechanisms that affect similarly infected teleosts. As agents of disease in teleosts, *Argulus* spp. cause tissue damage (including blood loss) and osmoregulatory problems via their feeding and attachment activities (Kabata, 1970; Lester and Roubal, 1995). In some instances they vector pathogenic viruses, bacteria, and nematodes (Moravec, 1978; Cusack and Cone, 1986; Molnar and Szekely, 1998; Moravec et al., 1999) and can cause lesions and stress that predispose hosts to opportunistic pathogens such as some species of bacteria and fungi (Lester and Roubal, 1995). Details regarding lesions associated with fish louse infections have been provided by Kabata (1970), Noga et al. (1991), and Lester and Roubal (1995).

Diagnosis, prevention, and treatment of infections

Fish louse infections are diagnosed by finding juvenile or adult fish lice on fish. However, finding eggs, juveniles, or adults away from hosts in a life support system is cause for obvious concern. Although no data exist regarding how fish louse infections affect chondrichthyan behavior, infections of teleosts by these parasites are sometimes betrayed by aberrant host behaviors such as lethargy, fasting, flashing, or darting about.

Quarantine is the first line of defense against fish louse infections. The captive environment and its fish should be regularly examined for the presence of fish lice, and this process should begin immediately upon quarantine to preclude the deposition of fish louse eggs. Husbandry staff should be aware of the cryptic coloration of fish lice as well as their ability to live away from the host for significant periods, to detach from the host when the host is disturbed, and to hide on the host in locations that are difficult to observe. Removal of fish lice using forceps is time consuming, but it can be an effective method to control or eradicate infections before they become problematic. Osmotic shock in the form of freshwater or saltwater dips, and water treatments using organophosphate pesticides or diflubenzuron at various concentrations can eradicate some fish louse infections (Stoskopf, 1993d; Benz et al., 1995; Noga, 1996; Ross, 1999). However, treating infected fishes without treating their entire environment may only provide temporary relief because fish lice deposit their eggs away from the host and can live away from their hosts for significant periods. Aquarium props, nets, and other equipment should be cleaned or sterilized, when appropriate, to prevent introducing or transferring fish louse larvae or eggs into or between life support systems. Nets used to capture infected fishes should have a fine mesh to retain fish lice and should be inspected for fish lice after every use because fish lice may abandon netted hosts. Fine-meshed dipnets can be used to capture free-swimming fish lice. In semi-open life support systems fish louse infections may be difficult to control. If the parasites entered the system from the immediate surroundings, or if infections can spread to nearby feral hosts, then repeated infections might be expected. Fine mesh screens and mechanical filtration can prevent immigration and emigration of fish lice, and properly maintained sand filters will retain juvenile and adult fish lice. Because fish lice may vector pathogens and open infection portals for opportunistic species of bacteria and fungi, concomitant health complications might arise when fish louse infections exist. In addition, husbandry staff should work cautiously when near infested life support systems because human eyes may become infected with fish lice (Hargis, 1958; Williams and Bunkley-Williams, 1996).

Fixation and taxonomic study

Fish lice are best prepared for traditional taxonomic study by fixing them in 10% n.b.f. and later transferring them into 70% ethanol (EtOH). Species' diagnoses are based on a variety of morphological characteristics of adult male and female specimens that are typically observed using a light microscope. Microscopic study is facilitated by the hanging-drop technique of Humes and Gooding (1964) as well as other methods detailed by Benz and Otting (1996).

Emerging problematic taxa

Emerging problematic taxa are herein considered to be metazoan parasites with suspected potential to cause the death of, or disease in, captive chondrichthyans. This group includes nematodes, copepods, and isopods. Of these, only some nematodes have been suspected of debilitating captive chondrichthyans based on clinical situations. However, based on the ever-expanding array of cartilaginous fishes maintained in captivity and ongoing efforts to artificially simulate nature within captive settings, the authors suspect that a growing cadre of species

representing the foregoing taxa will eventually cause problems for captive chondrichthyans. Prophylactic or parasiticidal treatments targeting other parasites have possibly been successful in eliminating undetected infections of some nematodes, copepods, and isopods.

Nematodes

Nematodes (Nematoda) are considered emerging problematic parasites of captive chondrichthyans because of the authors' familiarity with several cases of intense nematode infections of wild elasmobranchs (e.g., see Linton, 1900; Benz et al., 1987) and nematode infections in organs such as the brain or gills that were discovered during necropsies of captive elasmobranchs that had exhibited various abnormal characteristics before death (e.g., see Adamson et al., in preparation). Because the life cycles of many parasitic nematodes are complex (Dick and Choudhury, 1995b; Anderson, 2000), it is impossible for populations of these species to grow to problematic levels in captive settings devoid of necessary intermediate, paratenic, and definitive hosts. Nevertheless, hosts entering captivity with intense nematode infections may be prone to disease. The prevalence and intensity of nematode infections in elasmobranchs have probably been underestimated because some nematodes have histozoic larvae or adults that are difficult to detect during routine necropsies (Adamson, 1998).

Nematoda contains some 16,000 free-living and parasitic species that represent over 2,300 genera and 256 families, and it is estimated that 42,000 nematode species exist (Anderson, 1984). The nematode parasites of fishes are descendant species of terrestrial nematodes (Anderson, 1984). Although they are considered underrepresented in fishes (Anderson, 1984), there are at least ten nematode families (Kathlaniidae, Cucullanidae, Anisakidae, Acanthocheilidae, Philometridae, Micropleuridae, Gnathostomatidae, Physalopteridae, Cystidicolidae, Trichuridae) (Figures 24.40-24.60) that together hold approximately 100 species that infect chondrichthyans. Of these ten families, only Acanthocheilidae consists entirely of members that are restricted to these fishes. References by Yamaguti (1961), Anderson et al. (1974a, 1974b, 1975, 1976, 1978a, 1978b, 1980a, 1980b, 1982, 1983), Specian et al. (1975), Moravec (1987), and Moravec and Little (1988) are helpful in identifying many nematodes that infect chondrichthyans. Those challenged to interpret nematodes, and other parasitic metazoans, in tissue sections may benefit by referring to Chitwood and Lichtenfels (1972).

Also known as roundworms or threadworms, nematodes are typically long, tubular, nonsegmented worms with triradiate symmetry (Chitwood and Chitwood, 1950; Gibbons, 2002) (Figures 24.40-24.60). The body is covered by a nonliving proteinaceous cuticle (Figure 24.43) that functions as a hydrostatic skeleton. Beneath the cuticle the body is sheathed by a hypodermis and single layer of longitudinal muscle that mandates the characteristic sigmoid movements of nematodes. The mouth is usually surrounded by lips (Figure 24.41) that possess sensory organs; however, some groups that infect elasmobranchs lack well-defined lips (e.g., *Acanthocheilus* spp.) (Sprent, 1983). The alimentary tract consists of a muscular pharynx, intestine, and rectum. All nematode species that infect chondrichthyans are dioecious, and reproductive features associated with posterior portions of the body are often taxonomically important. Those seeking more information about the anatomy of nematodes will benefit by reading Chitwood and Chitwood (1950) and Gibbons (2002).

The life cycles of nematode species that infect chondrichthyans are poorly known (Anderson, 2000). Nevertheless, based on limited observations and a more complete understanding of the life cycles of related species, direct (in the minority) and indirect (predominantly) life cycles are assumedly possessed by these taxa (Anderson, 2000). Nematodes use chondrichthyans as definitive, intermediate, and paratenic hosts; and transmission between hosts is affected by predation (Anderson, 2000). Nematodes all pass through four larval stages (L1, L2, L3, and L4) before becoming adults, and each of these stages is separated by a molt (Chitwood and Chitwood, 1950). Capillariids (Capillariinae, Trichuridae) that infect chondrichthyans likely have direct life cycles (Dick and Choudhury, 1995b; Anderson, 2000); however, this has yet to be experimentally demonstrated. For taxa with indirect life cycles, crustaceans often serve as first intermediate hosts; second intermediate and paratenic hosts, if necessary, are typically fishes (Dick and Choudhury, 1995b; Anderson, 2000).

Nematodes infect sharks, batoids, and chimaeroids, and in elasmobranchs they are known from both marine and fresh water (Yamaguti, 1961; Deardorff et al., 1981). Because

the life cycles of nematodes are so reliant on trophic pathways, these parasites generally display only moderate levels of host specificity that appear mediated by ecological characteristics (Anderson, 1984, 2000). Nevertheless, nematode species that infect chondrichthyans appear to be somewhat restricted to these fishes. For example, Love and Moser (1983) listed 17 nematode species (representing six families) as infecting Californian chondrichthyans, and of these, 41% were collected from one host, 29% were collected from two hosts, 18% were collected from 3 hosts, and 12% were collected from four or more hosts. Of those species listed as having more than one host, 70% were restricted to chondrichthyans and 30% infected other fishes in addition to chondrichthyans. Of those species restricted to chondrichthyans, 71% infected various combinations of sharks, batoids, and chimaeroids, whereas 29% were confined to one of the foregoing chondrichthyan taxa.

Although nematodes have been collected from many tissues and organs within chondrichthyans, adult nematodes are moderately site specific within these fishes. Cucullanids, anisakids, acanthocheilids, physalopterids, cystidicolids, and gnathostomatids are typically found in the digestive tract (Williams and Richards, 1978; Moravec and Nagasawa, 2000; Knoff et al., 2001a; Moravec et al., 2001; Santos et al., 2004); philometrids are found in various tissues (Johnston and Mawson, 1943; Ruyck and Chabaud, 1960; Mudry and Dailey, 1969); and trichurids are found in the digestive tract and skin (Read, 1948; MacCallum, 1925, 1926; Moravec, 1987). Nematodes can cause the death of or disease in teleosts (Margolis, 1970; Sindermann, 1990; Dick and Choudhury, 1995b); however, little is known about the effects of these worms on their chondrichthyan hosts. Dick and Choudhury (1995b) listed several nematodes as causing disease in elasmobranchs, but the primary literature describing these cases (Linton, 1900; Schuurmans-Stekhoven and Botman, 1932; Williams, 1967; Williams and Richards, 1968; Rosa-Molinar et al., 1983) is vague regarding the health ramifications of lesions apparently caused by the parasites. Nevertheless, nematodes can cause lesions in important organs of chondrichthyans, such as the brain (Credille et al., 1993); pancreas (Borucinska and Heger, 1999; Borucinska and Frasca, 2002); ovary and uterus (Rosa-Molinar et al., 1983; Benz et al., 1987); stomach (Linton, 1900; Borucinska and Heger, 1999); spiral intestine (Williams and Richards, 1968; Borucinska and Heger, 1999); and gills, heart, spleen, and kidney (Borucinska and Heger, 1999). In addition, nematodes have been found within the lumen of the heart of a shark (Adamson and Caira, 1991).

The lack of reports of nematodes harming captive chondrichthyans may be linked to the complex life cycles of these worms and absence of required intermediate and paratenic hosts in captive communities. Without the ability for nascent infections to spread to new individuals or develop into parasite populations with life-threatening intensity, nematodes that have gained a foothold in captive environments cannot multiply to cause disease and produce epizootics. However, diagnosing the intensity of histozoic nematode infections is difficult (Adamson, 1998), and thus these worms have possibly played a greater-than-appreciated role in the health of their chondrichthyan hosts. Examinations of wild hosts (Benz et al., 1987; Adamson and Caira, 1991; Credille et al., 1993; Borucinska and Heger, 1999; Borucinska and Frasca, 2002) notably suggest that some individual chondrichthyans may enter captivity carrying high or possibly otherwise significant nematode burdens that eventually cause disease or condition loss. In spite of this, no justification exists for treatments, including prophylaxis, aimed at eradicating or preventing nematode infections not known to cause disease.

Treating enteric nematode infections is relatively simple, albeit not without risk to the host, via a variety of oral or injected anthelmintics such as heterocyclic compounds (e.g., piperazine), benzimidazoles (e.g., fenbendazole), imidazothiazoles (e.g., levamisole), and macrocyclic lactones (e.g., ivermectin) (Conder, 2002). Considerable anecdotes circulate regarding the toxicity of these and other chemotherapeutics to chondrichthyans; however, the validity of this conjecture remains unclear. When hosts perish during or shortly after a chemotherapeutic treatment, seldom do forensic studies reveal the proximate cause of death with certainty, yet chemotherapeutic toxicity is often suspected such that misinformation or hunches ultimately guide future husbandry decisions. This scenario seems particularly relevant when considering nematode infections in chondrichthyans because of the current lack of information regarding the pathogenicity of these worms. In some instances, eradication of worms may pose subsequent life-threatening problems for hosts. For example, successfully eradicating an intense histozoic nematode infection may create an acute immune challenge for the host that results in disease. The

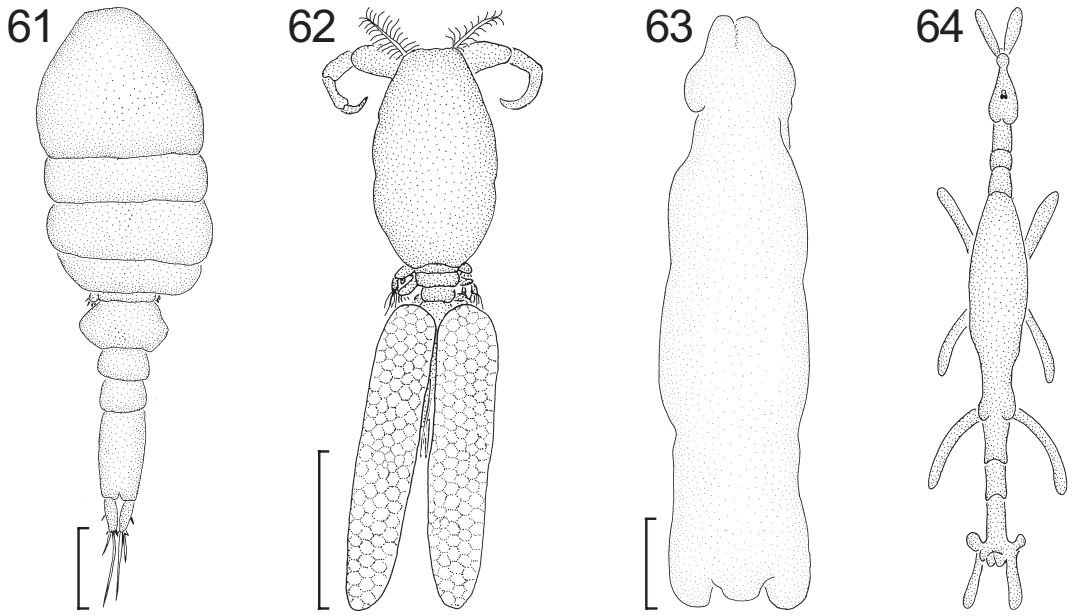
possibility that therapy may kill undetected histozoic nematodes with unpredictable consequences for the host can muddy decisions to treat nematode infections, or it can complicate the interpretation of mortalities subsequent to chemotherapeutic applications. Thus it should be stressed that until thorough studies address these and related matters, decisions to routinely treat hosts to eradicate or prevent nematode infections are not deeply rooted in science. Lastly here, it should be noted that nematodes can develop resistance to anthelmintics (Conder and Campbell, 1995; Sangster and Dobson, 2002).

In instances when nematodes are discovered upon necropsy, it is important to evaluate the possible impact they may have had on host health. When a nematode infection is linked to disease, the worms must be identified to species so that information regarding the parasite's life cycle and host range can be properly interpreted. For example, if the nematode species in question belongs to a higher taxon whose members possess indirect life cycles, and if no appropriate intermediate or paratenic hosts reside in the artificial environment and host food is not contaminated with viable nematode larvae, then it might be assumed that affected fishes transported their infections into captivity and that no subsequent increase in parasite intensity or prevalence has occurred since. Under such a hypothesis, the treatment of conspecifics and other known hosts to eradicate nematodes would not be warranted unless there was reason to expect that these individuals were similarly infected. It is notable that frozen marine fish were once implicated (Gaines and Rogers, 1972) in transmitting nematode infections when they were fed to hatchery-reared striped bass, *Morone saxatilis*, and that some nematodes are known to be vectored by fish lice (*Argulus* spp.) (Moravec, 1978; Molnar and Szekely, 1998; Moravec et al., 1999).

The preparation of nematodes for taxonomic study requires special care because nematodes possess a muscular body and thick cuticle that is often coated with host tissues, secretions, and other debris that can obfuscate taxonomically important features such as the lips and tail. Nematodes should be cleaned before fixation by placing them in a dish of saline. Live worms will often assist in casting off debris by writhing in the dish, and gentle petting with a fine artist's brush can speed this process or clean dead or moribund worms. Encysted nematodes should be carefully teased from their cysts using a pair of fine needles before cleaning. Special methods are required to prevent nematodes from twisting into practically useless coils upon fixation. To prevent coiling, clean worms are immersed in 95% glacial acetic acid for 5-10 minutes; particularly large or thick worms should be manually held uncoiled during this straightening and killing process. Nematodes <2-3 cm long can be uncoiled and killed by exposing them to near-boiling water, but this technique can blister or explode larger worms. Nematodes are fixed and stored in a solution of five parts glycerin and 95 parts 70% EtOH. A method to prepare small worms (e.g., larvae) for either morphological or molecular taxonomy study involves isolating them in a small volume of physiologic saline in a small container, swirling or shaking the container for about one minute, and then quickly but carefully pouring out the saline before adding a large volume of 95% EtOH and continuing to swirl the container for another minute. Nematodes are not typically stained when producing whole mounts for taxonomic study. Instead, whole specimens are submerged in a solution of five parts glycerin and 95 parts 70% EtOH in a loosely covered container. The glycerin penetrates and clears the specimen as the ethanol evaporates (a process that may take several days). Cleared specimens are mounted on glass slides using glycerin jelly (semipermanent mount) or lactic acid (temporary mount) (Deardorff and Overstreet, 1981; Pritchard and Kruse, 1982). Coverslips that cap glycerin-jelly mounts should be sealed about their perimeters with fingernail polish to protect the jelly from melting, and worms mounted in glycerin-jelly should be stored in a cool area. As a quick, temporary method to prepare nematodes for light microscopy, specimens may be cleared and mounted in lactic acid or PVA-lactophenol (Pritchard and Kruse, 1982). Finally, and as a last resort, nematodes may be relaxed in hot water and fixed in 10% n.b.f. However, these specimens should be transferred into 70% EtOH as soon as possible. Proper examination of taxonomically important features such as the lips can require thick sectioning and staining with Harris' hematoxylin and eosin (Presnell and Schreibman, 1997) or scanning electron microscopy (Gibbons, 1986). For those interested in the taxonomic identity of nematodes, but without the stamina or laboratory support to produce properly fixed specimens, nematodes can sometimes be shipped alive in saline to specialists for fixation.

Copepods

Copepods (Copepoda, Crustacea, Arthropoda) are considered emerging problematic parasites of

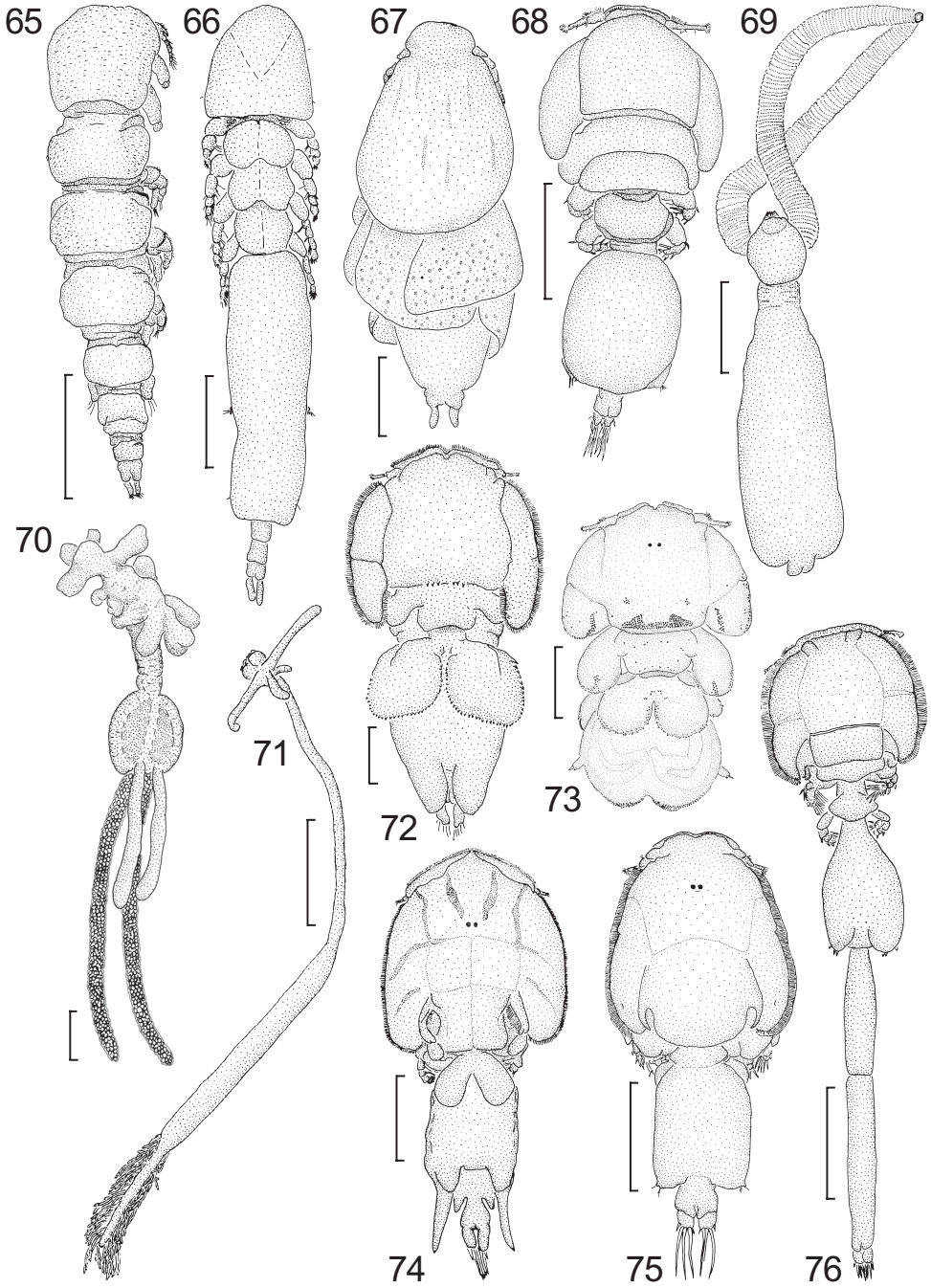


Figures 24.61-24.64. Poecilostomes (Poecilostomatoida, Copepoda) from chondrichthyans, adult females, dorsal views. **24.61.** *Taeniacanthodes dojirii* (Taeniacanthidae). Modified from Braswell et al. (2002), scale bar = 200 µm. **24.62.** *Ergasilus myctarothes* (Ergasilidae) with egg sacs. Drawn from Wilson (1913), scale bar = 1 mm. **24.63.** *Acanthochondrites annulatus* (Chondracanthidae). Drawn from Kabata (1979), scale bar = 2 mm. **24.64.** *Colobomatus lamnae* (Philichthyidae). Drawn from Yamaguti (1963b).

captive chondrichthyans because of their destructive feeding and attachment behaviors, direct life cycles, high levels of intensity in nature, and their potential ability to create entry lesions for opportunistic pathogens. Considering that some copepods (e.g., *Lernaea* spp.) can kill teleosts held in captivity, it is notable that species infecting chondrichthyans have to date been less problematic. This situation becomes bemusing when considering the great diversity and large number of copepod species that infect chondrichthyans in nature (see Yamaguti, 1963b). Most copepods that are regular pathogens of captive teleosts can complete their life cycles and reproduce in captivity; however, no unequivocal evidence exists of any chondrichthyan-infecting species being able to do so (but see Benz et al., 1992).

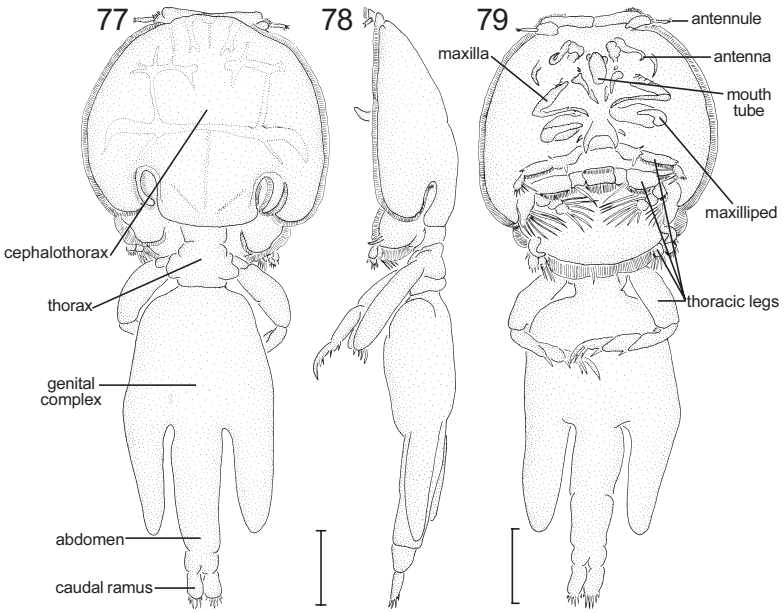
As treated here, Poecilostomatoida and Siphonostomatoida are the only copepod orders with representatives that infect chondrichthyans. Among the poecilostomes, the families Taeniacanthidae, Ergasilidae, Chondracanthidae, and Philichthyidae (Figures 24.61-24.64) possess members that infect chondrichthyans (Yamaguti, 1963b). Most species within these families infect teleosts, and those that infect sharks, batoids, and chimaeroids are restricted to marine waters (Yamaguti, 1963b). Among the siphonostomes, the families Eudactylinidae, Kroyeriidae, Dichelesthiidae, Pennellidae, Sphyriidae, Lernaeopodidae, Dissonidae, Pandaridae, Cecropidae, Trebiidae, Euryphoridae, and Caligidae (Figures 24.65-24.76) have representatives that infect chondrichthyans in marine and estuarine habitats (Yamaguti, 1963b; Kabata, 1979; Benz, 1993); however, the few records reporting infections by pennellids need verification. Together, the siphonostome species that infect chondrichthyans number in the hundreds and new species are constantly being described from sharks (e.g., see Dippenaar et al., 2000; Benz et al., 2001) and batoids (e.g., see Braswell et al., 2002; Dippenaar et al., 2004). Although Kroyeriidae is the only copepod family whose representatives are restricted to chondrichthyans (Yamaguti, 1963b; Benz, 1993; Deets, 1994), few copepod species infect chondrichthyans and nonchondrichthyans (Yamaguti, 1963b). Furthermore, the families Eudactylinidae, Pandaridae, Trebiidae, and Euryphoridae are predominantly composed of species that exclusively infect chondrichthyans, and siphonostomes infect representatives of all extant chondrichthyan orders (Yamaguti, 1963b; Benz, 1993).

Copepods display a wide array of body forms (Figures 24.61-24.76) and this as well as their complex morphology can make copepod identification difficult for nonspecialists. The



Figures 24.65-24.76. Siphonostomes (Siphonostomatoida, Copepoda) from chondrichthyans, adult females, dorsal views. **24.65.** *Eudactylinodes keratophagus* (Eudactylinidae). Drawn from Deets and Benz (1986), scale bar = 500 µm. **24.66.** *Kroeyerina deetsorum* (Kroyeriidae). Drawn from Benz et al. (2001), scale bar = 200 µm. **24.67.** *Anthosoma crassum* (Dichelesthiidae). Drawn from Kabata (1979), scale bar = 2 mm. **24.68.** *Dissonus pastinum* (Dissonidae). Drawn from Deets and Dojiri (1990), scale bar = 1 mm. **24.69.** *Ommatokoita elongata* (Lernaeopodidae). Drawn from Benz et al. (1998), scale bar = 5 mm. **24.70.** *Norkus cladocephalus* (Sphyriidae) with egg sacs. Drawn from Dojiri and Deets (1988), scale bar = 2 mm. **24.71.** *Pennella filosa* (Pennellidae). Drawn from Benz and Hogans (1993), scale bar = 1 mm. **24.72.** *Echthrogaleus disciarai* (Pandaridae). Drawn from Benz and Deets (1987), scale bar = 1 mm. **24.73.** *Entepherus laminipes* (Cecropidae). Drawn from Benz and Deets (1988), scale bar = 2 mm. **24.74.** *Alebion lobatus* (Euryphoridae). Drawn from Benz (1989), scale bar = 2mm. **24.75.** *Caligus curtus* (Caligidae). Drawn from Parker et al. (1968), scale bar = 2 mm. **24.76.** *Trebius longicaudatus* (Trebiidae). Drawn from Nagasawa et al. (1998), scale bar = 2 mm.

copepod body (Figures 24.77-24.79) primitively comprises a cephalosome of six somites, a postcephalic trunk of nine somites, and an anal somite; however, the boundaries between these regions may be obscured by tagmosis (Kabata, 1979; Huys and Boxshall, 1991). Kabata (1979) and Boxshall and Montú (1997) provided identification keys to families and Boxshall and



Figures 24.77-24.79. *Paralebion elongatus* (Caligidae), adult female. Modified from Benz et al. (1992), scale bars = 1 mm. **24.77.** Dorsal view. **24.78.** Lateral view. **24.79.** Ventral view.

Halsey (2004) provided identification keys to families, genera, and some species of parasitic copepods. Copepod taxonomy is largely based on the morphology of the adult female (Figures 24.77-24.79), and in addition to general body form, details regarding the appendages are often important in species' diagnoses. The males and larvae of many species are unknown or poorly known. Copepods are fixed in 10% n.b.f. and later transferred into 70% EtOH for traditional taxonomic study.

The life cycles of copepods that infect chondrichthyans are incompletely known at best (Benz, 1993). Based on scattered reports of copepod larvae collected from chondrichthyans (e.g., Benz, 1989, 1991; Benz et al., 1992, 2001, 2002a) and on knowledge of the complete life cycles of a handful of related species that infect teleosts (e.g., see Kabata and Cousens, 1973; Piasecki, 1989; Abdelhalim et al., 1991; Schram,1993), the life cycles of all species that infect chondrichthyans are probably direct (except for members of Pennellidae; Perkins, 1983; Benz, 1993) and involve a series of larval stages (nauplii and copepodids) each separated by a molt. A nauplius usually hatches from the egg. Dispersal is primarily achieved by the nauplii of most siphonostomes and by the nauplii, copepodids, and sometimes even adults of poecilostomes. Colonization is thought to be achieved primarily by the adults of poecilostomes and infective copepodids of most siphonostomes, although transfer among hosts may be possible for adult siphonostomes that can swim (e.g., at least some dissonids, trebiids, euryphorids, and caligids). The postcolonization copepodids of siphonostomes are parasitic. Copepods infect the general body surface, fins, cloaca, lateral line, buccal cavity, gills and branchial chamber, eyes, olfactory sacs, and uterus of chondrichthyans (Delamare-Deboutteville and Nunes-Ruivo, 1952; Benz, 1980, 1981, 1986, 1989; Benz and Deets, 1986, 1987, 1988; Benz and Dupre, 1987; Deets, 1987; Benz and Adamson, 1990; Borucinska et al., 1998; Nagasawa et al., 1998; Borucinska and Benz, 1999; Benz and Dippenaar, 2000; Benz et al., 2001, 2002a, 2002b; Braswell et al., 2002), and they have even been collected from the skin of shark embryos (Nagasawa et al., 1998). Most species are ectoparasites; however, some are mesoparasites that deeply penetrate their hosts (Benz and Deets, 1986; Deets and Ho, 1988; Dojiri and Deets, 1988), and a few are arguably endoparasites (Delamare-Deboutteville and Nunes-Ruivo, 1952; Nagasawa et al., 1998). The adults of many species of copepods infect narrow niches on chondrichthyans (Figure 24.6; Benz, 1993).

The effects of copepods on batoids and chimaeroids are only known from casual observations of lesions associated with infections, and information regarding the effects of copepods on sharks is limited to several more detailed studies. Regarding gill-dwelling copepods, Benz (1980) and Benz and Adamson

(1990) provided details regarding lesions caused by *Nemesis lamna* on shortfin makos, *Isurus oxyrinchus* Rafinesque, 1810, and *N. robusta* on thresher sharks, *Alopias vulpinus* (Bonnaterre, 1788), respectively. Adult female copepods were associated with considerable hyperplasia of the capping tissue that surrounded the efferent arterioles of the gill filaments of both host species, and the gills of thresher sharks also exhibited hyperplasia that totally occluded the respiratory water channels between gill lamellae (Benz, 1980; Benz and Adamson, 1990). The alteration of water flow that would presumably accompany the aforementioned lesions was suggested (Benz, 1980; Benz and Adamson, 1990) to diminish the overall respiratory efficiency of infected sharks, and it seems possible that intense infections of these parasites might seriously debilitate hosts. Infections of some gill-dwelling copepods can be extremely intense in nature, and this suggests that some sharks may enter captivity bearing considerable numbers of parasites. For example, Benz and Dupre (1987) estimated that the gills of one wild-caught blue shark, *Prionace glauca* (Linnaeus, 1758), were infected by over 1,200 *Kroyeria carchariaeglauci*, a copepod whose bright red color suggests it to be hemophagic. *Anthosoma crassum*, a dichelesthiid that principally infects lamniforms (Lamniformes), including the sand tiger and the smalltooth sand tiger shark, *Odontaspis ferox* (Risso, 1810), is a destructive copepod. Common in nature, *A. crassum* produces deep lesions about the jaws of its host that can result in severe subacute, necrotizing stomatitis in the mucosa and reactive lymphocytic infiltration of the submucosal skeletal muscle (Benz et al., 2002a). Complications associated with infection can result in loss of shark teeth and scales, and inflammation can be deep and involve host nerve tissue (Benz et al., 2002a). Based on these observations, and on the casual observations of others documenting high infection intensities resulting in sharks whose jaws were missing most of their teeth (Barnard, 1955) or whose tongues were apparently hollowed (Hewitt, 1968), Benz et al. (2002a) concluded that *A. crassum* might cause disease in wild sharks. In many instances, lesions associated with adult female copepods appear more chronic and severe than those associated with corresponding male copepods. For example, Borucinska and Benz (1999) reported this phenomenon regarding lesions caused by *Phyllothyreus cornutus* on the interbranchial septa of blue sharks, and they attributed the greater severity of lesions associated with female copepods to the relatively sessile nature of the adult female vs. the more vagile nature of adult males. In other instances, these differences may signal distinctions in the attachment mode (e.g., see Benz and Deets, 1986) or feeding habits of female vs. male copepods.

When assessing the impact of copepod infections it is important to consider distinctions between the presence of severe lesions and the existence of disabling disease, because the latter is not necessarily mandated by the presence of the former. For example, recent studies (Benz et al., 1998, 2002b; Borucinska et al., 1998) of ocular lesions caused by the lernaeopodid *Ommatokoita elongata* on Greenland sharks, *Somniosus microcephalus* (Bloch and Schneider, 1801), and Pacific sleeper sharks, *S. pacificus* Bigelow and Schroeder, 1944, indicated that severe corneal displasia associated with these infections may result in partial blindness. However, it was concluded (Borucinska et al., 1998; Benz et al., 2002b) that such a disability might have little effect on these hosts because sleeper sharks (Somniosidae) may not need to rely on acute vision to survive.

To avoid potential problems, chondrichthyans should be thoroughly examined for copepod infections during the early stages of captivity and again before they are released from quarantine. Mechanical removal of copepods using forceps can be an efficient method of eradicating infections of species in easily observed and accessible locations on hosts (e.g., skin); and because many copepods are readily noticeable, aesthetic appearance as well as health concerns prompt their removal. Infections by copepods that reside in relatively inaccessible regions (e.g., on the gills, in the buccal or branchial chambers, or in the olfactory sacs) would be difficult to diagnose without the use of special equipment such as an endoscope, and the resources needed for such examinations as well as the associated host manipulation involved does not generally warrant these searches. The broad-spectrum chemical treatments (e.g., organophosphate pesticides) commonly used during quarantine to control other parasites probably serve a dual purpose by also controlling copepods. However, because the impact of parasitic copepods on captive chondrichthyans seems primarily related to aesthetics rather than medical matters, decisions to disregard some copepod infections can be reasonable. In cases when copepods have been diagnosed as problematic or potentially so as, for example, when infection intensities seem high or when infections are associated with

significant lesions, more specific parasiticides (e.g., diflubenzuron or lufenuron) might be employed.

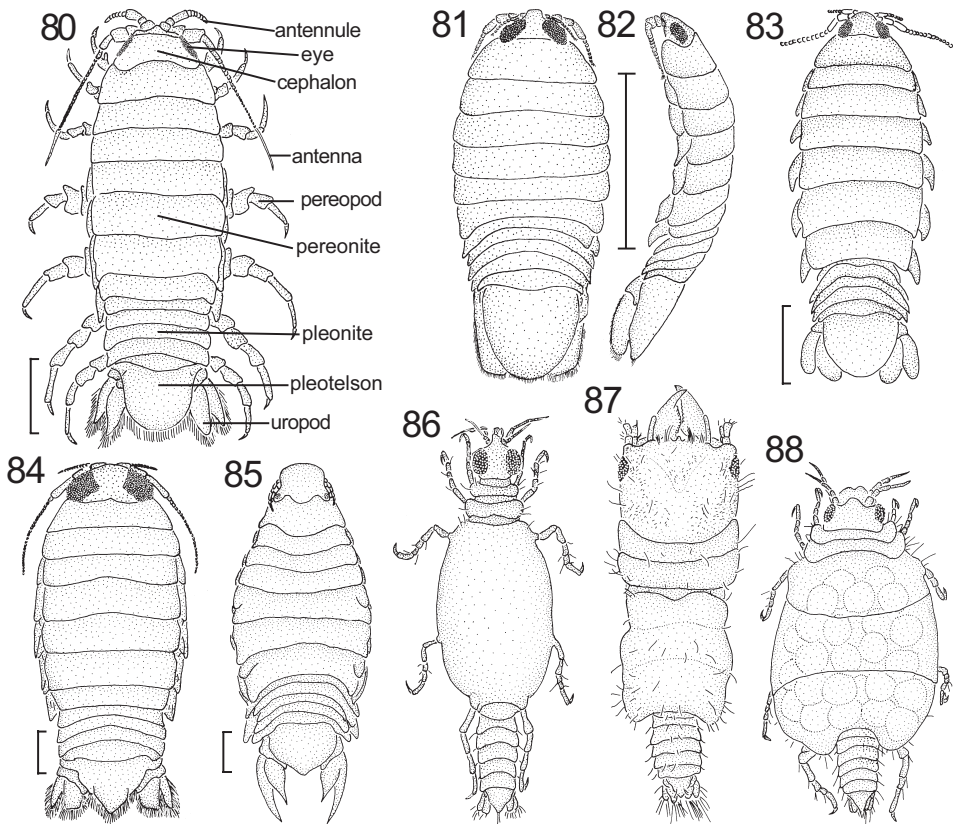
Most information regarding the control of parasitic copepods stems from efforts to control sea lice, i.e., various caligid species, or gill maggots, and various lernaeopodid species, that routinely impact marine aquaculture operations (Boxshall and Defaye, 1993; Roth et al., 1993a; Johnson and Heindel, 2001; Duston and Cusack, 2002; Roberts et al., 2004). To date, attempts at controlling sea lice have focused on a wide variety of methods, including environmental manipulation (Grant and Treasurer, 1993), chemotherapeutic application (Palmer et al., 1987; Bron et al., 1993; Costello, 1993; Roth et al., 1993a, 1993b; Smith et al., 1993; Thomassen, 1993; Bruno and Raynard, 1994; Johnson and Heindel, 2001; Duston and Cusack, 2002; Roberts et al., 2004), host vaccination (Costello, 1993; Jenkins et al., 1993; McAndrew et al., 1998), and biological control (Bjordal, 1988; Darwall et al., 1992; Costello, 1993; Treasurer, 1993). As noted by Costello (1993), each of the foregoing methods has its strengths and weaknesses. Although chemotherapeutics delivered by feed, injection, or as water treatments have been used in attempts to control sea lice in net-pen aquaculture operations (Costello, 1993; Roth et al., 1993a), water treatments seem advantageous in the closed life support systems typical of public aquariums. The advantages of using chemotherapeutics delivered via water treatments mainly stem from ease of application, dosing accuracy, and the potential to kill both free-living and parasitic life-history stages of copepods. Water applications of organophosphate pesticides have been widely used to control sea lice (Costello, 1993; Roth et al., 1993a), and the broad spectrum usefulness, wide availability, low cost, and widespread familiarity with these compounds make them a logical treatment option for public aquariums to control problematic copepods (for several examples regarding the dosing of organophosphate pesticides see Stoskopf, 1993d; Noga, 1996). For a somewhat more specific attack against copepods, water treatments using chitin inhibitors such as diflubenzuron and lufenuron can be employed (Stoskopf, 1993d; Noga, 1996).

Besides the environmental risks associated with using chemotherapeutics to control parasitic copepods, human health risks associated with the use of some parasiticides can be significant and warrant special application protocols and medical monitoring (Health and Safety Executive, 1987; Wells, 1989; Costello, 1993; Roth et al., 1993a). To reduce human exposure to hazardous chemicals, the salmon net-pen industry continues to explore alternative control measures for sea lice, such as the use of biological controls (see below), hydrogen peroxide treatments (McAndrew et al., 1998), and chemotherapeutics such as dichlorvos that are considered safer than trichlorfon (Grave et al., 1991; Costello, 1993). Some environmental concerns related to specific chemotherapeutics may be mitigated by altering the pH of treated water, exposing treated water to sunlight or elevated temperatures, retaining treated water until chemical transformation has occurred, discharging effluents into septic or sewer systems, or ozonating effluents (Costello, 1993). Overreliance on, or sublethal application of, chemotherapeutics can produce resistant populations of parasitic copepods, as evidenced by the documented resistance of *Lepeophtheirus salmonis* to the widely-used organophosphate parasiticide dichlorvos (Jones et al., 1992). In addition to chemotherapeutics, biological control of parasitic copepods using various wrasses (Labridae) is widely used in the salmon net-pen industry as one component of a combined biological and chemotherapeutic attack against sea lice (Bjordal, 1988; Darwall et al., 1992; Costello, 1993; Treasurer, 1993). Some cleaner fishes are known to consume parasitic copepods that infect elasmobranchs (Strasburg, 1959; Cressey and Lachner, 1970).

Isopods

Isopods (Isopoda, Crustacea, Arthropoda) (Figures 24.80-24.88) are considered to be emerging problematic associates of captive chondrichthyans because of their voracious tendencies,[10] reputed low degree of host specificity, direct life cycles, high mobility, ability to live away from their hosts for significant periods, potential to directly cause disease, potential to facilitate disease as vectors of pathogens, and potential to create portals for opportunistic pathogens. However, the authors are unaware of a report of an isopod being associated with the death of or disease in a captive chondrichthyan. Nevertheless, the potential exists for some isopods to go unnoticed on captive hosts and

[10] Some isopods that feed on fishes will also bite humans (Williams and Bunkley-Williams, 1996), as S.A.B. discovered when being repeatedly bitten on the scrotum by isopods while dissecting sharks in shallow water in the Gulf of California.



Figures 24.80-24.88. Isopods (Isopoda) from elasmobranchs. **24.80-24.85.** Flabelliferans. **24.80.** *Cirolana borealis* (Cirolanidae), adult female, dorsal view. Modified from Schultz (1969), scale bar = 24 mm. **24.81-24.82.** *Rocinela angustata* (Aegidae). Drawn from Rafi (1988), scale bar = 15 mm. **24.81.** Dorsal view. **24.82.** Lateral view. **24.83.** *R. propodialis*, adult female, dorsal view. Drawn from Rafi (1988), scale bar = 5 mm. **24.84.** *Excorallana tricornis* (Excorallanidae), adult female, dorsal view. Drawn from Schultz (1969), scale bar = 1 mm. **24.85.** *Nerocila acuminata* (Cirolanidae), adult female, dorsal view. Drawn from Brusca (1981), scale bar = 1 mm. **24.86-24.88.** *Gnathia puertoricensis* (Gnathiidae), dorsal views. Drawn from Kensley and Schotte (1989). **24.86.** Praniza larva. **24.87.** Adult male. **24.88.** Adult female.

become problematic, and it is probable that some isopods can reproduce in captivity. The isopod suborders Gnathiidea and Flabellifera contain species that infect chondrichthyans. Isopods are prepared for taxonomic study using a light microscope by fixing them in 10% n.b.f. and later transferring them into 70% EtOH.

Gnathiidea contains one family, Gnathiidae, whose representatives infect fishes in marine and estuarine habitats. Adult gnathiids (Figures 24.87-24.88) are benthic and have been found living in burrows, crevices, dead barnacles, sponges, dead coral, coral sand, bryozoans, and other structure suitable for hiding, wherein males sometimes guard harems of females (Wägele, 1988; Lester and Roubal, 1995; Grutter and Poulin, 1998). Females incubate eggs that hatch into swimming larvae (zuphae) that rapidly develop into parasitic larvae known as pranizae (Smit et al., 2003). Pranizae (Figure 24.86) are excellent swimmers and they feed on blood and other host body fluids (Monod, 1926; Lester and Roubal, 1995). Pranizae periodically detach from their hosts and swim to the benthos where they molt and then colonize new hosts (Monod, 1926; Upton, 1987; Klitgaard, 1991). Mature gnathiids are free-living, and they apparently do not feed or swim (Wägele, 1988; Grutter and Poulin, 1998). Few gnathiid life cycles have been demonstrated, and time to maturity for some species can be months to years; adults may live for several years (Stoll, 1962; Amanieu, 1963; Wägele, 1988; Smit et al., 2003).

Gnathiids are similar to other isopods in that they are most easily recognized by their bilaterally symmetrical body, T-shaped or triangulate pleotelson, terminally positioned uropods, and five pairs of pereopods (Schultz, 1969). Gnathiid taxonomy is largely based on adult male morphology, and adult males look quite dissimilar from their corresponding adult females and larvae (cf. Figure 24.87 with Figures 24.86 and 24.88; Schultz, 1969; Cohen and Poore, 1994) as well as from non-gnathiids. Adult female and larval gnathiids cannot be identified as species based on morphology, and certainly this has hampered

the understanding of gnathiid life history. Some researchers have maintained pranizae *in vitro* until males matured such that they could be identified (Stoll, 1962; Paperna and Por, 1977; Wägele, 1987, 1988; Smit et al., 2003). Molecular methods would probably be useful for identifying gnathiid larvae and adult females.

Gnathiids are known to infect members of Chimaeriformes, Squatiniformes, Orectolobiformes, Lamniformes, Carcharhiniformes, Rhinobatiformes, Torpediniformes, Rajiformes, and Myliobatiformes (Paperna and Por, 1977; Moreira and Sadowsky, 1978; Grutter and Poulin, 1998; Heupel and Bennett, 1999; Newbound and Knott, 1999; Smith et al., in preparation). Due to identification problems, the degree of host specificity exhibited by gnathiids is poorly understood. Pranizae infect many hosts, and some species are reported to infect teleosts as well as elasmobranchs (Monod, 1926; Paperna and Por, 1977; Grutter and Poulin, 1998). The intensity of gnathiid infections on elasmobranchs can be high in nature. For example, Grutter and Poulin (1998) reported an average of over 200 gnathiids per white-spotted wedgefish, *Rhynchobatus djiddensis* (Forsskael, 1775), and pink whip ray, *Himantura fai* Jordon and Seale, 1906, off Australia. Gnathiids infect the gills, olfactory sacs, buccal and branchial chamber walls, cloaca, and sometimes the skin of elasmobranchs (Honma and Chiba, 1991; Honma et al., 1991; Grutter and Poulin, 1998; Heupel and Bennett, 1999; Smit and Basson, 2002; Smith et al., in preparation). Although pranizae are able swimmers, some lesions associated with gnathiid infections of wild-caught elasmobranchs seemingly result from chronic infections of stationary pranizae (Honma and Chiba, 1991; Honma et al., 1991; Heupel and Bennett, 1999). Experimental results indicated that the feeding rate of some gnathiids was rapid (Grutter, 2003), and gnathiid infections have caused disease in and mortalities of confined teleosts (Paperna and Por, 1977). In addition, gnathiids have been reported to vector blood-borne parasites of teleosts (Davies, 1982; Davies et al., 1993; Davies and Smit, 2001; Davies et al., 2003).

Flabellifera contains four families (Aegidae, Cirolanidae, Corallanidae, and Cymothoidae) (Figures 24.80-24.85) that associate with or infect chondrichthyans (Moreira and Sadowsky, 1978; Bunkley-Williams and Williams, 1998), and together their representatives have been collected from Chimaeriformes, Hexanchiformes, Squaliformes, Squatiniformes, Heterodontiformes, Orectolobiformes, Lamniformes, Carcharhiniformes, Pristiformes, Rhinobatiformes, Torpediniformes, Rajiformes, and Myliobatiformes (Moreira and Sadowsky, 1978; Smith et al., in preparation). Although some flabelliferans associate with or infect freshwater teleosts (Thatcher, 1991), the present authors are unaware of any isopod that has been collected from an elasmobranch in freshwater.

Adult flabelliferans (Figure 24.80) possess a symmetrical body divided into a cephalon, pereon, and pleon; a well-developed pleotelson; uropods with flattened or pointed endopods and exopods that are attached lateral to and not folded above the pleotelson; and seven pairs of pereopods (Schultz, 1969). For more information on flabelliferan taxonomy and biology see Richardson (1904, 1905), Schultz (1969), Kensley and Schotte (1989), Brusca (1980), and the many contemporary references provided in Bunkley-Williams and Williams (1998).

Some flabelliferans found on fishes are obligate parasites (e.g., cymothoids) that remain permanently attached to a host as adults, whereas others are considered predators, facultative parasites, or associates (Bunkley-Williams and Williams, 1998). Bunkley-Williams and Williams (1998; page 893) defined an isopod associate as, "being in, or attached on, the host fish longer than is necessary to simply feed and drop off, as does a micropredator." Cymothoids feed on blood and other host body fluids, aegids feed on blood, cirolanids presumably feed on solid host tissues, and the diet of corallanids on fishes is unknown (Bunkley-Williams and Williams, 1998). The feeding and attachment activities of flabelliferans can cause considerable damage to their teleost hosts, and flabelliferans can stunt the growth of and kill their hosts (Lester and Roubal, 1995; Bunkley-Williams and Williams, 1998; Horton and Okamura, 2001, 2003). However, the effect of these isopods on chondrichthyans is largely unknown. Bird (1981) reported the interesting phenomenon of a high percentage of several carcharhiniform species infected by *Cirolana borealis* along the eastern coast of Florida. Isopods were found internally, and some had penetrated the pericardial chamber and heart (Bird, 1981). It was concluded that the high prevalence of infection was facilitated when a temporary local upwelling allowed the isopods to move into shallow water from their more typical deep-water habitat (Bird, 1981).

Flabelliferans exhibit direct life cycles and internal fertilization. Aegids, cirolanids, and corallanids are dioecious and cymothoids are protandrous

hermaphrodites. The adult female cymothoid incubates her eggs in a ventral brood pouch, and the young leave the pouch as juveniles that are sometimes referred to as mancae. The juveniles are excellent swimmers and they can immediately colonize a host (Bunkley-Williams and Williams, 1998). If a juvenile colonizes an uninfected host, it rapidly matures into an adult male and then into an adult female. If the juvenile colonizes a host infected by an adult female, its development is arrested as an adult male. If an adult female is lost from a host, the largest adult male replaces her by transforming into an adult female. Two types of sexual cannibalism play a role in the reproductive strategy of at least one cymothoid (Tsai and Dai, 2003). Unlike other flabelliferans, adult cymothoids cannot swim (Bunkley-Williams and Williams, 1998).

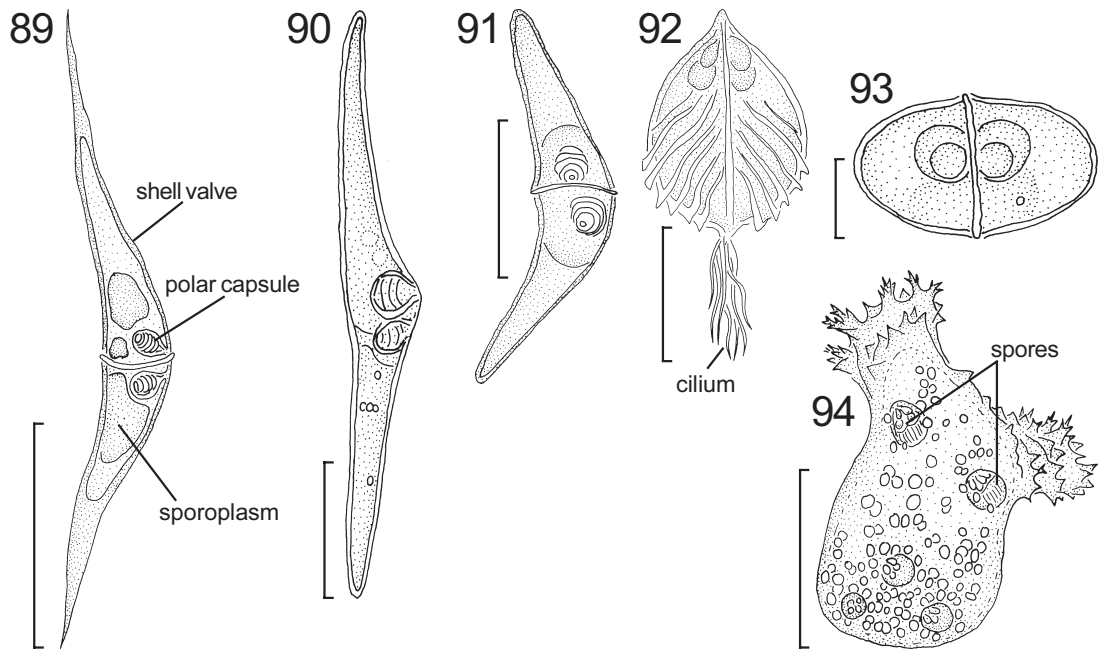
Chondrichthyans, particularly benthic species collected from gnathiid-infested localities, should be examined thoroughly for isopod infections during the early stages of captivity and again before they are released from quarantine. Many flabelliferans are conspicuous and can be removed using forceps. However, mechanical removal may be impractical if pranizae are numerous or if they infect inaccessible regions such as the olfactory sacs or branchial chambers, in which case osmotic shock or chemical treatments may be an effective means of control. Mugridge and Stallybrass (1983) controlled praniza infections on eels by altering the water supply from seawater to freshwater, and Lester and Roubal (1995) also cited the practice of using osmotic shifts to control flabelliferans. Regarding the chemical control of isopods, Lester and Roubal (1995) and Horton and Okamura (2001) noted cases in which infected teleosts were treated with pesticides or formalin, and Williams (1974) reported the use of formalin to control isopods. According to Bunkley-Williams and Williams (1998), Stoskopf's (1993d) mention of the use of praziquantel to control isopods was an error. Water treatments using diflubenzuron or lufenuron might be an effective means of controlling isopods, and some evidence suggests that the biological control of isopods may be possible. For example, Grutter (1997) reported that 90% of the diet items of the cleaner wrasse *Labroides dimidiatus* were gnathiids and that each fish ate an average of 1,200 parasites d^{-1} (Grutter, 1996). When confronting problematic isopod infections it should be remembered that many isopods are excellent swimmers that can live away from their hosts for extended periods, and that adult gnathiids reside and reproduce in the substrate. Hence, treating fishes infected with isopods without treating their surroundings might only provide temporary relief. Furthermore, husbandry staff should know that isopods may vector pathogens and open portals for secondary invaders such that adjunct therapies need to be considered.

Unproblematic taxa

Unproblematic parasites and associates of chondrichthyans are herein considered to be those metazoans whose presence is not associated with health problems, those that can be easily removed from their hosts, or those that are seldom encountered. This category includes a bewildering array of organisms, i.e., myxozoans, turbellarians, aspidogastreans, digeneans, cestodes, acanthocephalans, ostracods, barnacles, amphipods, gastropods, and fishes, that together demonstrate the multiphyletic success of symbiosis, and especially parasitism, as a way of life. A brief review of each group's biology is necessary to appreciate why these taxa are considered unproblematic; however, husbandry staff should be reminded that some members of these taxa may be harmful to captive elasmobranchs under unusual circumstances. Being able to identify unproblematic taxa is important regarding husbandry practices, as treating a chondrichthyan for a nonpathogenic infection squanders operational resources and may be detrimental to the health of the individual or its community.

Myxozoans

The literature suggests that myxozoans (Myxozoa) (Figures 24.89-24.94) seldom infect and do not cause disease in chondrichthyans, and that many myxozoans probably require intermediate hosts that are typically excluded from captive environments. Based on this, myxozoans are considered unproblematic parasites of chondrichthyans. Myxozoans historically were classified as aberrant protozoa, and they have only recently been accepted as metazoans that are related to cnidarians (Kent et al., 1994, 2001; Siddall et al., 1995; Schlegel et al., 1996). Of the more than 1,200 species of myxozoans that infect fishes, a handful have been collected from sharks (e.g., *Ceratomyxa lunata* from the tiger shark, *Galeocerdo cuvier* (Peron and Lesueur, in Lesueur, 1822); see Kudo, 1920), batoids (e.g., *Chloromyxum ovatum* from the Pacific torpedo, *Torpedo californica* Ayres, 1855; see Jameson,



Figures 24.89-24.94. Myxozoans (Myxozoa) of elasmobranchs. Figures 24.89, 24.92, and 24.94 modified from and Figures 24.90, 24.91, and 24.93 drawn from Kudo (1920). **24.89.** *Ceratomyxa sphaerulosa* (Ceratomyxidae), spore. Scale bar = 15 µm. **24.90.** *C. mesospora*, spore. Scale bar = 4 µm. **24.91.** *C. lunulata*, spore. Scale bar = 7 µm. **24.92.** *Leptotheca fusiformis* (Ceratomyxidae), spore. Scale bar = 4 µm. **24.93.** *Chloromyxum leydigi* (Chloromyxidae), spore. Scale bar = 2 µm. **24.94.** *C. leydigi*, plasmodium. Scale bar = 8 µm.

1929), and chimaeroids (e.g., *Leptotheca fisheri* from the spotted ratfish, *Hydrolagus colliei* Lay and Bennett, 1839; see Kudo, 1933). For additional records of myxozoans from chondrichthyans see Love and Moser (1983) and Cheung (1993).

Although no myxozoan collected from a chondrichthyan has been implicated in disease, lesions associated with myxozoan infections in elasmobranchs (Stoffregen and Anderson, 1990; Lom and Dyková, 1995; Heupel and Bennett, 1996) and myxozoan infections in captive elasmobranchs (Stoffregen and Anderson, 1990) have been reported. Life cycles of some myxozoans involve both invertebrate (e.g., oligochaetes, polychaetes, and bryozoans) and fish hosts. The parasite replicates in both hosts, and actinospores produced in invertebrates become trophozoites that disperse and colonize fish (Kent et al., 2001). Recently, oligochaetes imported into the United States as food for ornamental fishes were found to be infected by seven types of myxozoans (Lowers and Bartholomew, 2003). However, some myxozoans can disperse by direct transmission (Kent et al., 2001; Redondo et al., 2002). Redondo et al. (2002) experimentally demonstrated that direct transmission of *Enteromyxum scophthalmi* in the turbot *Scophthalmus maximus* can be achieved via cohabitation of uninfected and infected fishes, waterborne contamination, or the oral route using infected tissues.

Myxozoans are intracellular and intercellular parasites and as intercellular parasites they may infect tissues associated organ lumens or other body cavities (Blaylock et al., 2004). Myxozoans have caused disease in some wild or confined populations of teleosts (Lom and Dyková, 1995; Branson et al., 1999; Redondo et al., 2002; Gilbert and Granath, 2003). Most myxozoans from chondrichthyans have been collected from the lumen of the gall bladder. Although these parasites generally exhibit a moderate degree of host specificity (Lom and Dyková, 1995), exceptions exist. For example, Padrós et al. (2001) reported that 20 aquarium-reared Mediterranean fish species belonging to ten families were hosts to *Myxidium leei*, and Blaylock et al. (2004) reported seven species of fishes representing seven families infected with *Kudoa hypoepicardialis* even though these fishes did not represent a tight ecological group. Most myxozoan species collected from chondrichthyans seem restricted to these fishes (e.g., see Love and Moser, 1983). However, some myxozoans infect multiple elasmobranch hosts (Love and Moser, 1983), and at least one, *Zschokkella russelli*, infects an elasmobranch (*Mustelus mustelus* (Linnaeus, 1758)) and a teleost (*Gaidropsarus*

mediterraneus, Lotidae) (Lom and Dyková, 1995). Currently there are no chemotherapeutics that are widely accepted as being effective in controlling myxozoan infections in fishes; however, fumagillin DCH has been used successfully in this regard on several (Molnár et al., 1987; Hedrick et al., 1988; Wishkovsky et al., 1990; Yokoyama et al., 1990; El-Matbouli and Hoffmann, 1991) but not all (Staton et al., 2002) occasions. The water-borne dispersal stages of myxozoans might be controlled through water applications of formalin, and the eradication of intermediate and reservoir hosts has been used to control some myxozoans in freshwater hatchery systems. Exposure to UV light may also prove to be an effective means of eradicating the water-borne stages of myxozoans, as it has been shown to be for the microsporidian *Loma salmonae* (see Becker and Speare, 2004).

Taxonomy of myxozoans is based on spore morphology (Lom and Dyková, 1995; Kent et al., 2001), but genetic markers have also been used to distinguish some species (Blaylock et al., 2004). The microscopic multicellular spores of myxozoans (Figures 24.89-24.93) are typically 8-20 µm long and consist of two or more shell valves that surround one or more polar capsules and the sporoplasm (Lom and Dyková, 1995). Myxozoan infections are often betrayed by the presence of sporogonic plasmodia (Figure 24.94), some of which appear as macroscopic white cysts. Photographs and measurements of living spores prepared as wet mounts immersed in physiologic saline are essential to taxonomic study because spore fixation sometimes creates significant artifacts. Myxozoans are fixed in 10% n.b.f. for light microscopy; those intended for scanning electron microscopy are fixed in 10% n.b.f., Karnovsky's fixative, or Bouin's fixative; those intended for molecular taxonomy are fixed in 95% EtOH.

Turbellarians

Turbellarians form a grade of bilaterally symmetrical, acoelomate flatworms (Platyhelminthes) that includes several species that infect fishes, and one species that infects elasmobranchs (Cannon and Lester, 1988). *Micropharynx parasitica* (Tricladida) infects the thorny skate, *Amblyraja radiata* (Donovan, 1808), the gray skate, *Dipturus batis* (Linnaeus, 1758), and the thornback ray, *Raja clavata* Linnaeus, 1758, in the North Atlantic Ocean (Ball and Khan, 1976). *Micropharynx parasitica* (Figure 24.95) is about 7-mm long, and aside from its plicate pharynx, tripartite intestine, and lack of a haptor,

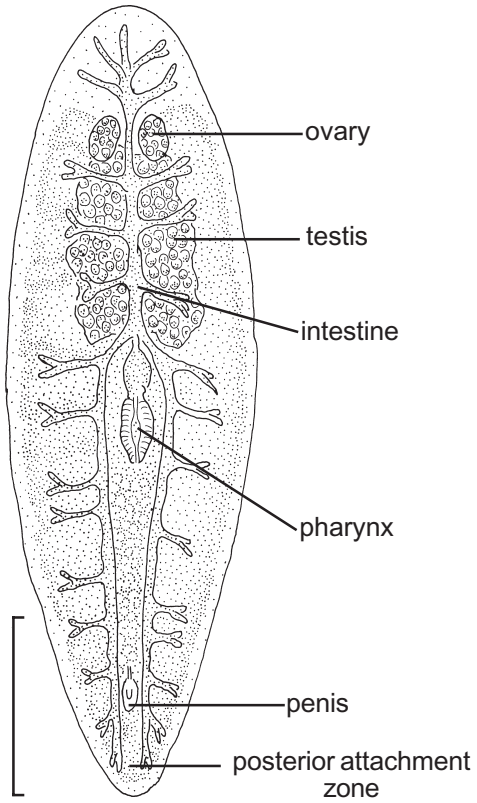
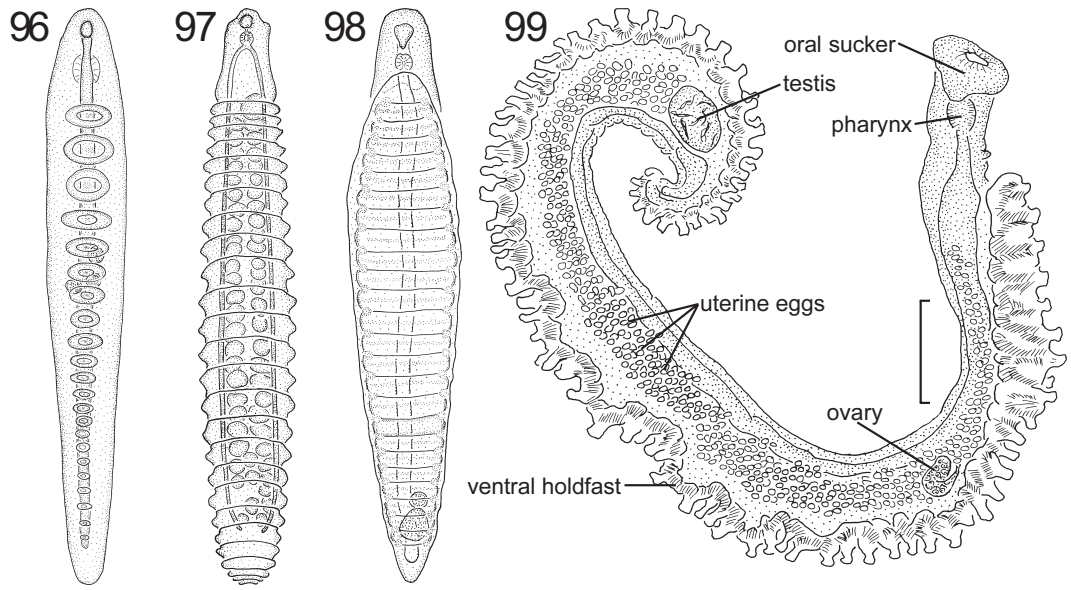


Figure 24.95. *Micropharynx parasitica* (Tricladida, Turbellaria), dorsal view. Modified from Ball and Khan (1976), scale bar = 1 mm.

it superficially resembles some monogeneans. *Micropharynx parasitica* is thought to feed on host skin, and individuals firmly grasp and often deeply embed themselves in the dorsal surface of rajids (Rajidae) by folding the posterior end of the body into an attachment clamp that has been referred to as the posterior attachment zone (Awerinzew, 1925; Ball and Khan, 1976). *Micropharynx parasitica* is monoecious and oviparous and its development does not require an intermediate host (Awerinzew, 1925). Ball and Khan (1976) reported intensities of 1-152 of these worms per infected host and a prevalence of infection of <6% in 425 rajids they examined off Newfoundland. *Micropharynx parasitica* is considered an unproblematic parasite of chondrichthyans because it infects few species and it has yet to be linked to a host health problem. Nevertheless, *M. parasitica* may be able to reproduce to problematic levels in captivity, as can *Paravortex* sp., a turbellarian that infects marine teleosts (Kent and Olson, 1986). Intense infections of *Paravortex* sp. have been associated with host death, acute focal dermatitis, and secondary infections of *Vibrio* sp. (Kent and Olson, 1986). Formalin water treatments, salinity shifts, and organophosphate pestiticide water treatments have been reported to



Figures 24.96-24.99. Aspidogastreans (Aspidogastrea) from chondrichthyans. **24.96.** *Stichocotyle nephropis* (Stichocotylidae), ventral view, diagrammatic. Drawn from Rohde (2002). **24.97.** *Rugogaster hydrolagi* (Rugogastridae), ventral view, diagrammatic. Drawn from Rohde (2002). **24.98.** *Multicalyx cristata* (Multicalycidae), ventral view, diagrammatic. Drawn from Rohde (2002). **24.99.** *M. elegans* (Multicalycidae), lateral view. Modified from Manter (1954), scale bar = 1 mm.

be effective in controlling *Paravortex* sp. epizootics (Blasiola, 1976; Kent and Olson, 1986), and praziquantel water treatments, as described above, to eradicate monogeneans would probably also provide effective control. Turbellarians intended for taxonomic study under a light microscope are killed and fixed using methods described above for monogeneans.

Aspidogastreans

The three aspidogastrean families Stichocotylidae, Multicalycidae, and Rugogastridae (Aspidogastrea, Trematoda, Platyhelminthes) (Figures 24.96-24.98) together hold four species that infect marine chondrichthyans.[11] These flatworms are considered unproblematic parasites because there is no record of an aspidogastrean causing a health problem for a chondrichthyan. The aspidogastreans that infect chondrichthyans are monoecious, oblong worms with ventral adhesive organs (Figure 24.99) composed of a series of separate suckers (*Stichocotyle nephropis*; Figure 24.96), transverse rugae (*Rugogaster hydrolagi*; Figure 24.97), or fused suckers (*Multicalyx elegans* and *M. cristata*; Figure 24.98). These worms are macroscopic as adults, and individuals of *M. cristata* can grow to at least 60-cm long (G. W. Benz, unpublished observations). Aspidogastreans are considered a sister group to the digeneans (Rohde, 2001), and thus it is not surprising that these two classes of flatworms share many morphological similarities. Aspidogastreans intended for taxonomic study under a light microscope are killed, fixed, and stained using methods described above for monogeneans. For more information on the morphology and taxonomy of aspidogastreans collected from chondrichthyans see Cunningham (1887), Jägerskiöld (1899), Odhner (1910), Manter (1954), Brinkmann (1957), Dollfus (1958), Stunkard (1962), Yamaguti (1963a), Schell (1973, 1985), Gibson and Chinabut (1984), Thoney and Burreson (1987, 1988), Rohde (1994, 2001, 2002), and Kearn (1998).

Life cycles of the aspidogastreans that infect chondrichthyans are incompletely known. However, based on complete information regarding other aspidogastreans (Rohde, 2001, 2002), it can be assumed that a life cycle similar to the following is shared by these worms. Adult worms shed eggs that pass from the definitive host into the environment. The eggs, each containing a larva known as a cotylocidium, are ingested by molluscs or crustaceans, or they hatch to release cotylocidia that infect these same invertebrate intermediate hosts. Cotylocidia develop into pre-adults, and if the intermediate host is consumed by a definitive host, the parasite typically migrates to the bile ducts or gall bladder

[11] Cheung (1993) erroneously included representatives of Multicalycidae and Rugogastridae as monogeneans and digeneans respectively in his widely used list of chondrichthyan parasites.

(*Stichocotyle nephropis*, *Multicalyx elegans*, and *M. cristata*) or the rectal gland (*Rugogaster hydrolagi*) and matures. Self-fertilization probably takes place in instances of single worm infections (Thoney and Burreson, 1986).

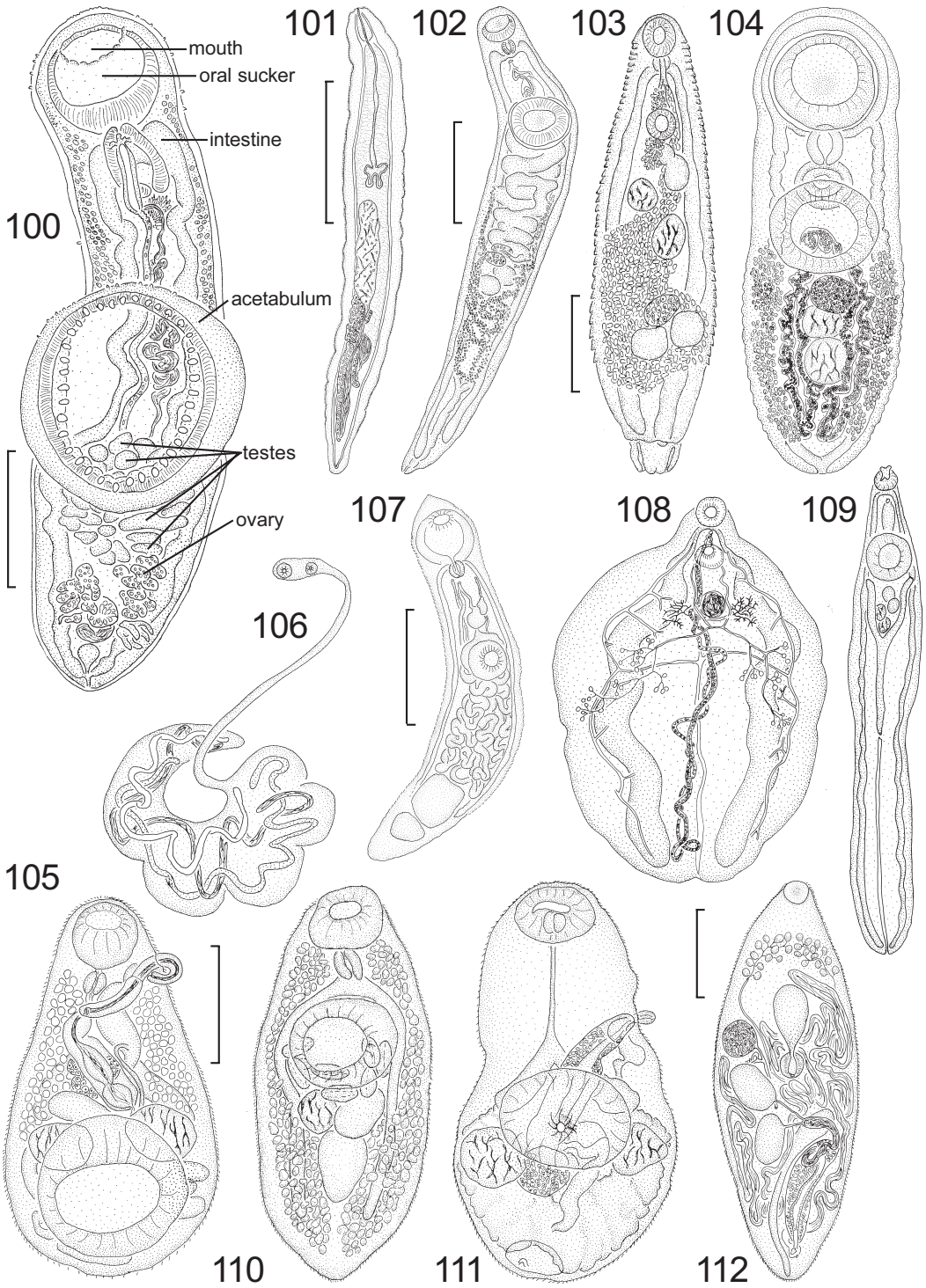
Several reports suggest that aspidogastreans occasionally bridge trophic gaps between invertebrate intermediate hosts and elasmobranch definitive hosts by using teleosts as paratenic hosts (Manter, 1931; Hendrix and Overstreet, 1977). Records of juvenile *Stichocotyle nephropis* collected from the intestines of lobsters (Cunningham, 1887; Nickerson, 1895; Odhner, 1910) may also represent cases of paratenic hosts. Evidence for this stems from observations that aspidogastreans infect species of molluscs that are eaten by lobsters; lobsters, in turn, are preyed on by rajids. Similarly, scalloped hammerheads, *Sphyrna lewini* (Griffith and Smith, in Cuvier, Griffith, and Smith, 1834), possibly become infected with *Multicalyx cristata* when they feed on infected stingrays (Myliobatiformes).

Although aspidogastreans exhibit a low degree of host specificity among teleost hosts, a more faithful pattern of host specificity may be recognizable for three of the four species that infect chondrichthyans. For example, *Stichocotyle nephropis* has only been reported (Linton, 1940; MacKenzie, 1964) from several rajids, including the barndoor skate, *Dipturus laevis* (Mitchill, 1817), and the thornback ray. *Rugogaster hydrolagi* has only been reported (Schell, 1973; Shvetsova, 1990; Machida and Araki, 1992; Rohde et al., 1992) from the spotted ratfish, Ogilby's ghostshark, *H. ogilbyi* (Waite, 1898), the silver chimaera, *Chimaera phantasma* Jordan and Snyder, 1900, and *Chimaera* sp. *Multicalyx elegans* has only been reported (Olsson, 1869; Manter, 1954; Stunkard, 1962; Szidat, 1966; Fernández et al., 1986) from several species of chimaeroids, i.e., the spotted ratfish, the ghost shark, *Callorhinchus milii* (Bory de Saint-Vincent, 1823), the elephantfish, *Callorhinchus callorhynchus* (Linnaeus, 1758), the rabbit fish, *Chimaera monstrosa* Linnaeus, 1758, and *C. phantasma*. Whether the relatively host specific associations for the aforementioned species are merely a result of inadequate sampling remains to be seen. Contrary to the above situation, *Multicalyx cristata* infects representatives of at least two orders of sharks and three orders of rays (Faust and Tang, 1936; Dollfus, 1958; Stunkard, 1962; Travassos et al., 1963; Hendrix and Overstreet, 1977; Parukhin and Tkachuk, 1980; Bray, 1984; Thoney and Burreson, 1986, 1988), including the sand tiger, the swell shark, *Cephaloscyllium ventriosum* (Garman, 1880), a smooth-hound, *Mustelus* sp., the scalloped hammerhead, the smalltooth sawfish, the blackchin guitarfish, *Rhinobatos cemiculus* Saint Hilaire, 1817, the Chola guitarfish, *R. percellens* (Walbaum, 1792), the roughtail stingray, the bluntnose stingray, the bullnose ray, *Myliobatis freminvillii* Lesueur, 1824, and the cow-nosed ray.

The aspidogastreans that infect chondrichthyans are remarkably specific regarding the organs that they infect (see above), and infection intensity is typically low (Brinkmann, 1957; Thoney and Burreson, 1986). Thoney and Burreson (1987) reported gross observations of the abnormal liver and bile of elasmobranchs infected by *Multicalyx cristata*; however, the health significance of this and other aspidogastrean infections in chondrichthyans remains unclear. Although they seem unlikely, problematic infections of aspidogastreans could probably be eradicated through oral treatments of praziquantel. In such instances, the presumed indirect life cycles of these worms would necessitate investigating the diets of infected species to determine if juvenile worms are colonizing the definitive hosts via their intended feed or through predation on tank mates that are serving as intermediate hosts.

Digeneans

Digeneans (Digenea, Trematoda, Platyhelminthes) (Figures 24.100-24.112) are considered unproblematic parasites because the authors are unaware of these flukes causing a health problem for a chondrichthyan. Digeneans are among the most morphologically and ecologically diverse platyhelminths (Kearn, 1998), and these flukes use representatives of all major vertebrate groups as definitive hosts (Olson et al., 2003). Of the 5,000 some odd digenean species that infect fishes, few infect chondrichthyans (Yamaguti, 1958; Bray and Cribb, 2003), and digeneans are regarded as being conspicuously underrepresented in these hosts (Olson et al., 2003). Nevertheless, there are digenean groups that exclusively infect chondrichthyans, for example, members of Ptychogonimidae, members of Anaporrhutinae (a subfamily of Gorgoderidae), and members of *Otodistomum* (Azygiidae) (see Bray and Moore, 2000; Bray and Cribb, 2003). In addition, some members of Syncoeliidae (Figure 24.100), especially those of the subfamily Otiotrematinae, infect sharks (Gibson and Bray, 1979). However,



Figures 24.100-24.112. Digeneans (Digenea) from chondrichthyans. General habitus of adults, ventral views. **24.100.** *Syncoelium vermilionensis* (Syncoeliidae). Modified from Curran and Overstreet (2000), scale bar = 500 µm **24.101.** *Selachohemecus olsoni* (Sanguinicolidae). Drawn from Short (1954), scale bar = 250 µm. **24.102.** *Otodistomum veliporum* (Azygiidae). Drawn from Curran and Overstreet (2000), scale bar = 4 mm. **24.103.** *Hemiurus levinseni* (Hemiuridae). Drawn from Schell (1985), scale bar = 300 µm. **24.104.** *Ptychogonimus megastomus* (Ptychogonimidae). Drawn from Yamaguti (1971). **24.105.** *Plectognathotrema hydrolagi* (taxonomic position uncertain). Drawn from Schell (1985), scale bar = 300 µm. **24.106.** *Tricharrhen okenii* (Didymozoidae). Drawn from Caira (1990). **24.107.** *Allocreadium annandalei* (likely a member of *Melogonimus*: Ptychogonimidae according to Bray and Cribb, 2003). Drawn from Southwell (1913), scale bar = 200 µm. **24.108.** *Anaporrhutum albidum* (Gorgoderidae). Drawn from Yamaguti (1971). **24.109.** *Aphanhystera monacensis* (Aphanhysteridae). Drawn from Caira (1990). **24.110.** *Spinoplagioporus minutus* (Opecoelidae). Drawn from Yamaguti (1971). **24.111.** *Diptherostomum betencourti* (Zoogonidae). Drawn from Bray and Moore (2000). **24.112.** *Prosorhynchus squamatus* (Bucephalidae). Drawn from Gibson (1996), scale bar = 200 µm.

as highlighted by Bray and Cribb (2003), some records of digeneans probably represent flukes that came to reside within the gut of an elasmobranch that consumed an infected prey item. Parasitologists refer to such occurrences as accidental or incidental infections, and while such assignments may appear arbitrary, they are typically based on data regarding the host range of closely related flukes and sometimes on data regarding host stomach contents and food habits. The biological significance of accidental infections has probably been underestimated, for the ability to live for even a brief period within another organism potentially has great evolutionary significance because it provides opportunity for phylogenetic and ecological change that can produce long-term host-parasite relationships (Caira et al., 1997a).

Bray and Cribb (2003) listed five digenean families as having members that exhibit long-term associations with elasmobranchs (i.e., Azygiidae, Gorgoderidae, Sanguinicolidae, Syncoeliidae, and Zoogonidae),[12] ten families as having members that have entered into accidental relationships with elasmobranchs (i.e., Acanthocolpidae, Bucephalidae, Derogenidae, Faustulidae, Hemiuridae, Hirudinellidae, Lecithasteridae, Lepocreadiidae, Opecoelidae, and Tandanicolidae), and two families with at least one species regarded as being in the early stages of potentially developing a long-term association within these fishes (i.e., Campulidae and Didymozoidae) (see Figures 24.100-24.112 for examples of digeneans from chondrichthyans).[13] Information provided by Cheung (1993), Cribb et al. (2001b), Bray and Cribb (2003), and Olson et al. (2003) reveal that approximately 20 of 150 families (ca. 11%), 28 of 2,700 genera (ca. 1%), and 48 of an estimated total of 18,000 nominal species (ca. 0.03%) of Digenea infect chondrichthyans.[14, 15, 16] For information regarding the general biology of digeneans see Yamaguti (1958, 1975), Williams and Jones (1994), Kearn (1998), Roberts and Janovy (2000), Cribb et al. (2001b), Gibson et al. (2002), and Bray and Cribb (2003).

The digeneans that infect chondrichthyans are morphologically diverse (Figures 24.100-24.112). These flukes are monoecious and they possess distinct anterior and posterior ends. Adult worms typically possess a conspicuous muscular, spheroid oral sucker that surrounds the mouth as well as a blind ventral sucker (acetabulum) located medially and posterior to the oral sucker (Figure 24.100). Exceptions to this generalized body form exist in the blood flukes of fishes (Sanguinicolidae), some of which infect chondrichthyans (Short, 1954; Bullard, 2002). Adult blood flukes infect the vascular system of their hosts, and they possess a thin, dorsoventrally flattened body lacking a conspicuous oral sucker and an acetabulum (Figure 24.101). These flukes apply their flat bodies to the smooth walls of blood vessels and may use their entire ventral body surface as a suction cup. Sanguinicolids also possess tegumental spines that allow them to grip uneven substrates such as the trabeculae of the heart. Digeneans intended for taxonomic study under a light microscope are prepared using methods described above for monogeneans. For more information on the general morphology and identification of digeneans from chondrichthyans see Dollfus (1937a, 1937b, 1937c, 1937d), Yamaguti (1958, 1971), Gibson and Bray (1979), Schell (1985), Gibson (1996), Kearn (1998), Curran and Overstreet (2000), and Gibson et al. (2002).

Life cycles of digeneans that infect chondrichthyans are not well known. However, based on incomplete information for these taxa (e.g., see Palombi, 1941, 1942a, 1942b; Yamaguti,

[12] Bray and Cribb (2003) did not list members of Ptychogonimidae as long-term parasites of elasmobranchs.

[13] In addition to the 17 digenean families listed by Bray and Cribb (2003) as being parasites of elasmobranchs, *Aphanhystera monacensis* (Aphanhysteridae) has been reported (see Yamaguti, 1958) from the stomach of the Portugese dogfish, *Centroscymnus coeloplepis* Bocage and Capello, 1864.

[14] Two citations of a heterophyid (Heterophyidae) from a chondrichthyan are dubious. Cheung (1993) without citation and Love and Moser (1983) citing Ginetsinskaya (1961) each noted the gray smooth hound, *Mustelus californicus* Gill, 1864, as a host for metacercaria of the heterophyid *Stictodora sawkinesis*. However, there is no mention of this association in Ginetsinskaya (1961).

[15] Contrary to the list of Cheung (1993), an allocreadiid (Allocreadiidae) has not been verified from a chondrichthyan. Cheung (1993) listed two digenean species of *Pedunculacetabulum* Yamaguti, 1934 from elasmobranchs without realizing that Yamaguti (1971) had previously transferred members of *Pedunculacetablum* from Allocreadiidae to Opecoelidae. Furthermore, Cheung (1993) listed *Opecoeloides vitellosus* as an apocreadiid (Apocreadiidae) when it is an opecoelid (see Yamaguti, 1971), and the present authors do not know of a valid record of an apocreadid from a chondrichthyan. In addition, *Allocreadium annandalei* Southwell, 1913 (see Figure 24.107), a species that infects an elasmobranch and which was originally placed in Allocreadiidae (see Southwell, 1913), was regarded as a species of uncertain taxonomic identity by Yamaguti (1971) and more recently considered as a likely member of *Melogonimus* (Ptychogonimidae) (Bray and Cribb, 2003).

[16] A report of a macroderoidid (Macroderoididae) from a batoid appears dubious (see Bray and Cribb, 2003).

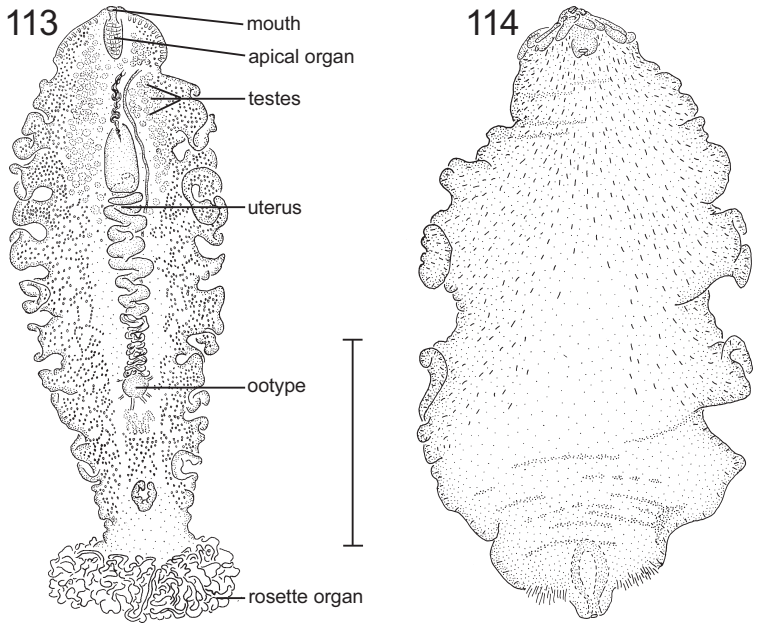
1975) and on known life cycles of other digeneans (Yamaguti, 1975; Williams and Jones, 1994; Kearn, 1998) it can be generalized that the following indirect life cycle is probably used by at least some of these flukes. Adult worms produce eggs that hatch in the environment to release a free-swimming ciliated larva called a miracidium. The miracidium infects the first intermediate host, probably a mollusk, wherein asexual reproduction takes place that produces many free-swimming larvae (cercariae) that move from the mollusc to seek a second intermediate host (e.g., a teleost). Cercariae develop into larvae known as metacercariae after penetrating the second intermediate host. If an appropriate species, i.e., a definitive host, eats this intermediate host, the metacercariae mature into adult digeneans. However, because digenean life cycles are so diverse, the foregoing example is a gross generalization, and the digeneans that infect chondrichthyans may have more or less elaborate life cycles. For example, the sanguinicolids that infect chondrichthyans probably have only a single intermediate host in their life cycle and lack an encysted metacercaria or second intermediate host (Bullard and Overstreet, 2002). Gibson and Bray (1994) regarded ptychogonimids to be underived (primitive) because they use scaphopods as intermediate hosts, they use elasmobranchs as the only definitive host, and they have a motile, free-living sporocyst (Olson et al., 2003). Chondrichthyans generally represent definitive hosts for most of the digenean taxa that infect them; however, some digeneans use these fishes as intermediate hosts (Bray and Cribb, 2003).

Adult digeneans generally exhibit a low degree of host specificity as compared to monogeneans or cestodes. Nevertheless, species considered to form long-term associations with chondrichthyans are usually restricted to this class of fishes and may only have been reported from one to several host species (e.g., see host records in Yamaguti, 1958, 1971; Bray and Cribb, 2003). Cheung (1993) listed 35 of 48 digenean species (ca. 73%) as having only one chondrichthyan species as a host, 6 of 48 digenean species (ca. 13%) were recorded from two chondrichthyan species, and 7 of 48 digenean species (ca. 15%) were recorded from more than two chondrichthyan species (see also Bray and Cribb, 2003). It has been suggested (Gibson, 1987; Cribb et al., 2001a; Bray and Cribb, 2003) that little coevolution has occurred between digeneans and their elasmobranch hosts and that long-term digenean infections in elasmobranchs, and probably in chimaeroids as well, have resulted from independent speciation episodes driven by host-switching accomplished over long geological periods.

Some digeneans that infect elasmobranchs exhibit a high degree of attachment site specificity, and it is notable that none of the groups that exclusively infect elasmobranchs, i.e., ptychogonimids, anaporrhutines, and *Otodistomum* spp., primarily infect the spiral intestine (Bray and Moore, 2000; Bray and Cribb, 2003). In fact, other than occasional reports of digeneans from the spiral intestine of elasmobranchs, only *Diphterostomum betencourti* (Zoogonidae) has been repeatedly reported from that location (Bray and Cribb, 2003). The attachment sites of digeneans considered to be nonaccidental parasites of elasmobranchs by Bray and Cribb (2003) can generally be summarized as follows regarding their locations within chondrichthyans: *Otodistomum* spp. (Azygiidae) seem restricted to the stomach or body cavity of chondrichthyans; a campulid was found in the liver of a shark; didymozoids seem restricted to tissues of elasmobranchs rather than within the lumen of digestive tract; gorgoderids seem primarily restricted to the body cavity but also to the ovary, rectum, and pericardium of elasmobranchs; ptychogonimids seem primarily restricted to the stomach of elasmobranchs; sanguinicolids are restricted to blood vessels, heart, and gill epithelium (S. A. Bullard, unpublished observations) of chondrichthyans; syncoeliids seem primarily restricted to the ends of the alimentary tract (e.g., buccal cavity, gill arches, cloaca) of elasmobranchs; and zoogonids seem restricted to the spiral intestine of sharks (Dollfus, 1937a, 1937b, 1937c, 1937d; Tandon, 1969; Yamaguti, 1971; Gibson and Bray, 1979, 1986; Cheung, 1993; Adams et al., 1998; Brickle et al., 2002; Bray and Cribb, 2003). For attachment locations of taxa considered to be accidental parasites of elasmobranchs see Bray and Cribb (2003). And regarding chimaeroids, *Plectognathotrema hydrolagi* infects the stomach of the spotted ratfish, *Hydrolagus colliei* (Lay and Bennet, 1839) (see Olson et al., 1970).[17]

Although they seem unlikely, problematic infections of digeneans in captive settings can probably be eradicated by using oral treatments

[17] Layman (1930) erected *Plectognathotrema* and did not assign it to a family, but some place it within Cephaloporidae. Bray (2002) stated that *Plectognathotrema* has affinities with Fellodistomidae but that *P. hydrolagi* may not belong in *Plectognathotrema*. Hence, to which family *P. hydrolagi* belongs is uncertain.



Figures 24.113-24.114. Gyrocotylideans (Gyrocotylidea, Cestoda) from holocephalans. Scale bar = 1 mm. **24.113.** *Gyrocotyle fimbriata*, ventral view. Modified from Xylander (2001). **24.114.** *Gyrocotyle confusa*, dorsal view. Drawn from van der Land and Dienske (1968).

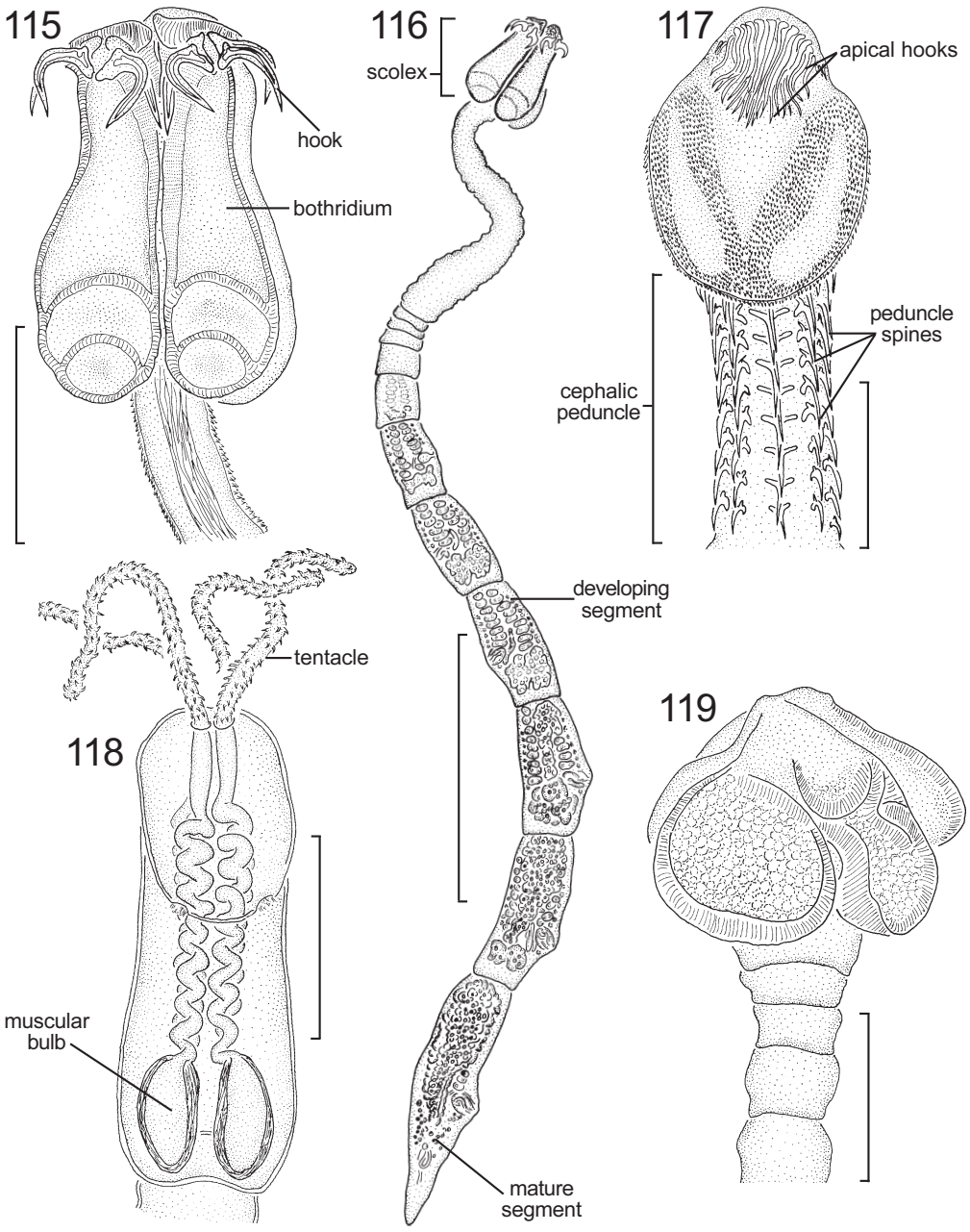
of praziquantel. Eradication of worms living in direct contact with life support water (e.g., syncoeliids attached within the branchial chambers of their hosts) might best be accomplished via water treatments using praziquantel. Finally, the elimination of intermediate hosts such as snails, bivalves, and polychaetes must also be considered when attempting to control digenean infections.

Cestodes

Cestodes (Cestoda, Cercomeromorpha, Platyhelminthes) that infect chondrichthyans comprise two subclasses, Gyrocotylidea and Eucestoda. These taxa are considered unproblematic parasites because they are not known to cause health problems for chondrichthyans. In the unlikely event that these worms become problematic, infections in the digestive tract can probably be eradicated by using oral or injected treatments of praziquantel (Moser and Sakanari, 1986). See Jones et al. (1994) for an identification key to orders of cestodes and Caira and Littlewood (2001) for a contemporary overview of tapeworm biology.

Gyrocotylidea is composed of one genus (*Gyrocotyle*) containing 13 nominal marine species that are only known to infect the spiral intestine of chimaeroids (Gibson, 1994) (Figures 24.113, 24.114). These monoecious flatworms are oblong, with a small, muscular, cup-shaped anterior holdfast, and a posterior attachment organ (Figures 24.113, 24.114). Gyrocotylideans absorb nutrients through the tegument and they lack a digestive tract. No gyrocotylidean life cycle has been experimentally demonstrated, and how hosts become infected with these worms is unknown. However, adult gyrocotylideans probably develop directly from a ciliated larva called a decacanth. Hosts are seldom infected by more than a couple of these worms in nature, and a density-dependant mechanism may control infection intensity (Bush et al., 2001). The present authors are unaware of a report of a lesion associated with a gyrocotylidean infection, or of a gyrocotylidean infection of a captive chimaeroid. The latter observation is surprising given that *Hydrolagus colliei*, a species that is routinely exhibited at public aquariums from California to British Columbia, is known to be infected by several species of *Gyrocotyle* (see Love and Moser, 1983). Gyrocotylideans intended for taxonomic study under a light microscope are fixed using methods described above for monogeneans.[18] For more information on

[18] Parasitologists must sometimes be resourceful. For example, an excellent whole mount of a gyrocotylidean was made from a specimen, collected on short notice by a herein unnamed but internationally well-known parasitologist, that was prefixed in the field in beer in a fortuitously found condom.



Figures 24.115-24.119. Eucestodes (Eucestoda, Cestoda) from elasmobranchs. **24.115-24.116.** *Calliobothrium hayhowi* (Tetraphyllidea). Modified from Nasin et al. (1997). **24.115.** Scolex. Scale bar = 600 µm. **24.116.** General habitus. Scale bar = 300 µm. **24.117.** *Echinobothrium hoffmanorum* (Diphyllidea). Modified from Tyler (2001), scale bar = 100 µm. **24.118.** *Otobothrium carcharidis* (Trypanorhyncha). Modified from Beveridge and Campbell (1998), scale bar = 200 µm. **24.119.** *Paraberrapex manifestus* (Lecanicephalidea). Drawn from Jensen (2001), scale bar = 50 µm.

gyrocotylideans see Bandoni and Brooks (1987), Williams et al. (1987), Gibson (1994), and Xylander (2001).

Eucestoda holds four orders of monoecious worms (Tetraphyllidea, Trypanorhyncha, Lecanicephalidea, and Diphyllidea; Littlewood and Bray, 2001 and references therein) that together include hundreds of species almost exclusively reported as adults from marine and freshwater elasmobranchs (Williams and Bray, 1984; Brooks and Amato, 1992; Cheung, 1993; Caira and Littlewood, 2001) (Figures 24.115-24.119). Based on the field observations of the authors and their colleagues, most elasmobranchs entering captivity are probably infected by eucestodes. However, the complex life cycles, high degree of host specificity, and benign nature of these worms assumedly keeps them from becoming problematic parasites in captive settings.

Eucestodes, commonly referred to as true tapeworms, are unique platyhelminths because they possess a segmented body as adults and lack a digestive tract during all life-history stages. The body (Figure 24.116) is composed of an anterior attachment organ (scolex) followed by a strobilla consisting of a series of segments (proglottids) that each contain a set of reproductive organs. Adult eucestodes range from several millimeters long to over 0.3-m long. For more information on the morphology and identification of representatives of the eucestode orders that infect chondrichthyans see Campbell and Beveridge (1994) for trypanorhynchs, Euzet (1994a) for tetraphyllideans, Khalil (1994) for diphyllideans, and Euzet (1994b) for lecanicephalideans. However, it should be noted that since publication of the foregoing citations, advancement has continued through taxonomic revision and the description of new eucestodes from chondrichthyans (e.g., see Ruhnke, 1994a, 1994b; Caira et al., 1997b; Nasin et al., 1997; McKenzie and Caira, 1998; Palm and Walter, 1999; Healy et al., 2001; Jensen, 2001; Marques et al., 2001; Caira and Tracy, 2002; Ivanov and Brooks, 2002; Ivanov and Campbell, 2002; Marques and Brooks, 2003).

Eucestoda is probably the most species-rich group of parasites infecting elasmobranchs, and eucestode species confined to elasmobranchs as adults likely outnumber these hosts. Why these parasites have radiated so in elasmobranchs remains unknown. In addition, eucestodes exhibit a high degree of host (e.g., see Yamaguti, 1959) and attachment site (e.g., see Williams, 1960, 1966, 1968; Williams et al., 1970; Carvajal and Dailey, 1975; Cislo and Caira, 1993; Curran and Caira, 1995; Nasin et al., 1997; Keeney and Campbell, 2001; Ivanov and Brooks, 2002) specificity in their elasomobranch hosts. Although studies have linked host-parasite associations to ecological factors such as diet (Testa and Dailey, 1977; Dailey and Vogelbein, 1982, 1990) and vicariance events including host-parasite coevolution (Nasin et al., 1997; Caira and Euzet, 2001; Caira and Jensen, 2001), the actual mechanisms facilitating host specificity in eucestode-elasmobranch systems are not understood. Some evidence exists that infection susceptibility may be mediated by immune factors (McVicar and Fletcher, 1970); however, it seems probable that patterns of host specificity are also influenced by the physical and chemical needs of specific eucestodes as they relate to the physical and chemical gut characteristics of prospective hosts (Williams et al., 1970). Within their elasmobranch hosts, adult eucestodes are usually confined to the spiral intestine; however, some species of trypanorhynchs infect the stomach or gall bladder as adults and eucestode larvae may infect the body cavity or viscera (Cheung, 1993; Caira and Littlewood, 2001).

Because each mature tapeworm segment typically contains a complete set of reproductive organs, the fecundity of these worms is unparalleled amongst the platyhelminthes. The life cycles of eucestodes that infect elasmobranchs are virtually unknown; however, they are assumed to be indirect, requiring one to several intermediate hosts (e.g., copepods, shrimps, mollusks, fishes, and mammals). In a hypothetical life cycle, eggs are voided in the feces or proglottids break free from the strobila and are passed into the environment where they rupture to liberate eggs. A free-swimming ciliated larva (coracidium) hatches from the egg and is eaten by a crustacean that serves as the first intermediate host. The tapeworm develops into a second stage larva (procercoid) within the crustacean, and if the infected crustacean is eaten by a second intermediate host (e.g., a cephalopod or fish), procercoids develop into third-stage larvae known as pleurocercoids (e.g., see Pascual et al., 1996; Palm and Overstreet, 2000). The pleurocercoid will mature if the second intermediate host is eaten by an appropriate elasmobranch. Pleurocercoids are sometimes found in elasmobranchs, indicating that these fishes may serve as intermediate hosts for some tapeworm species. In addition, some elasmobranchs that harbor eucestode larvae may be paratenic hosts. However, given the diversity exhibited within Eucestoda, the foregoing life cycle must be considered a mere example, and some species of tapeworms that infect elasmobranchs probably have less (e.g., see Dailey and Vogelbein, 1982) or more elaborate life cycles. Nevertheless, all of the tapeworms infecting elasmobranchs are assumed to enter these hosts through the food chain, and hence feeding fresh fish or invertebrates to captive elasmobranchs may facilitate infection. Southwell and Walker (1936) noted that larvae of *Phyllobothrium delphini* (a species presumed to mature in some sharks; Testa and Dailey, 1977; Dailey and Vogelbein, 1990) in seal blubber retained their viability for at least 11 days after the death of the host, and Testa and Dailey (1977) reported that *P. delphini* larvae in the blubber of marine mammals were viable after 1 month at 4 C. However, the present authors are unaware of any report of fresh or frozen food causing the

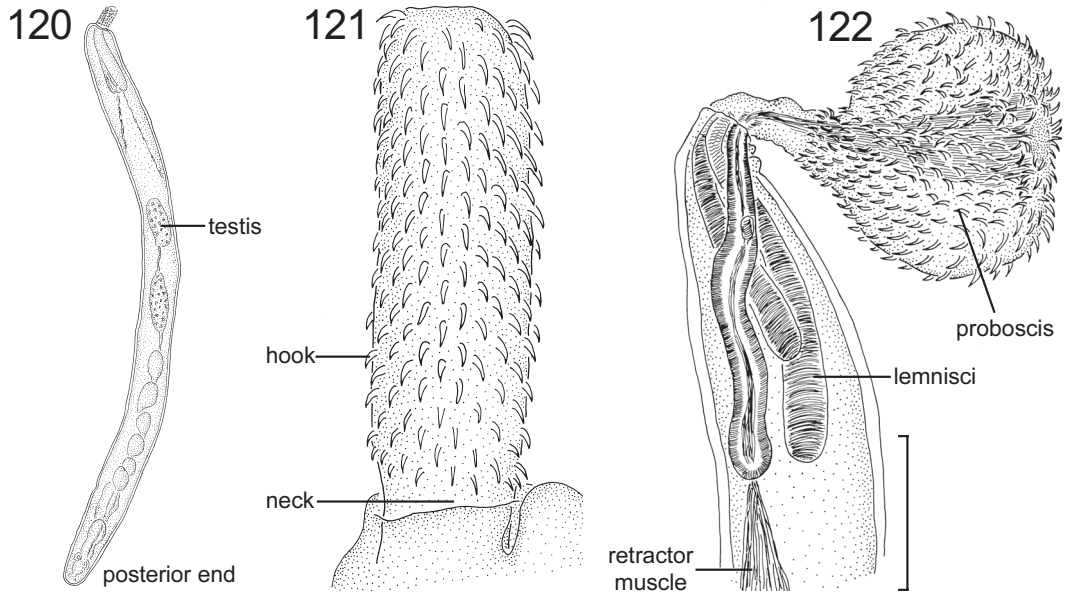
infection of a captive elasmobranch with tapeworms. For additional information regarding the life cycles of tapeworms that infect elasmobranchs see Mudry and Dailey (1971), Testa and Dailey (1977), Overstreet (1978, 1983), Dailey and Vogelbein (1982, 1990), Mattis (1986), Sakanari and Moser (1989), and Kent (1992).

Tapeworms apparently compete with their hosts for digested nutrients and vitamins, and large species can occlude the lumen of the gut (Bush et al., 2001). Nevertheless, a detailed study of these phenomena has not been completed for a eucestode that infects an elasmobranch, and no scientific evidence suggests that eucestode infections in elasmobranchs cause nutritional deficiencies. Closer study of eucestode-elasmobranch interactions may provide such evidence, as some adult tapeworms cause nutritional problems in teleosts (Dick and Choudhury, 1995a). There are several reports regarding lesions associated with eucestode infections in wild elasmobranchs; however, the significance of such lesions regarding the overall health of hosts is not understood. In general, larger worms and species that possess penetrating tentacles, i.e., trypanorhynchs, cause greater damage to the host than smaller and more superficially attached species. For example, Borucinska and Dunham (2000) reported that the duodeno-spiral intestine junction of a blue shark was obliterated by a mass of fibrous tissue associated with an infection of *Tentacularia* sp. (Trypanorhyncha). Campbell and Callahan (1998) reported on lesions caused by postlarvae of *Hepatoxylon trichiuri* (Trypanorhyncha) attached to the visceral surface of a blue shark. These lesions were marked by a rim of tissue surrounding a deep pit with holes corresponding to the penetration locations of tapeworm tentacles and hyperplasia of liver tissue about the attachment location (Campbell and Callahan, 1998). Borucinska and Caira (1993) studied lesions associated with the attachment of two tetraphyllidean species and two trypanorhynch species in the spiral intestine of the nurse shark, *Ginglymostoma cirratum* (Bonnaterre, 1788), reporting that larger tapeworms caused greater damage to the mucosa. Interestingly, although there is no report of an adult or larval eucestode causing the death of an elasmobranch, there are several reports of captive and wild teleost deaths associated with infections of tapeworm larvae that mature in sharks (Adjei et al., 1986; Kent et al., 1991).

Tapeworms intended for taxonomic study under a light microscope are prepared using methods described above for monogeneans. As tapeworm identification relies heavily on interpretations of scolex and reproductive system morphology, care should be taken to collect the scolex and mature proglottids of these worms. Some scoleces require special treatment to ensure proper fixation. For example, the trypanorhynch scolex (Figure 24.118) possesses four thorny, retractable tentacles that must be extruded before or during fixation to allow observations of taxonomically important features such as the shape, size, number, and arrangement of the tentacle armature. This is accomplished by immersing the worm in a few drops of salt solution (8.5 g NaCl L^{-1} of distilled water) or fresh water on a glass microscope slide with the specimen placed under coverslip pressure. Once the tentacles are extruded the specimen is relaxed by applying a flame underneath the slide for 1-2 seconds just before fixation in 5% n.b.f.

Acanthocephalans

Acanthocephalans (Acanthocephala), or spiny-headed worms, are dioecious, oblong worms that lack a mouth and digestive tract (Figures 24.120-24.122) and possess an eversible, spiny proboscis (Figures 24.121, 24.122) and a body cavity that is a pseudocoel (Nickol, 1995). Although acanthocephalan species richness is high among teleosts, few acanthocephalans infect elasmobranchs, and those that do have exclusively been collected from within the stomach or spiral intestine. *Echinorhynchus gadi* (Figures 24.120, 24.121) infects the piked dogfish and many teleost species in the northeastern Pacific Ocean (Golvan, 1969; Love and Moser, 1983). *Megapriapus ungriai* (Figure 24.122) infects the Porcupine River stingray, *Potamotrygon histrix* (Müller and Henle, in Orbigny, 1834), in freshwater rivers in Brazil and Venezuela (Golvan et al., 1964; Thatcher, 1991). Buckner et al. (1978) reported well-developed but immature specimens of *Tegorhynhcus furcatus* and *Dollfusentis chandleri* infecting fintail stingrays, *Dasyatis* spp., caught in the Gulf of Mexico off Mississippi. These rays may have represented dead-end hosts for these acanthocephalans because southern kingfish, *Menticirrhus americanus*, collected about the same time and locality were heavily infected with the adults of these parasites (Buckner et al., 1978). Three species of acanthocephalans have recently been reported (Knoff et al., 2001b) from sharks captured off the southern coast of Brazil: juveniles of *Corynosoma* sp. from an angel shark, *Squatina* sp., the shortnose spurdog, *Squalus*



Figures 24.120-24.122. Acanthocephalans (Acanthocephala) from elasmobranchs. **24.120-24.121.** *Echinorhynchus gadi* (Echinorhynchidae), adult male. Modified from Yamaguti (1963c). **24.120.** General habitus, lateral view. **24.121.** Extruded proboscis showing armature. **24.122.** *Megapriapus ungriai* (Gigantorhynchidae), extruded proboscis of female. Modified from Golvan et al. (1964), scale bar = 1 mm.

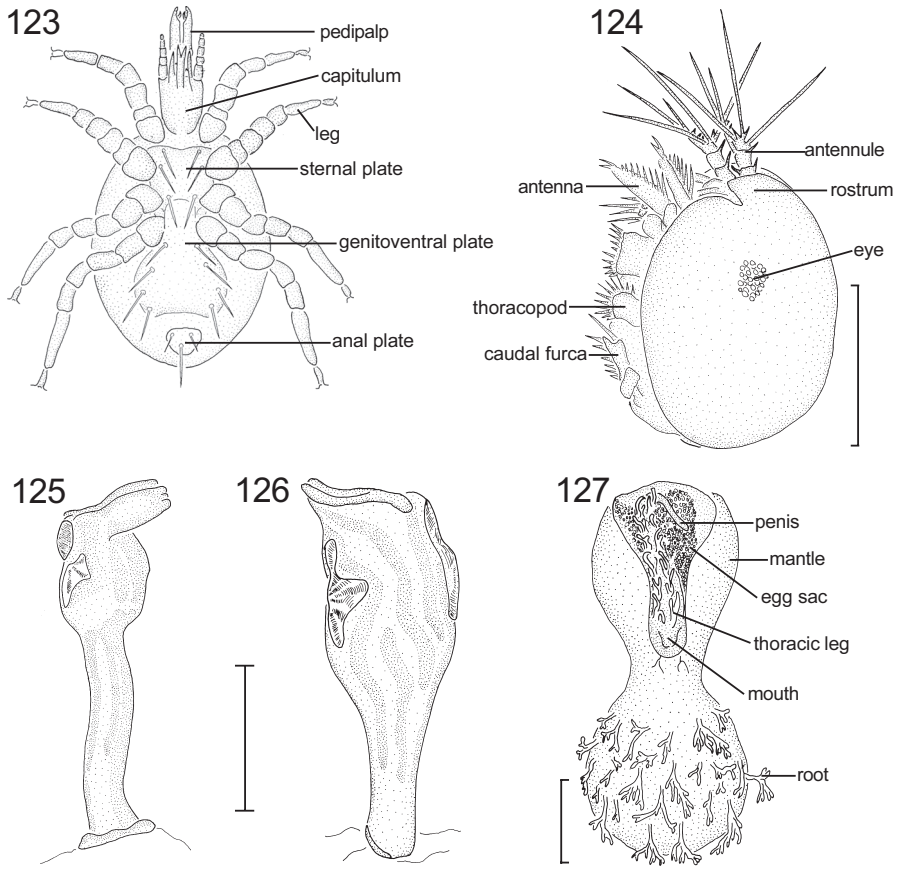
megalops (Macleay, 1881), and the tope shark, *Galeorhinus galeus* (Linnaeus, 1758); cystacanths of *Corynosoma australe* from the aforementioned hosts as well as from the bluntnose sixgill shark, *Hexanchus griseus* (Bonnaterre, 1788); and juveniles of *Gorgorhynchus* sp. from the smooth hammerhead, *Sphyrna zygaena* (Linnaeus, 1758).

No life cycle is known for an acanthocephalan species that infects a chondrichthyan; however, acanthocephalans that infect fishes as definitive hosts have multiple-host life cycles requiring crustacean intermediate hosts (Nickol, 1995). Buckner et al. (1978) reported that the amphipods *Lepidactylus* sp. and *Haustorius* sp. are intermediate hosts for *T. furcatus* and that the amphipods *Lepidactylus* sp., *Grandidierella bonnieroides*, and *Corophium lacustre* are intermediate hosts for *D. chandleri*. Acanthocephalans develop into a larva known as a cystacanth in the intermediate host, and the cyctacanth matures into an adult once the arthropod is eaten by a definitive fish host (Nickol, 1995). Some acanthocephalans infect paratenic hosts that bridge trophic gaps between intermediate and definitive hosts (Nickol, 1995). The acanthocephalan proboscis can cause lesions in fishes (Nickol, 1995), but any effect these parasites have on elasmobranchs is unknown. Acanthocephalans are considered unproblematic parasites of chondrichthyans because they rarely seem to infect these hosts and because their life cycles probably require intermediate hosts that are not present in captive environments.

Acanthocephalans intended for taxonomic study must be carefully removed from their hosts to ensure that the embedded proboscis is not damaged. In addition, because the armature pattern of the proboscis is taxonomically important, acanthocephalans are best fixed with the proboscis extruded. To do this, worms are gently swirled and allowed to sit in a dish of distilled water until the proboscis is extended due to an increase in internal pressure caused by osmosis. Specimens can then be transferred to and fixed in 70% EtOH.

Mites

Mites (Acari, Uniramia, Arthropoda) are small, dioecious, uniramous arthropods (Figure 24.123), some of which are parasites during at least a portion of their life cycle (Gledhill, 1985; Smith, 2001; Smith et al., 2001). Several mites have been reported as parasites of fishes (Bykhovskaya-Pavlovskaya et al., 1964; Hare and Burt, 1975; Fain and Belpaire, 1985; Fain and Lambrechts, 1985; Blaylock and Overstreet, 2003), but to the present authors' knowledge, only one mite has been collected in association with a chondrichthyan. This unidentified mite (seemingly a deutonymph or adult) was collected from the lumen of the heart of a nurse shark captured in



Figures 24.123-24.127. Parasites of chondrichthyans. **24.123.** Diagrammatic representation of an adult mite (Acari), ventral view. Modified from Roberts and Janovy (2000). **24.124.** *Vargula parasitica* (Cypridinidae, Ostracoda), lateral view. Modified from Williams and Bunkley-Williams (1996), scale bar = 1 mm. **24.125-24.127.** Barnacles (Cirripedia) from or associated with chondrichthyans. **24.125-24.126.** *Conchoderma* spp. (Lepadidae), lateral views. Drawn from Williams and Bunkley-Williams (1996), scale bar = 7 mm. **24.125.** *C. auritum*. **24.126.** *C. virgatum*. **24.127.** *Anelasma squalicola* (Anelasmatidae), anterior view. Modified from Lester and Roubal (1995), scale bar = 5 mm.

Florida Bay (G. W. Benz, unpublished observations). Although it is unknown how this mite came to reside within this heart, the authors do not think this case represented an atypical association. This opinion is based on more recent observations (G. W. Benz and S. A. Bullard, unpublished observations) of mites within the hearts of several teleosts from other regions. Furthermore, as noted by Adamson and Caira (1991) and accentuated by Bullard (2002), the circulatory system of fishes is seldom thoroughly inspected for small parasites. Hence, the lack of parasite records from the circulatory system of chondrichthyans may be more an indication of scientific neglect than that of a natural phenomenon. Together, members of Acari exhibit a variety of life cycles, but generally, an egg hatches to release a six-legged larva that later develops into an eight-legged deutonymph that eventually gives rise to an eight-legged adult (Smith et al., 2001). Although mites are known to cause lesions in fishes (Fain and Lambrechts, 1985), no record of a severe health problem has been associated with a mite infection in a fish. However, should mites become problematic within captive settings, water treatments similar to those described above to eradicate fish lice would probably provide effective control. Adult mites and deutonymphs intended for taxonomic study using a light microscope are fixed in a volume-to-volume mixture of five parts glycerin, four parts water, and one part glacial acetic acid (Smith et al., 2001).

Ostracods

Ostracods (Ostracoda, Maxillopoda, Crustacea, Arthropoda), or seed shrimp, are small, maxillopodan crustaceans with a dorsally-hinged bivalved carapace that encloses the body and head (Figure 24.124; Schram, 1986; Brusca and Brusca, 2003). Ostracods are dioecious and have direct life cycles with developmental stages punctuated by periods of molting. Although almost all ostracods are free living, several species are ectoparasites of elasmobranchs. Wilson (1913)

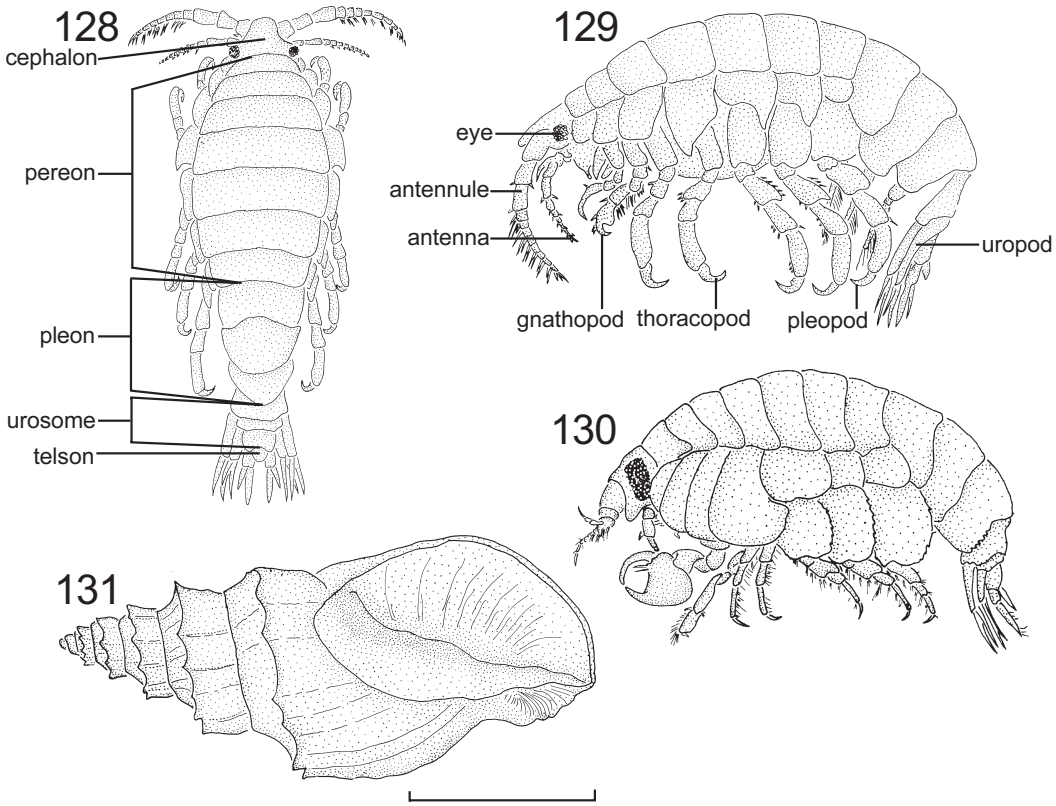
described *Vargula parasitica* (Figure 24.124) from specimens collected from the gills and olfactory sacs of smooth hammerheads and the gills of two species of teleosts captured off Jamaica, and he provided minor details regarding gill lesions associated with infections on *S. zygaena*. Williams and Bunkley-Williams (1996) reported that *V. parasitica* attached to the gills of a smooth hammerhead almost exclusively at the proximal end of the gill filaments, i.e., nearest the gill arch and between the bases of adjacent filaments, with only one ostracod found between any two filaments. Both male and female ostracods (each ca. 2-mm long) infected the host, and both sexes were partially surrounded by host tissue such that these lesions may have developed due to parasite-induced tissue erosion, tissue proliferation around the parasites, or both (Williams and Bunkley-Williams, 1996). Adult male and female *V. parasitica* scraped from the aforementioned gills could swim (Williams and Bunkley-Williams, 1996). Harding (1966) described *Sheina orri* from specimens collected from the gills of the epaulette shark, *Hemiscyllium ocellatum* (Bonnaterre, 1788), and the ribbon-tailed stingray, *Taeniura lymma* (Forsskael, 1775), captured off Queensland, Australia. Newbound and Knott (1999) reported, but did not identify, ostracods representing Cypridinidae on the gills of tiger sharks captured off western Australia. Too little is known about these seed shrimp to categorize them as either facultative or obligate parasites; however, given the scavenging habits of many ostracods, it is probable that other species also occasionally infect elasmobranchs. Bennett et al. (1997) reported that infections of *S. orri* on the gills of wild-caught epaulette sharks and ribbon-tailed stingrays caused lesions but not disease. However, Williams and Bunkley-Williams (1996) considered that intense infections of *V. parasitica* on smooth hammerheads could impair respiratory function over a considerable area of the gills. Ostracods are considered unproblematic taxa because few ostracod species are known to infect chondrichthyans. Nevertheless, in confined environments parasitic ostracods might reproduce to problematic levels. Water treatments as described above to control fish lice should kill them. Ostracods are best prepared for taxonomic study using a light microscope by fixing them in 10% n.b.f. and later transferring them into 70% EtOH.

Barnacles

Three barnacles (Cirripedia, Maxillopoda, Crustacea, Arthropoda), each with a cosmopolitan distribution, occasionally associate with or infect elasmobranchs. Although the rabbit-ear barnacle, *Conchoderma auritum*, (Figure 24.125) is not a parasite, it attaches to some inanimate objects, sharks, teleosts, and whales (Williams and Bunkley-Williams, 1996). The striped goose barnacle, *Conchoderma virgatum*, (Figure 24.126) also is not a parasite, but it attaches to some inanimate objects, fishes, sea turtles, sea snakes, whales, and crustacean ectoparasites that infect marine organisms (Williams and Bunkley-Williams, 1996). At least five cases of *C. virgatum* attached to pandarid (Pandaridae) copepods that infected sharks have been reported (Williams, 1978; Benz, 1984b). *Anelasma squalicola* (Figure 24.127) is an obligate parasite of several species of lantern sharks, *Etmopterus* spp., and combtooth dogfishes, *Centroscyllium* spp. (Hickling, 1963; Newman and Foster, 1987; Long and Waggoner, 1993; Fernández Ovies, 1995; Yano and Musick, 2000). The adults of *A. squalicola* are mesoparasites that typically embed themselves in the head, fins, and abdomen of their host (Yano and Musick, 2000), and several studies provided evidence that these infections may retard host growth and gonad development (Hickling, 1963; Yano and Musick, 2000). *Anelasma squalicola* is considered an unproblematic parasite because it infects few species and its hosts are not typically held in captivity. Furthermore, water treatments as described above to control fish lice should kill barnacles, and surgery to remove them from infected fishes seems uncomplicated. Barnacles are prepared for taxonomic study using a light microscope by fixing them in 10% n.b.f. and later transferring them into 70% EtOH.

Amphipods

Amphipods (Amphipoda, Malacostraca, Crustacea, Arthropoda) are small, dioecious, malacostracan crustaceans that are laterally compressed and lack a carapace (Figures 24.128-24.130; Schram, 1986; Bousfield and Kabata, 1988; Brusca and Brusca, 2003). Although most amphipods are free living, a few species are ectoparasites of elasmobranchs. *Lafystius sturionis* (Figures 24.128, 24.129) infects the gray skate and many other fishes in the North Atlantic Ocean (Kabata, 1970). *Lafystius morhuanus* infects the thorny skate, the smooth skate, *Malacoraja senta* (Garman, 1885), the little skate, *Leucoraja erinacea* (Mitchill, 1825), and several teleost species in Canadian Atlantic waters (Bousfield and Kabata, 1988). *Opisa tridentata* (Figure 24.130) infects the piked dogfish and several



Figures 24.128-24.131. Parasites of chondrichthyans. **24.128-24.129.** *Lafystius sturionis* (Lafystiidae, Amphipoda). Modified from Kabata (1970). **24.128.** Dorsal view. **24.129.** Lateral view. **24.130.** *Opisa tridentata* (Lysianassidae, Amphipoda), lateral view. Drawn from Bousfield and Kabata (1988). **24.131.** Shell of *Cancellaria cooperi* (Cancellariidae, Gastropoda), ventral view. Drawn from Abbott (1974), scale bar = 7 mm.

teleost species in the northeastern Pacific Ocean, and in the North Atlantic Ocean, *Trischizostoma raschi* infects the velvet belly lantern shark, *Etmopterus spinax* (Linnaeus, 1758), (Bousfield and Kabata, 1988). As noted by Kabata (1984), although little is known about the relationships between amphipods and their fish hosts, some records of amphipods feeding on hooked or netted fishes (including elasmobranchs) demonstrate how locally destructive these crustaceans can be. Amphipods are considered unproblematic taxa because few amphipod species are known to infect chondrichthyans. In confined environments, however, parasitic amphipods might reproduce to problematic levels. Water treatments, as described above, to control fish lice should kill them. Amphipods are prepared for taxonomic study using a light microscope by fixing them in 10% n.b.f. and later transferring them into 70% EtOH.

Gastropods

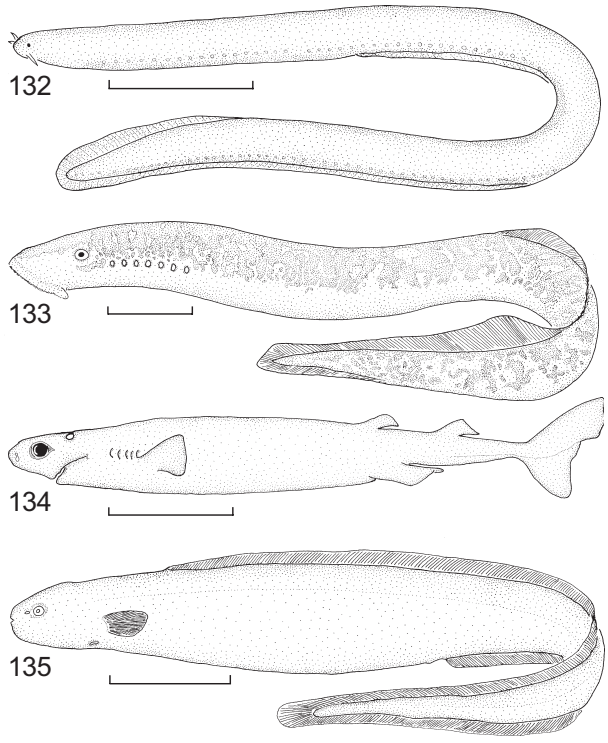
Although some boring gastropods prey on the eggs of elasmobranchs (Cox et al., 1999), Cooper's nutmeg, *Cancellaria cooperi*, (Neogastropoda, Gastropoda, Mollusca) (Figure 24.131) is the only mollusc that reportedly infects elasmobranchs. O'Sullivan et al. (1987) observed up to seven *C. cooperi* on the dorsal surface of partially buried Pacific torpedoes. In aquariums, *C. cooperi* bored into the ventral surface of a Pacific torpedo and a Pacific angel shark, apparently feeding on blood (O'Sullivan et al., 1987). Laboratory experiments indicated that these 5-8 cm long snails were chemically attracted to Pacific torpedoes, and snails remained stationary in the substrate for at least 12 days when a torpedo was not present (O'Sullivan et al., 1987). According to Morris (1966), *C. cooperi* ranges from off Monterey, California to Baja California, Mexico in moderately deep water. *Cancellaria cooperi* is considered an unproblematic parasite because few chondrichthyans host this snail, and also because snails would be easily recognized and removed from captive fishes or captive settings. For general morphological study, mollusks are relaxed before fixation in 10% n.b.f. and preservation in 75% EtOH (see Smith, 2001).

Craniates

Four craniate (Craniata, Chordata) classes include fishes that are either occasional parasites of elasmobranchs or that sometimes scavenge or prey on elasmobranchs in a manner bordering on parasitism. These fishes are considered unproblematic parasites and associates of chondrichthyans because no record exists of them causing problems in a captive setting, and certainly the size of these fishes precludes their inadvertent introduction into captivity. Nevertheless, those involved with capture operations may observe the characteristic wounds inflicted by these fishes, and proper recognition of them may prevent unnecessary medical treatments stemming from notions that they are lesions caused by infectious parasites. Fishes are fixed in 10% n.b.f. and later transferred into 70% isopropanol or 70% EtOH for general taxonomic study. Sometimes the body cavity is opened before fixation to facilitate the rapid penetration of formalin into the internal organs, and sometimes specimens are injected with formalin to ensure proper fixation.

Hagfishes (Myxinidae, Myxini, Agnatha) are jawless, eel-like fishes without vertebrae or paired fins, and with oral barbels and degenerate eyes (Figure 24.132; Nelson, 1994; Moyle and Cech, 1996; Helfman et al., 1997; Martini and Flescher, 2002). There are about 60 extant hagfish species worldwide (Fernholm, 1998), all primarily marine (although there are records of some from estuarine environments) and typically distributed in temperate regions with soft sediments (Nelson, 1994; Martini and Flescher, 2002). Hagfish primarily feed on invertebrates; however, they are also scavengers of fishes (Bigelow and Schroeder, 1953; Hart, 1973; Shelton, 1978; Martini and Flescher, 2002). Their ability to feed on unencumbered vertebrates is unknown, but they are well known to feed on dead, moribund, or otherwise compromised fishes and mammals, including hooked or netted sharks (Bigelow and Schroeder, 1953; Martini and Flescher, 2002). Hagfish can swallow small prey whole, and they can also use their horny tongue and mouth to pinch free pieces of flesh from larger food items (Martini and Flescher, 2002). Piecemeal feeding on large items is facilitated by a process known as knotting, which allows them to tear off bits of flesh through leveraging forces created by literally tying themselves into a knot (Adam, 1960; Hardisty, 1979; Martini and Flescher, 2002). When feeding on large fish, hagfish seek access to the viscera by entering soft tissues such as those about the anus, mouth, or branchial chamber; and scavenging on large carrion can result in hagfish occupying hollows within a corpse (Bigelow and Schroeder, 1953; Martini and Flescher, 2002).

Lampreys (Petromyzontiformes, Cephalaspidomorphi, Agnatha) are jawless, eel-like fishes without paired fins and with well-developed eyes and an oral disk studded with numerous horny teeth (Figure 24.133; Nelson, 1994; Moyle and Cech, 1996; Helfman et al., 1997; Gill et al., 2003). As adults, lamprey species are either parasitic or they do not feed, and to the authors' knowledge, Petromyzontiformes is the only taxon within which nonparasitic species have apparently evolved from parasitic species (Nelson, 1994; Gill et al., 2003). In marine waters there are about nine species of extant lampreys (Flescher and Martini, 2002); all anadromous, and hatching and passing through a larval stage (ammocoete) in rivers before transforming into juveniles and migrating to the sea (Nelson, 1994; Moyle and Cech, 1996;



Figures 24.132-24.135. Craniates (Craniata) that may harm chondrichthyans. All except Figure 24.134 drawn from Goode and Bean (1896), Figure 24.134 drawn from Compagno (1984). **24.132.** *Myxine glutinosa* (Myxinidae, Myxiniformes), adult, lateral view. Scale bar = 3 cm. **24.133.** *Petromyzon marinus* (Petromyzontidae, Petromyzontiformes), juvenile, lateral view. Scale bar = 3 cm. **24.134.** *Isistius brasiliensis* (Dalatiidae, Squaliformes), adult, lateral view. Scale bar = 8 cm. **24.135.** *Simenchelys parasiticus* (Synaphobranchidae, Anguilliformes), adult, lateral view. Scale bar = 4 cm.

Helfman et al., 1997). As juveniles and adults, marine lampreys are parasitic and they prey on a wide variety of cartilaginous and bony fishes as well as mammals (Beamish, 1980; Halliday, 1991). Feeding is accomplished by using the oral disk as a powerful attachment sucker while the rasping tongue penetrates the host. Food consists of blood and other host tissues (Bigelow and Schroeder, 1953; Helfman et al., 1997). Although lampreys can kill bony fishes (Beamish and Youson, 1987), almost nothing is known of their effects on chondrichthyans. However, hemorrhagic lesions associated with feeding lampreys and nonhemorrhagic scars caused by resting lampreys can be obvious on sharks. Some have speculated that basking sharks, *Cetorhinus maximus* (Gunnerus, 1765), breach in attempts to rid themselves of these hitchhikers, but no concrete evidence supports this (Fairfax, 1998). Lampreys can be abundant in some areas at certain times of year. For example, one of the authors (G. W. Benz, unpublished observations) observed that blue sharks in the vicinity of Hudson Canyon (northwestern Atlantic Ocean) were exclusively pestered during the fall by small lampreys. This phenomenon possibly occurred because juvenile lampreys may commonly follow river to submarine canyon pathways as they transition from fresh into marine waters.

Cookie-cutter sharks, *Isistius* spp., (Dalatiidae, Chondrichthyes, Gnathostomata) are small, pelagic dalatiids (Figure 24.134) circumglobally distributed in temperate and tropical seas (Compagno, 1984, 1999). These sharks are suspected of being ambush predators (Widder, 1998) that can feed on animals much larger than themselves by cleanly removing circular plugs of flesh with their powerful scoop-like lower jaw and its large teeth. Evidence of this feeding mechanism stems from chunks of tissue collected from the stomachs of *Isistius* spp. and the presence of corresponding crater-like lesions on their presumed hosts (Strasburg, 1963; Jones, 1971; Jahn and Haedrich, 1987; Le Boeuf et al., 1987). Because of this hypothesized feeding mode and evidence that at least one species, *I. brasiliensis* (Quoy and Gaimard, 1824), can consume small fishes and invertebrates entirely (Compagno, 1984), cookie-cutter sharks are considered predators by some (Widder, 1998) and facultative parasites by others (Jones, 1971; Compagno, 1984). In addition to feeding on squids, crustaceans, teleosts, and marine mammals, as well as attacking submarines and possibly humans (Strasburg, 1963; Jones, 1971; Compagno, 1984; Jahn and Haedrich, 1987; Le Boeuf et al., 1987), members of *Isistius* have been reported (Taylor et al., 1983; Berra and Hutchins, 1991) to bite megamouth sharks, *Megachasma pelagios* Taylor, Compagno, and Struhsaker, 1983.

Snubnose parasitic eels, *Simenchelys parasiticus* (Synaphobranchidae, Actinopterygii, Gnathostomata) are small anguilliforms (Anguilliformes) with large eyes and powerful jaws (Figure 24.135) that occur worldwide in tropical to temperate marine waters at depths between 365 and 2,620 m (Nelson, 1994). These eels are primarily considered scavengers of fishes; however, little is known about their food and feeding habits (Nelson, 1994). Nevertheless, the snubnose parasitic eel is justifiably considered a facultative parasite based on its ability to completely burrow into and feed on living fishes (Bigelow and Schroeder, 1953). A case of two snubnose parasitic eels from the lumen of the heart of a shortfin mako was mentioned first by Lampton (1995) and later detailed by Caira et al. (1997a). Although these eels had fed on blood, it was not determined beyond a doubt if they had penetrated the shark before its capture and death on a fishing line (Caira et al., 1997a).

Industry challenges and research opportunities

Maintaining healthy chondrichthyans in captivity depends on a commitment to provide or access the necessary expertise and facilities to appropriately confront parasite challenges. Inadequate understanding of parasites and parasitism or poor proficiency regarding parasitological techniques by husbandry and veterinary staff can seriously affect the fate of captive chondrichthyans. For example, the inability to locate and correctly assign metazoan parasites to one of the three aforementioned functional groups can result in hosts not being treated for a pathogen, or hosts being subjected to needless and possibly life-threatening manipulations. Regarding facilities, the following equipment and supplies are essential for parasite examinations: a quality low-power dissection microscope; a quality compound microscope; a complete set of dissection tools; fixatives, preservatives, stains, and mounting media as required to study various parasites (see sections above as well as Pritchard and Kruse, 1982); storage vials and assorted glassware; labeling supplies; and packing and mailing supplies. Because many chondrichthyans are relatively large, a well-lighted and comfortable work area of adequate size is necessary to efficiently

conduct physical examinations and necropsies. Rapid diagnoses and husbandry decisions can sometimes be facilitated by using microscopes equipped with digital cameras, such that images can be e-mailed to experts to obtain preliminary taxonomic identifications. Concerning staff education, in addition to formal training in Parasitology, proficiencies regarding necropsy and microscopy are critical.

Lack of information or reliance on misinformation creates many parasitological challenges regarding the captive maintenance of chondrichthyans. For example, beyond a general understanding of parasite life cycles, specific knowledge of how long it takes the eggs of a particular parasite species to hatch at a specific water temperature, or how long it takes the juveniles of specific parasites to mature into reproductively active adults at various water temperatures, is usually unknown. And likewise, the minimum effective doses of parasiticides as well as the comparative efficacy of various parasiticides regarding specific problematic parasites are often unknown. Lacking pertinent information requires some husbandry decisions to be based on hunches rather than on scientific data.

Because scientific research is an effective and rigorous way to gather information, husbandry practices can benefit from research initiatives. However, when parasites are discovered and blamed for health problems, seldom are even casual experiments performed on them. This is unfortunate because, and as examples, holding parasites to see how long they can live away from their hosts, determining what dosages of various chemical treatments are lethal to parasites, observing the movement capabilities of parasites, or harvesting and incubating parasite eggs to see how fast they hatch, all provide insight applicable to future infection episodes. Without proper experimental design, observations stemming from activities such as the aforementioned will be flawed regarding their scientific rigor. However, if the scientific limits of observations are understood, casual experiments may yield information that rules out hunch-based husbandry decisions. In addition, casual experiments can sometimes provide compelling insight that justifies the added time necessary to carry out more formal experimentation.

Institutions that hold chondrichthyans have ample opportunities to contribute to, and benefit from, an understanding of metazoan parasites through applied and basic research. To date, however, the commitment made by these institutions to formally conduct such research has been relatively minor and primarily represented by studies best described as serendipitous rather than designed. Resulting from these studies, case histories, descriptions of new species or unknown life stages of parasites, topic reviews, and reports of parasite control measures have been published. But despite the sanctifying nature of publication, in some instances these efforts have unfortunately been suboptimal and have resulted in the validation of parasitological lapses and errors because appropriate professional collaborations were not established to ensure scientific rigor.

The field of Parasitology spans the panoply of biological subdisciplines, but even among parasitologists, the scope of individual expertise is typically narrow. Thus interest and general expertise should be tempered with realistic assessments of abilities when planning parasite research and preparing manuscripts intended for publication. Because the animal care exigencies at most public aquariums typically prevent staff from acquiring or maintaining a level of expertise that allows efficient independent research, husbandry staff and veterinarians usually benefit from collaborations with bonafide parasitologists when pursuing parasite studies. Likewise, parasitologists often benefit from collaborations with one another as well as with others possessing synergistic expertise (e.g., aquarists, veterinarians, pathologists, etc.). Yet no matter how many authors' names are to appear on publications, it is wise to adopt a philosophy that if research or publication products cannot minimally equal those of accomplished others working within the field, then additional expertise, equipment, or both should be sought. In addition, research should be executed with the same high level of professionalism regardless of the intended publication vehicle, and thus shopping for journals in which to publish should not be primarily based on the desire to publish or the robustness of the research.

Institutions maintaining captive chondrichthyans can theoretically conduct investigations of metazoan parasites through studies of free-ranging or captive hosts. Although the former category is outside the scope of the present chapter, it should be noted that some public aquariums have participated in field studies of elasmobranch parasites (e.g., see Benz et al., 1998, 2001, 2002a, 2002b, 2003; Borucinska and Benz, 1999; Borucinska et al., 1998; Braswell et

al., 2002; Bullard et al., 2000a, 2000c, 2001) and that parasitological information gathered under natural conditions is often valuable regarding husbandry operations. Minimally in this regard, hosts that perish during capture operations or during the initial stages of captivity, such as during transport and quarantine, should be thoroughly examined to document their natural parasite faunas, especially as these examinations may reveal parasites that also infect other fishes captured concurrently. Regarding areas of Parasitology that could be advanced through the study of captive chondrichthyans, four general research categories offer the greatest self-serving opportunities for those interested in chondrichthyan husbandry: parasite taxonomy and distributions; host-parasite interactions; parasite life histories; and parasite control.

Research on the taxonomy and distributions of parasites is important to the captive care of chondrichthyans because the success of husbandry operations can be directly linked to the ability of husbandry staff to identify parasites and where they live. The identification of some metazoan parasites of chondrichthyans can be exceedingly difficult because some species descriptions are inadequate. Furthermore, chondrichthyans are infected by many species of parasites that await formal description. Thus, describing and redescribing parasites are important taxonomic tasks that allow the recognition of biodiversity while bolstering the practical foundations of chondrichthyan husbandry. Documenting patterns of biodiversity by reporting new geographic or ecosystem records of parasites provides valuable data that can be applied to ecological and biogeographical studies, and also to husbandry decisions such as field collection areas to avoid because of the presence of particularly problematic parasites.

Research on host-parasite interactions is important to the captive care of chondrichthyans because the success of husbandry operations can depend on knowledge of how various parasites affect their hosts. For example, studies of how host physiology is affected by parasite infections may provide specific details useful to those formulating health care. Research can also investigate the physical nature and severity of lesions associated with metazoan infections to gain an understanding of the potential health risks that parasites pose. Studies of host and infection site specificity can facilitate parasite risk assessments and parasite diagnoses, and they can also provide insight regarding how infections are regulated.

Research on the life history of parasites is important to the captive care of chondrichthyans because the success of husbandry efforts can be tied to the ability to recognize the various life stages of parasites and to understand the factors that mediate parasite reproduction and transmission in captive settings. The early life stages of many parasites look strikingly different from their corresponding adults, and the literature often contains no species-level information on the early life stages of particular parasites that infect chondrichthyans. This lack of information can cause missed opportunities for important diagnoses in captive settings as, for example, when a parasite population is rapidly reproducing and early life stages of the parasite are not recognized by husbandry staff. Studies investigating the physical and chemical factors that influence the tempo of parasite life cycles can help husbandry staff avoid or control infections, and behavioral studies of parasite feeding, mobility, transmission, and reproduction can provide information allowing staff to anticipate and prepare for the challenges that various infections may pose.

Research on parasite control is important to the captive care of chondrichthyans because the ability to kill or otherwise limit the impact of parasites can determine the health and appearance of hosts. In addition, research regarding the possible toxic effects of chemotherapeutics is similarly important and sorely needed. Given the applied nature of such research, it is puzzling to the authors that so little research in these areas has been carried out or otherwise supported by public aquariums in particular.

The ability of institutions to participate in each of the aforementioned research areas depends on many factors. Studies in some areas require the design of controlled experiments involving rigorous replication, the repeated manipulation of live hosts, and the killing of experimental animals. Thus, the compatibility between science and industry, as mediated by the physical, operational, fiscal, and philosophical underpinnings of institutions, will continue to determine the contribution that industry can muster regarding this important field of science.

ACKNOWLEDGEMENTS

The authors thank the Tennessee Aquarium Research Institute, the Tennessee Aquarium, Middle Tennessee State University, and the Gulf Coast Research Laboratory (GCRL, Department of Coastal Sciences, The University of Southern

Mississippi [USM]) for general support that expedited the production of this chapter, Robin M. Overstreet and Ronnie G. Palmer (both GCRL, USM Department of Coastal Sciences) for sharing information regarding parasite fixation, R. M. Overstreet for providing expert counsel regarding several important taxonomic matters, Catherine Schloss and Joyce Shaw (both USM Department of Coastal Sciences) for library assistance, Stephen S. Curran (GCRL, USM Department of Coastal Sciences) and Thomas A. Tarpley for allowing the authors to modify and use several of their unpublished sketches of parasites (S. S. C. Figures 24.30 and 24.33, T. A. T. Figures 24.35-24.38), Dan Holiday (GCRL, USM Department of Coastal Sciences) for digitizing Figures 24.5 and 24.6, and the editors of this volume for their patience and for getting this chapter into print. The junior author's efforts in producing this chapter were supported by his advisor R. M. Overstreet through National Marine Fisheries Service (National Oceanic and Atmospheric Association) (NMFS/NOAA) Award NA-03NMF4720320. The authors' participation at the First International Elasmobranch Husbandry Symposium, where an overview of this chapter was presented, was facilitated for S.A.B. by R. M. Overstreet through NMFS/NOAA Award NA-03NMF4720320 and for G.W.B. by Doug Warmolts (Columbus Zoo) and Jackson Andrews (Tennessee Aquarium).

REFERENCES

Abbott, R. T. (1974) American seashells: the marine mollusca of the Atlantic and Pacific coasts of North America, second edition. Van Nostrand Reinhold Company, New York, New York, 663 pp.

Abdelhalim, A. I., Lewis, J. W. and Boxshall, G. A. (1991) The life-cycle of *Ergasilus sieboldi* Nordmann (Copepoda: Poecilostomatoida), parasitic on British freshwater fish. Journal of Natural History, 25, 559-582.

Adam, H. (1960) Different types of body movement in the hagfish, *Myxine glutinosa* L. Nature, 188, 595-596.

Adams, A. M., Hoberg, E. P., McAlpine, D. F. and Clayden, S. L. (1998) Occurrence and morphological comparisons of *Campula oblonga* (Digenea: Campulidae), including a report from an atypical host, the thresher shark, *Alopias vulpinus*. Journal of Parasitology, 84, 435-438.

Adamson, M. L. (1998) Nematodes of elasmobranchs from the Gulf of California. Seventy-third Annual Meeting Program Guide and Abstracts, The American Society of Parasitologists, Kona, Hawaii, 58 p.

Adamson, M. L., Benz, G. W., Jenkins, J. L. and Henningsen, A. D. (in preparation) *Lockenloia aetobati* n. sp. (Nematoda; Dracunculoidea) from brain and gills of the spotted eagle ray, *Aetobatus narinari* (Myliobatidae; Elasmobranchii). Journal of Parasitology.

Adamson, M. L. and Caira, J. N. (1991) *Lockenloia sanguinis* n. gen., n. sp. (Nematoda: Dracunculoidea) from the heart of a nurse shark, *Ginglyostoma cirratum*, in Florida. Journal of Parasitology, 77, 663-665.

Adamson, M. L., Deets, G. B. and Benz, G. W. (1987) Description of male and redescription of female *Phlyctainophora squali* Mudry and Dailey, 1969 (Nematoda; Dracunculoidea) from elasmobranchs. Canadian Journal of Zoology, 65, 3006-3010.

Adjei, E. L., Barnes, A. and Lester, R. J. G. (1986) A method for estimating possible parasite-related host mortality, illustrated using data from *Callitetrarhynchus gracilis* (Cestoda: Trypanorhyncha) in lizardfish (*Saurida* spp.). Parasitology, 92, 227-243.

Aken'Ova, T. O. and Lester, R. J. G. (1996) *Udonella myliobati* n. comb. (Platyhelminthes: Udonellidae) and its occurrence in eastern Australia. Journal of Parasitology, 82, 1017-1023.

Allum, M. O. and Hugghins, E. J. (1959) Epizootics of fish lice, *Argulus biramosus*, in two lakes of eastern South Dakota. Journal of Parasitology, 45(4) section 2, 33-34.

Amanieu, M. (1963) Evolution des populations de *Paragnathia formica* (Hesse) au cours d'un cycle annuel. Bulletin of the Institute of Oceanography, Monaco, 60(261), 1-12.

Anderson, R. C. (1984) The origins of zooparasitic nematodes. Canadian Journal of Zoology, 62, 317-328.

Anderson, R. C. (2000) Nematode parasites of vertebrates: their development and transmission, second edition. CABI Publishing, Wallingford, United Kingdon, 650 pp.

Anderson, R. C., Chabaud, A. G. and Willmott, S. (Eds.) (1974a) CIH keys to the nematode parasites of vertebrates, number 1. CAB International, Wallingford, United Kingdom, 17 pp.

Anderson, R. C., Chabaud, A. G. and Willmott, S. (Eds.) (1974b) CIH keys to the nematode parasites of vertebrates, number 2. CAB International, Wallingford, United Kingdom, 15 pp.

Anderson, R. C., Chabaud, A. G. and Willmott, S. (Eds.) (1975) CIH keys to the nematode parasites of vertebrates, number 3, parts 1-3. CAB International, Wallingford, United Kingdom, 116 pp.

Anderson, R. C., Chabaud, A. G. and Willmott, S. (Eds.) (1976) CIH keys to the nematode parasites of vertebrates, number 4, CAB International, Wallingford, United Kingdom, 30 pp.

Anderson, R. C., Chabaud, A. G. and Willmott, S. (Eds.) (1978a) CIH keys to the nematode parasites of vertebrates, number 5, CAB International, Wallingford, United Kingdom, 40 pp.

Anderson, R. C., Chabaud, A. G. and Willmott, S. (Eds.) (1978b) CIH keys to the nematode parasites of vertebrates, number 6, CAB International, Wallingford, United Kingdom, 71 pp.

Anderson, R. C., Chabaud, A. G. and Willmott, S. (Eds.) (1980a) CIH keys to the nematode parasites of vertebrates, number 7, CAB International, Wallingford, United Kingdom, 41 pp.

Anderson, R. C., Chabaud, A. C. and Willmott, S. (Eds.) (1980b) CIH keys to the nematode parasites of vertebrates, number 8, CAB International, Wallingford, United Kingdom, 26 pp.

Anderson, R. C., Chabaud, A. G. and Willmott, S. (Eds.) (1982) CIH keys to the nematode parasites of vertebrates, number 9, CAB International, Wallingford, United Kingdom, 26 pp.

Anderson, R. C., Chabaud, A. G. and Willmott, S. (Eds.) (1983) CIH keys to the nematode parasites of vertebrates, number 10, CAB International, Wallingford, United Kingdom, 86 pp.

Avenant-Oldewage, A. and Swanepoel, J. H. (1993) The male reproductive system and mechanism of sperm transfer in *Argulus japonicus* (Crustacea: Branchiura). Journal of Morphology, 215, 51-63.

Awerinzew, S. A. (1925) Über eine neue Art von parasitären Tricladen. Zoologischer Anzeiger, 64, 81-84.

Ball, I. R. and Khan, R. A. (1976) On *Micropharynx parasitica* Jägerskiöld, a marine planarian ectoparasitic on thorny skate, *Raja radiata* Donovan, from the north Atlantic Ocean. Journal of Fish Biology, 8, 419-426.

Bandoni, S. M. and Brooks, D. R. (1987) Revision and phylogenetic analysis of the Gryocotylidae Poche, 1922 (Platyhelminthes: Cercomeria: Cercomeromorphae). Canadian Journal of Zoology, 65, 2369-2389.

Barnard, K. H. (1955) South African parasitic Copepoda. Annals of the South African Museum, 41, 223-312.

Beamish, F. W. H. (1980) Biology of the North American anadromous sea lamprey, *Pertromyzon marinus*. Canadian Journal of Fisheries and Aquatic Science, 37, 1924-1943.

Beamish, R. J. and Youson, J. H. (1987) Life history and abundance of young adult *Lampetra ayresi* in the Fraser River and their possible impact on salmon and herring stocks in the Strait of Georgia. Canadian Journal of Fisheries and Aquatic Sciences, 44, 525-537.

Becker, J. A. and Speare, D. J. (2004) Ultraviolet light control of horizontal transmission of *Loma salmonae*. Journal of Fish Diseases, 27, 177-180.

Bennett, M. B., Heupel, M. R., Bennett, S. M. and Parker, A. R. (1997) *Sheina orri* (Myodocopa: Cypridinidae) an ostracod parasitic on the gills of the epaulette shark, *Hemiscyllium ocellatum* (Bonnaterre, 1788) (Elasmobranchii: Hemiscyllidae). International Journal of Parasitology, 27, 275-281.

Benz, G. W. (1980) Tissue proliferations associated with *Nemesis lamna* Risso, 1826 (Copepoda: Eudactylinidae) infestations on the gill filaments of shortfin makos (*Isurus oxyrinchus* Rafinesque). Journal of Fish Diseases, 3, 443-446.

Benz, G. W. (1981) Observations on the attachment scheme of the parasitic copepod *Pandarus satyrus* (Copepoda: Pandaridae). Journal of Parasitology, 67, 966-967.

Benz, G. W. (1984a) On the conservative nature of the gill filaments of sharks. Environmental Biology of Fishes, 10, 111-116.

Benz, G. W. (1984b) Association of the pedunculate barnacle, *Conchoderma virgatum* (Spengler, 1790), with pandarid copepods (Siphonostomatoida: Pandaridae). Canadian Journal of Zoology, 62, 741-742.

Benz, G. W. (1986) Distributions of siphonostomatoid copepods parasitic upon large pelagic sharks in the western North Atlantic. Syllogeus, 58, 211-219.

Benz, G. W. (1987) *Dermophthirius penneri* sp. n. (Monogenea: Microbothriidae) an ectoparasite of carcharhinid sharks, *Carcharhinus brevipinna* and *Carcharhinus limbatus*. Proceedings of the Helminthological Society of Washington, 54, 185-190.

Benz, G. W. (1989) Developmental stages of *Alebion lobatus* Cressey, 1970 (Copepoda: Euryphoridae) found parasitic on the sandbar shark (*Carcharhinus plumbeus* (Nardo, 1827)) in the western North Atlantic, and a phylogenetic analysis of the genus *Alebion* Krøyer, 1863. Canadian Journal of Zoology, 67, 1578-1598.

Benz, G. W. (1991) Description of some larval stages and augmented description of adult stages of *Albionella etmopteri* (Copepoda: Lernaeopodidae), a parasite of deep-water lanternsharks (*Etmopterus*: Squalidae). Journal of Parasitology, 77, 666-674.

Benz, G. W. (1993) Evolutionary biology of Siphonostomatoida (Copepoda) parasitic on vertebrates. Ph.D. Dissertation, The University of British Columbia, Vancouver, British Columbia, Canada, 173 pp.

Benz, G. W. (1995) Biodiversity: are we neglecting half of Earth's fauna? *In* Proceedings of the Third International Aquarium Congress. (Ed. Barrett, C.) Boyd Press, Wheeling, West Virginia, 90-96 pp.

Benz, G. W. and Adamson, S. A. M. (1990) Disease caused by *Nemesis robusta* (van Beneden, 1851) (Eudactylinidae: Siphonostomatoida: Copepoda) infections on gill filaments of thresher sharks (*Alopias vulpinus* (Bonnaterre, 1758)), with notes on parasite ecology and life history. Canadian Journal of Zoology, 68, 1180-1187.

Benz, G. W., Borucinska, J. D. and Greenwald, S. A. (2002a) First descriptions of early- and middle-stage copepodids of *Anthosoma crassum* (Dichelesthiidae: Siphonostomatoida) and lesions on shortfin makos (*Isurus oxyrinchus*) infected with *A. crassum*. Journal of Parasitology, 88, 19-26.

Benz, G. W., Borucinska, J. D., Lowry, L. F. and Whiteley, H. E. (2002b) Ocular lesions associated with attachment of the copepod *Ommatokoita elongata* (Lernaeopodidae: Siphonostomatoida) to corneas of Pacific sleeper sharks *Somniosus pacificus* captured off Alaska in Prince William Sound. Journal of Parasitology, 88, 474-481.

Benz, G. W. and Deets, G. B. (1986) *Kroyeria caseyi* sp. nov. (Kroyeriidae: Siphonostomatoida), a parasitic copepod infesting gills of night sharks (*Carcharhinus signatus* (Poey, 1868)) in the western North Atlantic. Canadian Journal of Zoology, 64, 2492-2498.

Benz, G. W. and Deets, G. B. (1987) *Echthrogaleus disciarai* sp. nov. (Siphonostomatoida: Pandaridae), a parasitic copepod of the devil ray, *Mobula lucasana* Beebe and Tee Van, 1938 from the Sea of Cortez. Canadian Journal of Zoology, 65, 685-690.

Benz, G. W. and Deets, G. B. (1988) Fifty-one years later: an update on *Entepherus*, with a phylogenetic analysis of Cecropidae Dana, 1849 (Copepoda: Siphonostomatoida). Canadian Journal of Zoology, 66, 856-865.

Benz, G. W. and Dippenaar, S. M. (2000) Putting the bite on jaws: copepods as enemies of sharks. *In* The shark tagger 1998. (Eds. Kohler, N., Natanson, L. and Pratt, H. W., Jr.) National Marine Fisheries Service, Narragansett, Rhode Island, 12, 18-19 pp.

Benz, G. W. and Dupre, K. S. (1987) Spatial distribution of the parasite *Kroyeria carchariaeglauci* Hesse, 1879 (Copepoda: Siphonostomatoida: Kroyeriidae) on gills of the blue shark (*Prionace glauca* (L., 1758)). Canadian Journal of Zoology, 65, 1275-1281.

Benz, G. W. and Hogans, W. E. (1993) *Pennella filosa* (L., 1758) (Copepoda: Siphonostomatoida) from the escolar *Lepidocybium flavobrunneum* (Smith, 1849) in the north-west Atlantic. Systematic Parasitology, 26, 121-131.

Benz, G. W., Lucas, Z. and Lowry, L. F. (1998) New host and ocean records for the copepod *Ommatokoita elongata* (Siphonostomatoida: Lernaeopodidae), a parasite of the eyes of sleeper sharks. Journal of Parasitology, 84, 1271-1274.

Benz, G. W., Mohan, P. J. and Crow, G. L. (1992) Developmental stages of *Paralebion elongatus* from aquarium-held reef whitetip sharks (*Triaenodon obesus*) captured in Hawaiian waters. Journal of Parasitology, 78, 1027-1035.

Benz, G. W., Mollet, H. F., Ebert, D. A., Davies, C. R. and Van Sommeran, S. R. (2003) Five species of parasitic copepods (Siphonostomatoida: Pandaridae) from the body surface of a white shark captured in Morro Bay, California. Pacific Science. 57, 39-43.

Benz, G. W. and Otting, R. L. (1996) Morphology of the fish louse (*Argulus:* Branchiura). Drum and Croaker, 27, 15-22.

Benz, G. W., Otting, R. L. and Case, A. (1995) Redescription of *Argulus melanostictus* (Branchiura: Argulidae), a parasite of California grunion (*Leuresthes tenuis*:

Atherinidae), with notes regarding chemical control of *A. melanostictus* in a captive host population. Journal of Parasitology, 81, 754-761.

Benz, G. W., Pratt, H. L., Jr. and Adamson, M. L. (1987) Larval philometrid nematodes (Philometridae) from the uterus of a sandbar shark, *Carcharhinus plumbeus*. Proceedings of the Helminthological Society of Washington, 54, 154-155.

Benz, G. W., Smith, B. E. and Bullard, S. A. (2001) *Kroeyerina deetsorum* n. sp. (Copepoda: Kroyeriidae) from the olfactory sacs of Atlantic sharpnose sharks (*Rhizoprionodon terraenovae*) captured in the Gulf of Mexico and northwestern Atlantic and first report of copepodids representing Kroyeriidae. Journal of Parasitology, 87, 1279-1290.

Bere, R. (1936) Parasitic copepods from Gulf of Mexico fish. American Midland Naturalist, 17, 577-625.

Berra, T. M. and Hutchins, B. (1991) Natural history notes on the megamouth shark, *Megachasma pelagios*, from western Australia. Western Australian Naturalist, 18, 224-233.

Beveridge, I. and Campbell, R. A. (1998) Re-examination of the trypanorhynch cestode collections of A. E. Shipley, J. Hornell and T. Southwell, with erection of a new genus, *Trygonicola*, and redescriptions of seven species. Systematic Parasitology, 39, 1-34.

Bigelow, H. B. and Schroeder, W. C. (1953) Fishes of the Gulf of Maine. Fishery Bulletin 53, Fish and Wildlife Service, United States Printing Service, Washington, D.C., 577 pp.

Bird, P. M. (1981) The occurrence of *Cirolana borealis* (Isopoda) in the hearts of sharks from Atlantic coastal waters of Florida. Fishery Bulletin, 79, 376-383.

Bjordal, Å. (1988) Cleaning symbiosis between wrasses (Labridae) and lice infested salmon (*Salmo salar*) in mariculture. ICES Committee Meeting (C.M.) Paper. International Council for the Exploration of the Sea, 1988/F:17, 8 pp.

Blasiola, G. C. (1976) Ectoparasitic turbellaria. Marine Aquarist, 7, 53-58.

Blasiola, G. C. (1992) Diseases of ornamental marine fishes. *In* Aquariology: the science of fish health management. (Eds. Gratzek, J. B. and Matthews, J. R.) Tetra Press, Morris Plains, New Jersey, 275-300 pp.

Blaylock, R. B. and Overstreet, R. M. (2003) Diseases and parasites of the spotted seatrout. *In* Biology of the spotted seatrout. (Ed. Bortone, S. A.) CRC Press, Boca Raton, Florida, 197-225 pp.

Blaylock, R. B., Bullard, S. A. and Whipps, C. M. (2004) *Kudoa hypoepicardialis* n. sp. (Myxozoa: Kudoidae) and associated lesions from the heart of seven perciform fishes in the northern Gulf of Mexico. Journal of Parasitology, 90, 584-593.

Boeger, W. A. and Kritsky, D. C. (1989) Phylogeny, coevolution, and revision of the Hexabothriidae Price, 1942 (Monogenea). International Journal for Parasitology, 19, 425-440.

Boeger, W. A. and Kritsky, D. C. (1993) Phylogeny and revised classification of the Monogenoidea Bychowsky, 1937 (Platyhelminthes). Systematic Parasitology, 26, 1-32.

Boeger, W. A. and Kritsky, D. C. (2001) Phylogenetic relationships of the Monogenoidea. *In* Interrelationships of the Platyhelminthes. (Eds. Littlewood, D. T. J. and Bray, R. A.) Taylor and Francis, London, United Kingdom, 92-102 pp.

Borucinska, J. D. and Benz, G. W. (1999) Lesions associated with attachment of the parasitic copepod *Phyllothyreus cornutus* (Pandaridae: Siphonostomatoida) to interbranchial septa of blue sharks. Journal of Aquatic Animal Health, 11, 290-295.

Borucinska, J. D., Benz, G. W. and Whiteley, H. E. (1998) Ocular lesions associated with attachment of the parasitic copepod *Ommatokoita elongata* (Grant) to corneas of Greenland sharks, *Somniosus microcephalus* (Bloch & Schneider). Journal of Fish Diseases, 21, 415-422.

Borucinska, J. D. and Caira, J. N. (1993) A comparison of mode of attachment and histopathogenicity of four tapeworm species representing two orders infecting the spiral intestine of the nurse shark, *Ginglymostoma cirratum*. Journal of Parasitology, 79, 238-246.

Borucinska, J. D. and Dunham, A. (2000) Lesions associated with attachment of the parasitic cestode *Tentacularia* sp. to the duodeno-spiral junction in blue sharks (*Prionace glauca*) (Linnaeus, 1758). Journal of Fish Diseases, 23, 353-359.

Borucinska, J. D. and Frasca, S., Jr. (2002) Naturally occurring lesions and micro-organisms in two species of free-living sharks: the spiny dogfish, *Squalus acanthias* L., and the smooth dogfish, *Mustelus canis* (Mitchill), from the north-western Atlantic. Journal of Fish Diseases, 25, 287-298.

Borucinska, J. D. and Heger, K. (1999) Disseminated granulomas associated with nematode larvae in a shortfin mako shark. Journal of Wildlife Diseases, 35, 98-100.

Bousfield, E. L. and Kabata, Z. (1988) Amphipoda. *In* Guide to the parasites of fishes of Canada, part II. Crustacea. (Eds. Margolis, L. and Kabata, Z.) Department of Fisheries and Oceans, Ottawa, Canada, 149-163 pp.

Boxshall, G. A. and Defaye, D. (Eds.) (1993) Pathogens of wild and farmed fish: sea lice. Ellis Horwood, New York, New York, 378 pp.

Boxshall, G. A. and Halsey, S. H. (2004) An introduction to copepod diversity, parts I and II. The Ray Society, London, United Kingdom, 966 pp.

Boxshall, G. A. and Montú, M. A. (1997) Copepods parasitic on Brazilian coastal fishes: a handbook. Nauplius, Rio Grande, 5, 1-255.

Branson, E., Riaza, A. and Alvarez-Pellitero, P. (1999) Myxosporean infection causing intestinal disease in farmed turbot, *Scophthalmus maximus* (L.), (Teleostei: Scophthalmidae). Journal of Fish Diseases, 22, 395-399.

Braswell, J. S., Benz, G. W. and Deets, G. B. (2002) *Taeniacanthodes dojirii* n. sp. (Copepoda: Poecilostomatoida: Taeniacanthidae), from Cortez electric rays (*Narcine entemedor*: Torpediniformes: Narcinidae) captured in the Gulf of California, and a phylogenetic analysis of and key to species of *Taeniacanthodes*. Journal of Parasitology, 88, 28-35.

Bray, R. A. (1984) Some helminth parasites of marine fishes and cephalopods of South Africa: Aspidogastrea and the digenean families Bucephalidae, Haplosplanchnidae, Mesometridae and Fellodistomidae. Journal of Natural History, 18, 271-292.

Bray, R. A. (2002) Family Fellodistomidae Nicoll, 1909. *In* Keys to the Trematoda, volume 1. (Eds. Gibson, D. I., Jones, A. and Bray, R. A.) CAB International, Wallingford, United Kingdom, 261-293 pp.

Bray, R. A. and Cribb, T. H. (2003) The digeneans of elasmobranchs— distribution and evolutionary significance. *In* Taxonomy, ecology and evolution of metazoan parasites. (Eds. Tome, I., Combes, C. and Jourdane, J.) PUP, Perpignan, France, 67-96 pp.

Bray, R. A. and Moore, A. B. M. (2000) The first record of the elasmobranch parasite *Diphterostomum betencourti* (Monticelli, 1893) (Digenea, Zoogonidae) in the coastal waters of southern England. Acta Parasitologica, 45, 299-302.

Brickle, P., Pompert, J. and Poulding, D. (2002) The occurrence of *Otodistomum plunketi* Fyfe, 1953 (Digenea: Azygiidae), in rays (Chondrichthyes: Rajidae) around the Falkland Islands. Comparative Parasitology, 69, 86-89.

Brinkmann, A., Jr. (1952) Some Chilean monogenetic trematodes. Reports of the Lund University Chile Expedition 1948-1949 (6). Lunds University årsskrift neue folge avdelning 2, band 47(11), 26 pp.

Brinkmann, A., Jr. (1957) Fish trematodes from Norwegian waters. IIa. The Norwegian species of the orders Aspidogastrea and Digenea (Gasterostomata). Universitetet i Bergen, Årbok 1957, Naturvitenskapelig Rekke, 4, 1-31.

Bron, J. E., Sommerville, C., Wootten, R. and Rae, G. H. (1993) Influence of treatment with dichlorvos on the epidemiology of *Lepeophtheirus salmonis* (Krøyer, 1837) and *Caligus elongatus* Nordmann, 1832 on Scottish salmon farms. *In* Pathogens of wild and farmed fish: sea lice. (Eds. Boxshall, G. A. and Defaye, D.) Ellis Horwood, New York, New York, 263-274 pp.

Brooks, D. R. and Amato, J. F. R. (1992) Cestode parasites in *Potamotrygon motoro* (Natterer) (Chondrichthyes: Potamotrygonidae) from southwestern Brazil, including *Rhinebothroides mclennanae* n. sp. (Tetraphyllidea: Phyllobothriidae), and a revised host-parasite checklist for helminths inhabiting neotropical freshwater stingrays. Journal of Parasitology, 78, 393-398.

Brooks, G. L. (1934) Some new ectoparasitic trematodes (Onchocotylinae) from the gills of American sharks. Parasitology, 26, 259-267.

Bruno, D. W. and Raynard, R. S. (1994) Studies on the use of hydrogen peroxide as a method for the control of sea lice on Atlantic salmon. Aquaculture International, 2, 10-18.

Brusca, R. C. (1980) Common intertidal invertebrates of the Gulf of California, second edition. University of Arizona Press, Tucson, Arizona, 513 pp.

Brusca, R. C. (1981) A monograph on the Isopoda Cymothoidae (Crustacea) of the eastern Pacific. Zoological Journal of the Linnean Society, 73, 117-199.

Brusca, R. C. and Brusca, G. J. (2003) Invertebrates, second edition. Sinauer Associates, Sunderland, Massachusetts, 936 pp.

Buckner, R. L., Overstreet, R. M. and Heard, R. W. (1978) Intermediate hosts for *Tegorhynchus furcatus* and *Dollfusentis chandleri* (Acanthocephala). Proceedings of the Helminthological Society of Washington, 45, 195-201.

Bullard, S. A. (2002) Blood flukes (Digenea: Sanguinicolidae) from commercially and recreationally important fishes in the Gulf of Mexico. M.Sc. Thesis, The University of Southern Mississippi, Hattiesburg, Mississippi, 129 pp.

Bullard, S. A., Benz, G. W. and Braswell, J. S. (2000a) *Dionchus* postoncomiracidia (Monogenea: Dionchidae) from the skin of blacktip sharks, *Carcharhinus limbatus* (Carcharhinidae). Journal of Parasitology, 86, 245-250.

Bullard, S. A., Benz, G. W., Overstreet, R. M., Williams, E. H., Jr. and Hemdal, J. (2000b) Six new host records and an updated list of wild hosts for *Neobenedenia melleni* (MacCallum) (Monogenea: Capsalidae). Comparative Parasitology, 67, 190-196.

Bullard, S. A. and Dippenaar, S. (2003) *Branchotenthes robinoverstreeti* n. gen. and n. sp. (Monogenea: Hexabothriidae) from gill filaments of the bowmouth guitarfish, *Rhina ancylostoma* (Rhynchobatidae), in the Indian Ocean. Journal of Parasitology, 89, 595-601.

Bullard, S. A., Dippenaar, S. M., Hoffmayer, E. R. and Benz, G. W. (2004) New locality records for *Dermophthirius carcharhini* (Monogenea: Microbothriidae) and *Dermophthirius maccallumi* and a list of hosts and localities for species of *Dermophthirius*. Comparative Parasitology, 71, 78-80.

Bullard, S. A., Frasca, S., Jr. and Benz, G. W. (2000c) Skin lesions caused by *Dermophthirius penneri* (Monogenea: Microbothriidae) on wild-caught blacktip sharks (*Carcharhinus limbatus*). Journal of Parasitology, 86, 618-622.

Bullard, S. A., Frasca, S., Jr. and Benz, G. W. (2001) Gill lesions associated with *Erpocotyle tiburonis* (Monogenea: Hexabothriidae) on wild and aquarium-held bonnethead sharks (*Sphyrna tiburo*). Journal of Parasitology, 87, 972-977.

Bullard, S. A. and Overstreet, R. M. (2000) *Calicotyle californiensis* n. sp. and *Calicotyle urobati* n. sp. (Monogenea: Calicotylinae) from elasmobranchs in the Gulf of California. Journal of Parasitology, 86, 939-944.

Bullard, S. A. and Overstreet, R. M. (2002) Potential pathological effects of blood flukes (Digenea: Sanguinicolidae) on pen-reared marine fishes. *In* Proceedings of the fifty-third annual Gulf and Caribbean Fisheries Institute. (Ed. Creswell, R. L.) Fort Pierce, Florida, 10-25 pp.

Bullard, S. A., Payne, R. and Braswell, J. S. (in press). New genus with two new species of capsalid monogeneans from dasyatids in the Gulf of California. Journal of Parasitology.

Bunkley-Williams, L. and Williams, E. H., Jr. (1998) Isopods associated with fishes: a synopsis and corrections. Journal of Parasitology, 84, 893-896.

Burreson, E. M. (1995) Phylum Annelida: Hirudinea as vectors and disease agents. *In* Fish diseases and disorders, volume 1. Protozoan and metazoan infections. (Ed. Woo, P. T. K.) CAB International, Wallingford, United Kingdom, 599-629 pp.

Burreson, E. M. and Kearn, G. C. (2000) *Rhopalobdella japonica* n. gen., n. sp. (Hirudinea, Piscicolidae) from *Dasyatis akajei* (Chondrichthyes: Dasyatididae) in the northwestern Pacific. Journal of Parasitology, 86, 696-699.

Burreson, E. M. and Zwerner, D. E. (1982) The role of host biology, vector biology and temperature in the distribution of *Trypanoplasma bullocki* infections in the lower Chesapeake Bay. Journal of Parasitology, 68, 306-313.

Bush, A. O., Fernández, J. C., Esch, G. W. and Seed, J. R. (2001) Parasitism: the diversity and ecology of animal parasites. Cambridge University Press, Cambridge, United Kingdom, 566 pp.

Bush, A. O., Lafferty, K. D., Lotz, J. M. and Shostak, A. W. (1997) Parasitology meets ecology on its own terms: Margolis et al. revisited. Journal of Parasitology, 83, 575-583.

Bychowsky, B. E. (1961) Monogenetic trematodes, their classification and phylogeny. (Eds. Hargis, W. J. and Oustinoff, P. C.) American Institute of Biological Sciences, Washington, D.C., 626 pp. [English translation of original Russian volume]

Bykhovskaya-Pavlovskaya, I. E., Gusev, A. V., Dubinina, M. N., Izyumova, N. A., Smirnova, T. S., Sokolovskaya, I. L., Shtein, G. A., Shul'man, S. S. and Epshtein, G. A. (1964) Key to parasites of freshwater fish of the U.S.S.R. (Ed. Pavlovskii, E. N.) Israel Program for Scientific Translations, Jerusalem, Israel, 919 pp. [English translation of original Russian volume]

Caira, J. N. (1990) Metazoan parasites as indicators of elasmobranch biology. *In* Elasmobranchs as living resources: advances in the biology, ecology, systematics, and the status of the fisheries. (Eds. Pratt, H. L., Jr., Gruber, S. H. and Taniuchi, T.) United States Department of Commerce, National Oceanic and Atmospheric Association Technical Report 90, 71-96 pp.

Caira, J. N., Benz, G. W., Borucinska, J. and Kohler, N. E. (1997a) Pugnose eels, *Simenchelys parasiticus* (Synaphobranchidae) from the heart of a shortfin mako, *Isurus oxyrinchus* (Lamnidae). Environmental Biology of Fishes, 49, 139-144.

Caira, J. N. and Euzet, L. (2001) Age of association between the nurse shark, *Ginglymostoma cirratum*, and

tapeworms of the genus *Pedibothrium* (Tetraphyllidea: Onchobothriidae): implications from geography. Biological Journal of the Linnean Society, 72, 609-614.

Caira, J. N. and Jensen, K. (2001) An investigation of the co-evolutionary relationships between onchobothriid tapeworms and their elasmobranch hosts. International Journal for Parasitology, 31, 960-975.

Caira J. N., Jensen, K., Yamane, Y., Isobe, A. and Nagasawa, K. (1997b) On the tapeworms of *Megachasma pelagios*: description of a new genus and species of lecanicephalidean and additional information on the trypanorhynch *Mixodigma leptaleum*. *In* Biology of the megamouth shark. (Eds. Yano, K., Morrissey, J. F., Yabumoto, Y. and Nakaya, K.) Tokai University Press, Tokyo, Japan, 181-191 pp.

Caira, J. N. and Littlewood, D. T. J. (2001) Worms, Platyhelminthes. *In* Encyclopedia of biodiversity, volume 5. (Ed. Levine, S. A.) Academic Press, San Diego, California, 863-899 pp.

Caira, J. N. and Tracy, R. (2002) Two new species of *Yorkeria* (Tetraphyllidea: Onchobothriidae) from *Chiloscyllium punctatum* (Elasmobranchii: Hemiscyllidae) in Thailand. Journal of Parasitology, 88, 1172-1180.

Campbell, R. A. and Beveridge, I. (1994) 7. Order Trypanorhyncha. *In* Keys to cestode parasites of vertebrates. (Eds. Khalil, L. F., Jones, A. and Bray, R. A.) CAB International, Wallingford, United Kingdom, 51-148 pp.

Campbell, R. A. and Callahan, C. (1998) Histopathological reactions of the blue shark, *Prionace glauca*, to postlarvae of *Hepatoxylon trichuri* (Cestoda: Trypanorhyncha: Hepatoxylidae) in relationship to scolex morphology. Folia Parasitologica, Praha, 45, 47-52.

Cannon, L. R. G. and Lester, R. J. G. (1988) Two turbellarians parasitic in fish. Diseases of Aquatic Organisms, 5, 15-22.

Carvajal, J. and Dailey, M. D. (1975) Three new species of *Echeneibothrium* (Cestoda: Tetraphyllidea) from the skate, *Raja chilensis* Guichenot, 1848, with comments on mode of attachment and host specificity. Journal of Parasitology, 61, 89-94.

Cheung, P. (1993) Parasitic diseases of elasmobranchs. *In* Fish medicine. (Ed. Stoskopf, M. K.) W. B. Saunders, Philadelphia, Pennsylvania, 782-807 pp.

Cheung, P. J. and Nigrelli, R. F. (1983) *Dermophthiriodes pristidis* n. gen., n. sp. (Microbothriidae) from the skin and *Neoheterocotyle ruggierii* n. sp. (Monocotylidae) from the gills of the smalltooth sawfish, *Pristis pectinata*. Transactions of the American Microscopical Society, 102, 366-370.

Cheung, P. J., Nigrelli, R. F., Ruggieri, G. D. and Cilia, A. (1982) Treatment of skin lesions in captive lemon sharks, *Negaprion brevirostris* (Poey), caused by monogeneans (*Dermophthirius* sp.). Journal of Fish Diseases, 5, 167-170.

Cheung, P. J., Nigrelli, R. F., Ruggieri, G. D. and Crow, G. L. (1988) A new microbothriid (monogenean) causing skin lesion on the Pacific blacktip reef shark, *Carcharhinus melanopterus* (Quoy and Gaimard). Journal of Aquariculture and Aquatic Sciences, 5(2), 21-25.

Cheung, P. J. and Ruggieri, G. D. (1983) *Dermophthirius nigrellii* n. sp. (Monogenea: Microbothriidae), an ectoparasite from the skin of the lemon shark, *Negaprion brevirostris*. Transactions of the American Microscopical Society, 102, 129-134.

Cheung, P. and Whitaker, B. (1993) A new dendromonocotylinid (monogenean) from the skin of the roughtail stingray, *Dasyatis centroura* Mitchill. Journal of Aquariculture and Aquatic Sciences, 6, 63-68.

Chisholm, L. A., Hansknecht, T. J., Whittington, I. D. and Overstreet, R. M. (1997a) A revision of the Calicotylinae Monticelli, 1903 (Monogenea: Monocotylidae). Systematic Parasitology, 38, 159-183.

Chisholm, L. A., Wheeler, T. A. and Beverley-Burton, M. (1995) A phylogenetic analysis and revised classification of the Monocotylidae Taschenberg, 1879 (Monogenea). Systematic Parasitology, 32, 159-191.

Chisholm, L. A. and Whittington, I. D. (1995) A revision of *Dendromonocotyle* Hargis, 1955 (Monogenea: Monocotylidae) with a description of a new species from *Pastinachus sephen* (Forsskål) (Myliobatiformes: Dasyatidae) from the Great Barrier Reef, Australia. Journal of Natural History, 29, 1093-1119.

Chisholm, L. A. and Whittington, I. D. (1997b) A revision of *Neoheterocotyle* (Monogenea: Monocotylidae) with descriptions of the larvae of *N. rhinobatis* and *N. rhynchobatis* from Heron Island, Great Barrier Reef, Australia. International Journal for Parasitology, 27, 1041-1060.

Chisholm, L. A. and Whittington, I. D. (2000) Egg hatching in three species of monocotylid monogenean parasites from the shovelnose ray *Rhinobatos typus* at Heron Island, Australia. Parasitology, 121, 303-313.

Chisholm, L. A. and Whittington, I. D. (2002) Efficacy of praziquantel bath treatments for monogenean infections of the *Rhinobatos typus*. Journal of Aquatic Animal Health, 14, 230-234.

Chisholm, L. A. and Whittington, I. D. (2004) Two new species of *Dendromonocotyle* Hargis, 1955 (Monogenea: Monocotylidae) from the skin of *Taeniura meyeni* (Dasyatidae) and *Aetobatus narinari* (Myliobatidae) from aquaria in Queensland, Australia. Systematic Parasitology, 57, 221-228.

Chitwood, B. G. and Chitwood, M. B. (1950) Introduction to nematodology. University Park Press, Baltimore, Maryland, 334 pp.

Chitwood, M. and Lichtenfels, J. R. (1972) Identification of parasitic metazoa in tissue sections. Experimental Parasitology, 32, 407-519.

Cislo, P. R. and Caira, J. N. (1993) The parasite assemblage in the spiral intestine of the shark *Mustelus canis*. Journal of Parasitology, 79, 886-899.

Cohen, B. F. and Poore, G. C. B. (1994) A phylogeny of the Gnathiidae (Crustacea: Isopoda) with descriptions of new genera and species from south-eastern Australia. Memoires of the Museum of Victoria, 54, 271-397.

Combes, C. (2001) Parasitism: the ecology and evolution of intimate interactions. University of Chicago Press, Chicago, Illinois, 728 pp. [English translation of original French volume]

Compagno, L. J. V. (1984) Sharks of the world: Hexanchiformes to Lamniformes. Fishery Synopsis number 125, volume 4, part 1. Food and Agriculture Organization of the United Nations, Rome, Italy, 249 pp.

Compagno, L. J. V. (1999) Checklist of living elasmobranchs. *In* Sharks, skates, and rays. (Ed. Hamlett, W. C.) The Johns Hopkins University Press, Baltimore, Maryland, 471-498 pp.

Conder, G. A. (2002) Chemical control of animal-parasitic nematodes. *In* The biology of nematodes. (Ed. Lee, D. L.) Taylor and Francis, London, United Kingdom, 521-529 pp.

Conder, G. A. and Campbell, W. C. (1995) Chemotherapy of nematode infections of veterinary importance, with special reference to drug resistance. Advances in Parasitology, 35, 1-84.

Costello, M. J. (1993) Review of methods to control sea lice (Caligidae: Crustacea) infestations on salmon (*Salmo salar*) farms. *In* Pathogens of wild and farmed fish: sea lice. (Eds. Boxshall, G. A. and Defaye, D.) Ellis Horwood, New York, New York, 219-252 pp.

Cox, D. L., Walker, P. and Koob, T. J. (1999) Predation on eggs of the thorny skate. Transactions of the American Fisheries Society, 128, 380-384.

Credille, K. M., Johnson, L. K. and Reimschuessel, R. (1993) Parasitic meningoencephalitis in nurse sharks (*Ginglymostoma cirratum*). Journal of Wildlife Diseases 29, 502-506.

Cressey, R. F. (1976) The genus *Argulus* (Crustacea: Branchiura) of the United States. Water Pollution Control Research Series 18050 ELDO2/72, United States Environmental Protection Agency, Washington, D.C., 14 pp.

Cressey, R. F. (1978) Marine flora and fauna of the northeastern United States. Crustacea: Branchiura. National Oceanic and Atmospheric Association Technical Report, National Marine Fisheries Service Circular 413, United States Department of Commerce, Washington, D.C., 10 pp.

Cressey, R. F. and Lachner, E. A. (1970) The parasitic copepod diet and life history of diskfish (Echeneidae). Copeia, 1970, 310-318.

Cribb, T. H., Bray, R. A. and Littlewood, D. T. J. (2001a) The nature and evolution of the association between digeneans, molluscs and fishes. International Journal for Parasitology, 31, 997-1011.

Cribb, T. H., Bray, R. A., Littlewood, D. T. J., Pichelin, S. P. and Herniou, E. H. (2001b) The Digenea. *In* Interrelationships of the Platyhelminthes. (Eds. Littlewood, D. T. J. and Bray, R. A.) Taylor and Francis, New York, New York, 168-185 pp.

Cruz-Lacierda, E. R., Toledo, J. D., Tan-Fermin, J. D. and Burreson, E. M. (2000) Marine leech (*Zeylanicobdella arugamensis*) infestation in cultured orange-spotted grouper, *Epinephelus coioides*. Aquaculture, 185, 191-196.

Cunningham, J. T. (1887) *Stichocotyle nephropis*, a new trematode. Transactions of the Royal Society of Edinburgh, 32, 273-280.

Curran, S. and Caira, J. N. (1995) Attachment site specificity and the tapeworm assemblage in the spiral intestine of the blue shark (*Prionace glauca*). Journal of Parasitology, 81, 149-157.

Curran, S. S. and Overstreet, R. M. (2000) *Syncoelium vermilionensis* sp. n. (Hemiuroidea: Syncoeliidae) and new records for members of Azygiidae, Ptychogonimidae, and Syncoeliidae parasitizing elasmobranchs in the Gulf of California. *In* Metazoan parasites in the neotropics: a systematic and ecological perspective. (Eds. Salgado-Maldonado, G., García Aldrete, A. N. and Vidal-Martínez, V. M.) Instituto de Biología, U.N.A.M., México, 117-133 pp.

Curran, S. S., Overstreet, R. M., Benz, G. W. and Henningsen, A. D. (in preparation) *Pseudaustrobdella cairae* n. gen., n. sp. (Hirudinea: Piscicolidae) from banded guitarfish, *Zapteryx exasperata*, in the Gulf of California and Pacific Ocean. Journal of Parasitology.

Cusack, R. and Cone, D. K. (1986) A review of parasites as vectors of viral and bacterial diseases of fish. Journal of Fish Diseases, 9, 169-171.

Dailey, M. D. and Vogelbein, W. (1982) Mixodigmatidae, a new family of cestode (Trypanorhyncha) from a deep sea, planktivorous shark. Journal of Parasitology, 68, 145-149.

Dailey, M. D. and Vogelbein, W. (1990) *Clistobothrium carcharodoni* gen. et sp. n. (Cestoda: Tetraphyllidea) from the spiral valve of the great white shark (*Carcharodon carcharias*). Journal of the Helminthological Society of Washington, 57, 108-112.

Darwall, W., Costello, M. and Lysaght, S. (1992) Wrasse — how well do they work? Aquaculture Ireland, 50, 26-29.

Davies, A. J. (1982) Further studies on *Haemogregarina bigemina* Laveran & Mesnil, the marine fish *Blennius pholis* L., and the isopod *Gnathia maxillaris* Montagu. Journal of Protozoology, 29, 576-583.

Davies, A. J., Eiras, J. C. and Austin, R. T. E. (1993) The transmission of *Haemogregarina bigemina* Laveran & Mesnil (Apicomplexa: Adeleorina) between fishes in Portugal. *In* Diseases of fish and shellfish, Sixth International Conference, European Association of Fish Pathologists, Brest, France, 64 p.

Davies, A. J., Reed, C. C. and Smit, N. J. (2003) An unusual intraerythrocytic parasite of *Parablennius cornutus* from South Africa. Journal of Parasitology, 89, 913-917.

Davies, A. J. and Smit, N. J. (2001) The life cycle of *Haemogregarina bigemina* (Adeleina: Haemogregarinidae) in South African hosts. Folia Parasitologica, Praha, 48, 169-177.

Deardorff, T. L., Brooks, D. R. and Thorson, T. B. (1981) A new species of *Echinocephalus* (Nematoda: Gnathostomidae) from neotropical stingrays with comments on *E. diazi*. Journal of Parasitology, 67, 433-439.

Deardorff, T. L. and Overstreet, R. M. (1981) Larval *Hysterothylacium* (=*Thynnascaris*) (Nematoda: Anisakidae) from fishes and invertebrates in the Gulf of Mexico. Proceedings of the Helminthological Society of Washington, 48, 113-126.

Deets, G. B. (1987) Phylogenetic analysis and revision of *Kroeyerina* Wilson, 1932 (Siphonostomatoida: Kroyeriidae), copepods parasitic on chondrichthyans, with descriptions of four new species and the erection of a new genus, *Prokroyeria*. Canadian Journal of Zoology, 65, 2121-2148.

Deets, G. B. (1994) Copepod-chondrichthyan coevolution: a cladistic consideration. Ph.D. Dissertation, The University of British Columbia, Vancouver, British Columbia, Canada, 448 pp.

Deets, G. B. and Benz, G. W. (1986) *Eudactylinodes keratophagus* sp. nov., the first record of Eudactylinidae Kabata, 1979 (Copepoda: Siphonostomatoida) from horn sharks (*Heterodontus francisci* (Girard, 1854), and *H. mexicanus* Taylor and Castro-Aguirre, 1972). Canadian Journal of Zoology, 64, 2499-2502.

Deets, G. B. and Dojiri, M. (1990) *Dissonus pastinum* n. sp. (Siphonostomatoida: Dissonidae), a copepod parasitic on a horn shark from Japan. Beaufortia, 41(8), 49-54.

Deets, G. B. and Ho, J.-S. (1988) Phylogenetic analysis of the Eudactylinidae (Crustacea: Copepoda: Siphonostomatoida), with descriptions of two new genera. Proceedings of the Biological Society of Washington, 101, 317-339.

Delamare-Debouteville, C. and Nunes-Ruivo, L. (1952) Copépodes parasites des poissons de Banuyls (deuxième série). Vie et Milieu, 3, 292-300.

Dick, T. A. and Choudhury, A. (1995a) Cestoidea (Phylum Platyhelminthes). *In* Fish diseases and disorders, volume 1. Protozoan and metazoan infections. (Ed. Woo, P. T. K.) CAB International, Wallingford, United Kingdom, 391-414 pp.

Dick, T. A. and Choudhury, A. (1995b) Phylum Nematoda. *In* Fish diseases and disorders, volume 1. Protozoan and metazoan infections. (Ed. Woo, P. T. K.) CAB International, Wallingford, United Kingdom, 415-473 pp.

Didier, D. A. (1995) Phylogenetic systematics of extant chimaeroid fishes (Holocephali, Chimaeroidei). American Museum Novitates, 3119, 1-86.

Diebakate, C., Raibaut, A. and Kabata, Z. (1997) *Thamnocephalus cerebrinoxius* n. g., n. sp. (Copepoda: Sphyriidae), a parasite in the nasal capsules of *Leptocharias smithii* (Müller & Henle, 1839) (Pisces: Leptochariidae) off the coast of Senegal. Systematic Parasitology, 38, 231-235.

Dippenaar, S. M., Benz, G. W. and Olivier, P. A. S. (2000) *Kroyeria deetsi* n.sp. (Kroyeriidae: Siphonostomatoida), a parasitic copepod infecting gills of spinner sharks,

Carcharhinus brevipinna (Müller & Henle, 1839), in the Indian Ocean. African Zoology, 35, 185-192.

Dippenaar, S. M., Olivier, P. A. S. and Benz, G. W. (2004) *Schistobrachia jordaanae* n. sp. (Copepoda: Siphonostomatoida: Lernaeopodidae) from gill filaments of a diamond ray (*Gymnura natalensis*) captured in the Indian Ocean and a key to species of *Schistobrachia*, *Dendrapta* and *Brianella*. Journal of Parasitology, 90, 481-484.

Dojiri, M. and Deets, G. B. (1988) *Norkus cladocephalus*, new genus, new species (Siphonostomatoida: Sphyriidae), a copepod parasitic on an elasmobranch from southern California waters, with a phylogenetic analysis of the Sphyriidae. Journal of Crustacean Biology, 8, 679-687.

Dollfus, R. Ph. (1937a) Les trématodes Digenea des sélachiéns (plagiostomes) catalogue par hotes. Distribution géographique. Annales de Parasitologie Humaine et Comparée, 15, 57-73.

Dollfus, R. Ph. (1937b) Les trématodes Digenea des sélachiéns (plagiostomes) catalogue par hotes. Distribution géographique. (Suite). Annales de Parasitologie Humaine et Comparée, 15, 164-176.

Dollfus, R. Ph. (1937c) Les trématodes Digenea des sélachiéns (plagiostomes) catalogue par hotes. Distribution géographique. (Suite et fin). Annales de Parasitologie Humaine et Comparée, 15, 259-281.

Dollfus, R. Ph. (1937d) Trematodes de sélaciens et de cheloniens. Parasitologia Mauritanica Helmintha (III). Bulletin du Comité d'Etudes Historiques et Scientifiques de l'Afrique Occidentale Française, 19, 397-519.

Dollfus, R. Ph. (1958) Sur *Macraspis cristata* (E.-C. Faust et C.-C. Tang 1936) H.-W. Manter 1936 et sur une emendation nécessaire a ma définition de la famille des Aspidogastridæ (Trematoda). Annales de Parasitologie Humaine et Comparée, 33, 227-231.

Duston, J. and Cusack, R. R. (2002) Emamectin benzoate: an effective in-feed treatment against the gill parasite *Salmincola edwardsii* on brook trout. Aquaculture, 207, 1-9.

Ellis, E. P. and Watanabe, W. O. (1993) The effects of hyposalinity on eggs, juveniles and adults of the marine monogenean, *Neobenedenia melleni* treatment of ecto-parasitosis in seawater-cultured tilapia. Aquaculture, 117, 15-27.

El-Matbouli, M. and Hoffmann, R. W. (1991) Prevention of experimentally induced whirling disease in rainbow trout *Oncorhynchus tshawytscha* by fumagillin. Diseases of Aquatic Organisms, 10, 109-113.

Esch, G. W. and Fernández, J. C. (1993) A functional biology of parasitism. Chapman and Hall, London, United Kingdom, 337 pp.

Euzet, L. (1994a) 8. Order Tetraphyllidea. *In* Keys to cestode parasites of vertebrates. (Eds. Khalil, L. F., Jones, A. and Bray, R. A.) CAB International, Wallingford, United Kingdom, 149-194 pp.

Euzet, L. (1994b) 9. Order Lecanicephalidea. *In* Keys to cestode parasites of vertebrates. (Eds. Khalil, L. F., Jones, A. and Bray, R. A.) CAB International, Wallingford, United Kingdom, 195-204 pp.

Euzet, L. and Combes, C. (1998) The selection of habitats among the monogenea. International Journal for Parasitology, 28, 1645-1652.

Euzet, L. and Maillard, C. (1967) Parasites de poissons de mer ouest-africains, récoltés par J. Cadenat. VI. Monogènes de Sélaciens. Bulletin de l'Institut Fondamental d'Afrique Noire, 29, 1435-1494.

Euzet, L. and Raibaut, A. (1960) Le développement postlarvaire de *Squalonchocotyle torpedinis* (Price, 1942) (Monogenea, Hexabothriidae). Bulletin de la Société Neuchâteloise des Sciences Naturelles, 83, 101-108.

Fain, A. and Belpaire, C. (1985) A new mite (Acari, Anoetidae) parasitizing the gills of young eels *Anguilla anguilla* (L.). Bulletin and Annales de la Societe Royale Belge d'Entomologie, 121, 285-292.

Fain, A. and Lambrechts, L. (1985) A new anoetid mite parasitic in the swim-bladder of the aquarium fish *Pangasius sutchi*. Bulletin and Annales de la Societe Royale Belge d'Entomologie, 121, 119-126.

Fairfax, D. (1998) The basking shark in Scotland. Tuckwell Press, East Lothian, Scotland, 206 pp.

Faust, E. C. and Tang, C.-C. (1936) Notes on new aspidogastrid species, with a consideration of the phylogeny of the group. Parasitology, 28, 487-501.

Fernández, J. C., Villalba, C. S. and Alviña, A. (1986) Parasitos del pejegallo, *Callorhynchus callorhynchus* (L.), en Chile: aspectos biologicos y sistematicos. Biología Pesquera, 15, 63-73.

Fernández Ovies, C. L. (1995) Crustáceos parásitos sobre tiburones bentopelágicos del talud continental Asturiano. 2. *Anelasma squalicola* (Loven) (Cirripedia: Thoracica: Anelasmatidae). Boletino Ciencias Naturales R.I.D.E.A., 43, 7-14.

Fernholm, B. (1998) Hagfish systematics. *In* The biology of hagfishes. (Eds. Jórgensen, J. M., Lomholt, J. P., Weber, R. E. and Malte, H.) Chapman and Hall, London, United Kingdom, 33-44 pp.

Flescher, D. and Martini, F. H. (2002) Lampreys. Family Petromyzontidae. *In* Fishes of the Gulf of Maine, third edition. (Eds. Collette, B. B. and Klein-MacPhee, G.) Smithsonian Institution Press, Washington, D.C., 16-19 pp.

Gaines, J. L., Jr. and Rogers, W. A. (1972) Fish mortalities associated with *Goezia* sp. (Nematoda: Ascaroidea) in central Florida. *In* Proceedings of the twenty-fifth annual conference southeastern association of game and fish commissioners, (Ed. Webb, J. W.) Columbia, South Carolina, 496-497 pp.

Gibbons, L. M. (1986) SEM guide to the morphology of nematode parasites of vertebrates. CAB International, London, United Kingdom, 199 pp.

Gibbons, L. M. (2002) General organisation. *In* The biology of nematodes. (Ed. Lee, D. L.) Taylor and Francis, London, United Kingdom, 31-59 pp.

Gibson, D. I. (1987) Questions in digenean systematics and evolution. Parasitology, 95, 429-460.

Gibson, D. I. (1994) 3. Order Gyrocotylidea Poche, 1926. *In* Keys to cestode parasites of vertebrates. (Eds. Khalil, L. F., Jones, A. and Bray, R. A.) CAB International, Wallingford, United Kingdom, 11-13 pp.

Gibson, D. I. (1996) Trematoda. *In* Guide to the parasites of fishes of Canada, part IV. (Eds. Margolis, L. and Kabata, Z.) Department of Fisheries and Oceans, Ottawa, Canada, 1-373 pp.

Gibson, D. I. and Bray R. A. (1979) The Hemiuroidea: terminology, systematics, and evolution. Bulletin of the British Museum (Natural History) (Zoology Series), 32, 167-245.

Gibson, D. I. and Bray R. A. (1986) The Hemiuridae (Digenea) of fishes from the north-east Atlantic. Bulletin of the British Museum (Natural History), 51, 1-125.

Gibson, D. I. and Bray, R. A. (1994) The evolutionary expansion and host-parasite relationships of the Digenea. International Journal of Parasitology, 24, 1213-1226.

Gibson, D. I. and Chinabut, S. (1984) *Rohdella siamensis* gen. et sp. nov. (Aspidogastridae: Rohdellinae subfam.nov.) from freshwater fishes in Thailand, with a reorganization of the classification of the subclass Aspidogastrea. Parasitology, 88, 383-393.

Gibson, D. I., Jones, A. and Bray, R. A. (Eds.) (2002) Keys to the Trematoda, volume 1. CAB International, Wallingford, United Kingdom, 544 pp.

Gilbert, M. A. and Granath, W. O., Jr. (2003) Whirling disease of salmonid fish: life cycle, biology, and disease. Journal of Parasitology, 89, 658-667.

Gill, H. S., Renaud, C. B., Chapleau, F., Mayden, R. L. and Potter, I. C. (2003) Phylogeny of living parasitic lampreys (Petromyzontiformes) based on morphological data. Copeia, 2003, 687-703.

Ginetsinskaya, T. A. (1961) The life cycles of fish helminths and the biology of their larval stages. *In* Parasitology of fishes. (Eds. Dogiel, V. A., Petrushevski, G. K. and Polyanski, Yu. I.) Oliver and Boyd, Edinburg, United Kingdom, 140-179 pp. [English translation of original Russian volume]

Gledhill, T. (1985) Water mites – predators and parasites. Freshwater Biological Association, Annual Report, 53, 45-59.

Goldstein, R. J. and Wells, H. W. (1966) Note on the incidence of a marine leech, *Branchellion ravenellii*. Journal of Parasitology, 52, 690.

Golvan, Y. J. (1969) Systematique des Acanthocephales. Premiere partie. L'ordre des Palaeacanthocephala Meyer 1931. Premier fascicule. La super-famille des Echinorhynchoidea (Cobbold 1876) Golvan et Houin 1963. Memoires du Museum National d'Histoire Naturelle, série A, Zoologie, 57, 1-373.

Golvan, Y. J., Garcia-Rodrigo, A. and Díaz-Ungría, C. (1964) *Megapriapus ungriai* (Garcia-Rodrigo, 1960) n. gen. (Palaeacanthocephala) parasite d'une Pastenague d'eau douce du Vénézuéla (*Pomatotrygon hystrix*). Annales de Parasitologie Humain et Comparee, 39, 53-59.

Goode, G. B. and Bean, T. H. (1896) Oceanic ichthyology, a treatise on the deep-sea and pelagic fishes of the world, based chiefly upon the collections made by the steamers *Blake, Albatross,* and *Fish Hawk* in the northwestern Atlantic, with an Atlas containing 417 figures. United States National Museum, Special Bulletin, 2, 1-553.

Goven, B. A., Gilbert, J. P. and Gratzek, J. B. (1980) Apparent drug resistance to the organophosphate dimethyl (2,2,2-trichloro-1-hydroxyethyl) phosphonate by monogenetic trematodes. Journal of Wildlife Diseases, 16, 343-346.

Grant, A. N. and Treasurer, J. W. (1993) The effects of fallowing on caligid infestations on farmed Atlantic salmon (*Salmo salar* L.) in Scotland. *In* Pathogens of wild and farmed fish: sea lice. (Eds. Boxshall, G. A. and Defaye, D.) Ellis Horwood, New York, New York, 255-260 pp.

Gratzek, J. B., Shotts, E. B., Jr. and Dawe, D. L. (1992) Infectious diseases and parasites of freshwater ornamental fish. *In* Aquariology: the science of fish health management. (Eds. Gratzek, J. B. and Matthews, J. R.) Tetra Press, Morris Plains, New Jersey, 228-274 pp.

Grave, K., Engelstad, M. and Söli, N. E. (1991) Utilization of dichlorvos and trichlorfon in salmon farming in Norway during 1981-1988. Acta Veterinaria Scandinavica, 32, 1-7.

Gresty, K. A., Boxshall, G. A. and Nagasawa, K. (1993) The fine structure and function of the cephalic appendages of the branchiuran parasite, *Argulus japonicus* Thiele. Philosophical Transactions of the Royal Society of London (B), 339, 119-135.

Grimes, D. J., Brayton, P., Colwell, R. R. and Gruber, S. H. (1985a) Vibrios as autochthonous flora of neritic sharks. Systematic and Applied Microbiology, 6, 221-226.

Grimes, D. J., Gruber, S. A. and May, E. B. (1985b) Experimental infection of lemon sharks, *Negaprion brevirostris* (Poey), with *Vibrio* species. Journal of Fish Diseases, 8, 173-180.

Gruber, S. H. (1980) Keeping sharks in captivity. Journal of Aquariculture, 1, 6-14.

Gruber, S. H. and Keyes, R. S. (1981) Keeping sharks for research. *In* Aquarium systems. (Ed. Hawkins, A. D.) Academic Press, London, United Kingdom, 373-402 pp.

Grutter, A. S. (1996) Parasite removal rates by the cleaner wrasse *Labroides dimidiatus*. Marine Ecology Progress Series, 130, 61-70.

Grutter, A. S. (1997) Spatio-temporal variation and feeding selectivity in the diet of the cleaner fish *Labroides dimidiatus*. Copeia, 1997, 346-355.

Grutter, A. S. (2003) Feeding ecology of the fish ectoparasite *Gnathia* sp. (Crustacea: Isopoda) from the Great Barrier Reef, and its implications for fish cleaning behaviour. Marine Ecology Progress Series, 259, 295-302.

Grutter, A. S. and Poulin, R. (1998) Intraspecific and interspecific relationships between host size and the abundance of parasitic larval gnathiid isopods on coral reef fishes. Marine Ecology Progress Series, 164, 263-271.

Halliday, R. G. (1991) Marine distribution of the sea lamprey (*Petromyzon marinus*) in the northwest Atlantic. Canadian Journal of Fisheries and Aquatic Science, 48, 832-842.

Harding, J. P. (1966) Myodocopan ostracods from the gills and nostrils of fishes. *In* Contemporary studies in marine science. (Ed. Barnes, H.) George Allen and Unwin, London, United Kingdom, 369-374 pp.

Hardisty, M. W. (1979) Biology of cyclostomes. Chapman and Hall, London, United Kingdom, 428 pp.

Hare, G. M. and Burt, M. D. B. (1975) Identification, host sites and biology of parasites infecting juvenile Atlantic salmon (*Salmo salar*) in the Miramichi River system, New Brunswick. Fisheries and Marine Service Research and Development Technical Report, 581, 1-34.

Hargis, W. J., Jr. (1955) Monogenetic trematodes of Gulf of Mexico fishes. Part V. The superfamily Capsaloidea. Transactions of the American Microscopical Society, 74, 203-225.

Hargis, W. J., Jr. (1958) The fish parasite *Argulus laticauda* as a fortuitous human epizoon. Journal of Parasitology, 44, 45.

Hart, J. L. (1973) Pacific fishes of Canada. Bulletin 180, Fisheries Research Board of Canada, Ottawa, Canada, 740 pp.

Health and Safety Executive (1987) Biological monitoring of workers exposed to organophosphorus pesticides. Health Safety Executive Guidance Note MS17, Department of the Environment, London, United Kingdom.

Healy, C. J., Scholz, T. and Caira, J. N. (2001) *Erudituncus* n. gen. (Tetraphyllidae: Onchobothriidae) with a redescription of *E. musteli* (Yamaguti, 1952) n. comb. and comments on its hook homologies. Journal of Parasitology, 87, 833-837.

Hedrick, R. P., Groff, J. M., Foley, P. and McDowell, T. (1988) Oral administration of Fumagillin DCH protects chinook salmon *Oncorhynchus tshawytscha* from experimentally-induced proliferative kidney disease. Diseases of Aquatic Organisms, 4, 165-168.

Helfman, G. S., Collette, B. B. and Facey, D. E. (1997) The diversity of fishes. Blackwell Scientific, Malden, Massachusetts, 528 pp.

Hendrix, S. S. (1994) Marine flora and fauna of the eastern United States, Platyhelminthes: Monogenea. (Ed. Orr, J. W.) United States Department of Commerce, National Oceanic and Atmospheric Association, National Marine Fisheries Service Technical Report, 121, 1-107.

Hendrix, S. S. and Overstreet, R. M. (1977) Marine aspidogastrids (Trematoda) from fishes in the northern Gulf of Mexico. Journal of Parasitology, 63, 810-817.

Heupel, M. R. and Bennett, M. B. (1996) A myxosporean parasite (Myxosporea: Multivalvulida) in the skeletal muscle of epaulette sharks, *Hemiscyllium ocellatum* (Bonnaterre), from the Great Barrier Reef. Journal of Fish Diseases, 19, 189-191.

Heupel, M. R. and Bennett, M. B. (1999) The occurrence, distribution and pathology associated with gnathiid

isopod larvae infecting the epaulette shark, *Hemiscyllium ocellatum*. International Journal for Parasitology, 29, 321-330.

Hewitt, G. C. (1968) Some New Zealand parasitic Copepoda of the family Anthosomidae. Zoology Publications from Victoria University of Wellington, 47, 1-31.

Hewitt, G. C. (1979) Eight species of parasitic copepods on a white shark. New Zealand Journal of Marine and Freshwater Research, 13, 171.

Hickling, C. F. (1963) On the small deep-sea shark *Etmopterus spinax* L., and its cirripede parasite *Anelasma squalicola* (Lovén). Journal of the Linnaean Society, Zoology, 45, 2-24.

Hoberg, E. P., Brooks, D. R., Molina-Ureña, H. and Erbe, E. (1998) *Echinocephalus janzeni* n. sp. (Nematoda: Gnathostomatidae) in *Himantura pacifica* (Chondrichthyes: Myliobatiformes) from the Pacific coast of Costa Rica and Mexico, with historical biogeographic analysis of the genus. Journal of Parasitology, 84, 571-581.

Honma, Y. and Chiba, A. (1991) Pathological changes in the branchial chamber wall of stingrays, *Dasyatis* spp., associated with the presence of juvenile gnathiids (Isopoda, Crustacea). Gyobyo Kenkyu, 26, 9-16.

Honma, Y., Tsunaki, S., Chiba, A. and Ho, J.-S. (1991) Histological studies on the juvenile gnathiid (Isopoda, Crustacea) parasitic on the branchial chamber wall of the stingray, *Dasyatis akajei*, in the Sea of Japan. Report of the Sado Marine Biological Station, Niigata University, 21, 37-47.

Horton, T. and Okamura, B. (2001) Cymothoid isopod parasites in aquaculture— a review and case study of a Turkish sea bass (*Dicentrarchus labrax*) and sea bream (*Sparus auratus*) farm. Diseases of Aquatic Organisms, 46, 181-188.

Horton, T. and Okamura, B. (2003) Post-haemorrhagic anaemia in sea bass, *Dicentrarchus labrax* (L.), caused by blood feeding of *Ceratothoa oestroides* (Isopoda: Cymthoidae). Journal of Fish Diseases, 26, 401-406.

Humes, A. G. and Gooding, R. U. (1964) A method for studying the external anatomy of copepods. Crustaceana, 6, 238-240.

Huys, R. and Boxshall, G. A. (1991) Copepod evolution. The Ray Society, London, United Kingdom, 468 pp.

Ivanov, A. V. (1952) Morphology of *Udonella caligorum* Johnston, 1835, and the position of Udonellidae in the systematics of platyhelminths. Parazitologicheskii Sbornik, 14, 112-163. [English translation, 1981, edited by Simmons, J. E., Hargis, W. J. and Zwerner, D. E., University of California at Berkeley, Berkeley, California]

Ivanov, V. A. and Brooks, D. R. (2002) *Calliobothrium* spp. (Eucestoda: Tetraphyllidea: Onchobothriidea) in *Mustelus schmitti* (Chondrichthyes: Carcharhiniformes) from Argentina and Uruguay. Journal of Parasitology, 88, 1200-1213.

Ivanov, V. A. and Campbell, R. A. (2002) *Notomegarhynchus navonae* n. gen. and n. sp. (Eucestoda: Tetraphyllidea), from skates (Rajidae: Arhynchobatinae) in the southern hemisphere. Journal of Parasitology, 88, 340-349.

Iwama, G. and Nakanishi, T. (Eds.) (1996) The fish immune system: organism, pathogen, and environment. Academic Press, San Diego, California, 380 pp.

Jägerskiöld, L. A. (1899) Uber den bau von *Macraspis elegans* Olsson. Öfversigt af Kongelige Vetenskaps-Akademiens Förhandlingar, 56, 197-214.

Jahn, A. E. and Haedrich, R. L. (1987) Notes on the pelagic squaloid shark *Isistius brasiliensis*. Biological Oceanography, 5, 297-309.

Jameson, A. P. (1929) Myxosporidia from California fishes. Journal of Parasitology, 16, 59-68.

Jenkins, P. G., Grayson, T. H., Hone, J. V., Wrathmell, A. B., Gilpin, M. L., Harris, J. E. and Munn, C. B. (1993) The extraction and analysis of potential candidate vaccine antigens from the salmon louse *Lepeophtheirus salmonis* (Krøyer, 1837). *In* Pathogens of wild and farmed fish: sea lice. (Eds. Boxshall, G. A. and Defaye, D.) Ellis Horwood, New York, New York, 311-322 pp.

Jensen, K. (2001) Four new genera and five new species of lecanicephalideans (Cestoda: Lecanicephalidea) from elasmobranchs in the Gulf of California, Mexico. Journal of Parasitology, 87, 845-861.

Johnson, K. A. and Heindel, J. A. (2001) Efficacy of manual removal and ivermectin gavage for control of *Salmincola californiensis* (Wilson) infestation in chinook salmon, *Oncorhynchus tschawytscha* (Walbaum), captive broodstocks. Journal of Fish Diseases, 24, 197-203.

Johnston, T. H. and Mawson, P. M. (1943) Some nematodes from Australian elasmobranchs. Transactions of the Royal Society of South Australia, 67, 187-190.

Jones, A., Bray, R. A. and Khalil, L. F. (1994) 1. Key to the orders of the Cestoda. *In* Keys to cestode parasites of vertebrates. (Eds. Khalil, L. F., Jones, A. and Bray, R. A.) CAB International, Wallingford, United Kingdom, 1-2 pp.

Jones, E. C. (1971) *Isistius brasiliensis*, a squaloid shark, the probable cause of crater wounds on fishes and cetaceans. Fisheries Bulletin, 69, 791-798.

Jones, M. W., Sommerville, C. and Wootten, R. (1992) Reduced sensitivity of the salmon louse, *Lepeophtheirus salmonis*, to the organophosphate dichlorvos. Journal of Fish Diseases, 15, 197-202.

Kabata, Z. (1970) Crustaceans as enemies of fishes. *In* Diseases of fishes. (Eds. Snieszko, S. F. and Axelrod, H. R.) Tropical Fish Hobbiest Publications, Jersey City, New Jersey, 1-171 pp.

Kabata, Z. (1973) Distribution of *Udonella caligorum* Johnston, 1835 (Monogenea: Udonellidae) on *Caligus elongatus* Nordmann, 1832 (Copepoda: Caligidae). Journal of the Fisheries Research Board of Canada, 30, 1793-1798.

Kabata, Z. (1979) Parasitic Copepoda of British fishes. The Ray Society, London, United Kingdom, 468 pp.

Kabata, Z. (1984) Diseases caused by metazoans: crustaceans. *In* Diseases of marine animals, volume 4. (Ed. Kinne, O.) Biologische Anstalt Helgoland, Hamburg, Germany, 321-399 pp.

Kabata, Z. (1988) Copepoda and Branchiura. *In* Guide to the parasites of fishes of Canada, part II. Crustacea. (Eds. Margolis, L. and Kabata, Z.) Department of Fisheries and Oceans, Ottawa, Canada, 3-127 pp.

Kabata, Z. and Cousens, B. (1973) Life cycle of *Salmincola californiensis* (Dana, 1852) (Copepoda: Lernaeopodidae). Journal of the Fisheries Research Board of Canada, 30, 881-903.

Kearn, G. C. (1965) The biology of *Leptocotyle minor*, a skin parasite of the dogfish, *Scyliorhinus canicula*. Parasitology, 55, 473-480.

Kearn, G. C. (1967) The life-cycle and larval development of some acanthocotylids (Monogenea) from Plymouth rays. Parasitology, 57, 157-167.

Kearn, G. C. (1978) Early development and microhabitat of the monogenean *Horricauda rhinobatidis*, with observations on the related *Troglocephalus rhinobatidis*, from *Rhinobatos batillum* from Queensland, Australia. International Journal for Parasitology, 8, 305-311.

Kearn, G. C. (1986) The eggs of monogeneans. Advances in Parasitology, 25, 175-273.

Kearn, G. C. (1987) The site of development of the monogenean *Calicotyle kröyeri*, a parasite of rays. Journal of the Marine Biological Association of the United Kingdom, 67, 77-87.

Kearn, G. C. (1994) Evolutionary expansion of the Monogenea. International Journal for Parasitology, 24, 1227-1271.

Kearn, G. C. (1998) Parasitism and the platyhelminthes. Chapman and Hall, London, United Kingdom, 544 pp.

Kearn, G. C. and Gowing, R. (1990) Vestigial marginal hooklets in the oncomiracidium of the microbothriid monogenean *Leptocotyle minor*. Journal of Helminthology, 64, 340-342.

Kearn, G. C., Ogawa, K. and Maeno, Y. (1992) Egg production, the oncomiracidium and larval development of *Benedenia seriolae*, a skin parasite of the yellowtail, *Seriola quinqueradiata*, in Japan. Publications of the Seto Marine Biological Laboratory, 35, 351-362.

Kearn, G. C. and Whittington, I. D. (1991) Swimming in a sub-adult monogenean of the genus *Entobdella*. International Journal for Parasitology, 21, 739-741.

Keeney, D. B. and Campbell, R. A. 2001. *Grillotia borealis* sp. n. (Cestoda: Trypanorhyncha) from five species of *Bathyraja* (Rajiformes: Arhynchobatidae) in the North Pacific Ocean with comments on parasite enteric distribution. Folia Parasitologica, Praha, 48, 21-29.

Kensley, B. and Schotte, M. (1989) Guide to the marine isopod crustaceans of the Caribbean. Smithsonian Institution Press, Washington, D.C., 308 pp.

Kent, M. L. (1992) Diseases of seawater netpen-reared salmonid fishes in the Pacific Northwest. Canadian Special Publication of Fisheries and Aquatic Sciences, 116, 1-76.

Kent, M. L., Andree, K. B., Bartholomew, J. L., El-Matbouli, M., Desser, S. S., Devlin, R. H., Feist, S. W., Hedrick, R. P., Hoffmann, R. W., Khattra, J., Hallett, S. L., Lester, R. J. G., Longshaw, M., Palenzeula, O., Siddall, M. E. and Xiao, C. (2001) Recent advances in our knowledge of the Myxozoa. Journal of Eukaryotic Microbiology, 48, 395-413.

Kent, M. L., Margolis, L. and Corliss, J. O. (1994) The demise of a class of protists: taxonomic and nomenclatural revisions proposed for the protist phylum Myxozoa Grassé, 1970. Canadian Journal of Zoology, 72, 932-937.

Kent, M. L., Margolis, L. and Fournie, J. W. (1991) A new eye disease in pen-reared chinook caused by metacestodes of *Gilquinia squali* (Trypanoryncha). Journal of Aquatic Animal Health, 3, 134-140.

Kent, M. L. and Olson, A. C., Jr. (1986) Interrelationships of a parasitic turbellarian, (*Paravortex* sp.) (Graffillidae, Rhabdocoela) and its marine fish hosts. Fish Pathology, 21, 65-72.

Khalil, L. F. (1994) 6. Order Diphyllidea. *In* Keys to cestode parasites of vertebrates. (Eds. Khalil, L. F., Jones, A. and Bray, R. A.) CAB International, Wallingford, United Kingdom, 45-49 pp.

Klitgaard, A. B. (1991) *Gnathia abyssorum* (G. O. Sars, 1872) (Crustacea, Isopoda) associated with sponges. Sarsia, 76, 33-40.

Knoff, M., São Clemente, S. C., Pinto, R. M. and Gomes, D. C. (2001a) Nematodes of elasmobranch fishes from the southern coast of Brazil. Memorias do Instituto Oswaldo Cruz, Brazil, 96, 81-87.

Knoff, M., São Clemente, S. C., Pinto, R. M. and Gomes, D. C. (2001b) Digenea and Acanthocephala of elasmobranch fishes from the southern coast of Brazil. Memorias do Instituto Oswaldo Cruz, Brazil, 96, 1095-1101.

Kolipinski, M. C. (1969) Gar infested by *Argulus* in the Everglades. Quarterly Journal of the Florida Academy of Sciences, 32, 39-49.

Ktari, M. H. (1977) Le parasitisme d'*Echineis naucrates* L. (poisson-téléostéen) par deux monogènes (Monopisthocotylea) du genre *Dionchus*: *D. agassizi* Goto, 1899 et *D. remorae* Mac Callum, 1916. Excerta Parasitológica en Memoria del doctor Eduardo Caballero y Caballero Instituto de Biología, Universidad Nacional Autónoma de Mexico, Publicaciones Especiales, 4, 61-67.

Kudo, R. (1920) Studies on Myxosporidia. A synopsis of genera and species of Myxosporidia. Illinois Biological Monographs, 5, 1-265.

Kudo, R. (1933) A taxonomic consideration of Myxosporidia. Transactions of the American Microscopical Society, 52, 195-216.

Kurochkin, Y. V. (1985) Applied and scientific aspects of marine parasitology. *In* Parasitology and pathology of marine organisms of the world ocean. (Ed. Hargis, W. J., Jr.) United States Department of Commerce, National Oceanic and Atmospheric Association Technical Report, National Marine Fisheries Service 25, 15-18 pp.

LaMarre, E. and Cochran, P. A. (1992) Lack of host species selection by the exotic parasitic crustacean, *Argulus japonicus*. Journal of Freshwater Ecology, 7, 77-80.

Lampton, C. (1995) Sharks, rays, and eels. Western Publishing Company, Racine, Wisconsin, 36 pp.

Lawler, A. R. (1981) Zoogeography and host-specificity of the superfamily Capsaloidea Price, 1936 (Monogenea: Monopisthocotylea): an evaluation of the host-parasite locality records of the superfamily Capsaloidea Price, 1936, and their utility in determinations of host-specificity and zoogeography. Special Papers in Marine Science number 6, Virginia Institute of Marine Science, Gloucester Point, Virginia, 650 pp.

Layman, E. M. 1930. Parasitic worms from the fishes of Peter the Great Bay. Bulletin of the Pacific Scientific Fisheries Research Station, 3, 1-120.

Le Boeuf, B. J., McCosker, J. E. and Hewitt, J. (1987) Crater wounds on northern elephant seals: the cookiecutter shark strikes again. Fishery Bulletin, 85, 387-392.

Lester, R. J. G. and Roubal, F. R. (1995) Phylum Arthropoda. *In* Fish diseases and disorders, volume 1. Protozoan and metazoan infections. (Ed. Woo, P. T. K.) CAB International, Wallingford, United Kingdom, 475-598 pp.

Lincoln, R., Boxshall, G. and Clark, P. (1998) A dictionary of ecology, evolution and systematics, second edition. Cambridge University Press, Cambridge, United Kingdom, 361 pp.

Linton, E. (1900) Fish parasites collected at Woods Hole in 1898. United States Fish Commission Bulletin, 1899, 267-304.

Linton, E. (1940) Trematodes from fishes, mainly from the Woods Hole region. Proceedings of the United States National Museum, 88(3078), 1-172.

Littlewood, D. T. J. and Bray, R. A. (Eds.) (2001) Interrelationships of the Platyhelminthes. Taylor and Francis, New York, New York, 356 pp.

Llewellyn, J. (1960) Amphibdellid (Monogenean) parasites of electric rays (Torpedinidae). Journal of the Marine Biological Association of the United Kingdom, 39, 561-589.

Llewellyn, J. (1963) Larvae and larval development in monogeneans. Advances in Parasitology, 1, 287-326.

Llewellyn, L. C. (1966) Pontobdellinae (Piscicolidae: Hirudinea) in the British Museum (Natural History) with a review of the subfamily. Bulletin of the British Museum (Natural History), Zoology, 14, 391-439.

Lom, J. and Dyková, I. (1995) Myxosporea (Phylum Myxozoa). *In* Fish diseases and disorders, volume 1. Protozoan and metazoan infections. (Ed. Woo, P. T. K.) CAB International, Wallingford, United Kingdom, 97-148 pp.

Long, D. J. and Waggoner, B. M. (1993) The ectoparasitic barnacle *Anelasma* (Cirripedia, Thoracica, Lepadomorpha) on the shark *Centroscyllium nigrum* (Chondrichthyes, Squalidae) from the Pacific sub-Antarctic. Systematic Parasitology, 26, 133-136.

Love, M. S. and Moser, M. (1983) A checklist of parasites of California, Oregon, and Washington marine and estuarine fishes. National Oceanic and Atmospheric

Association Technical Report, National Marine Fisheries Service SSRF-777, United States Department of Commerce, Washington, D.C., 576 pp.

Lowers, J. M. and Bartholomew, J. L. (2003) Detection of myxozoan parasites in oligochaetes imported as food for ornamental fish. Journal of Parasitology, 89, 84-91.

Lund, R. and Grogan, E. D. (1997) Relationships of the Chimaeriformes and the basal radiation of the Chondrichthyes. Reviews in Fish Biology, 7, 65-123.

MacCallum, G. A. (1926) Deux nouveaux trématodes parasites de *Carcharhinus commersonii*: *Philura orata* et *Dermophthirius carcharhini*. Annales de Parasitologie, 4, 162-171.

MacCallum, G. A. (1925) Eggs of a new species of nematoid worm from a shark. Proceedings of the United States National Museum, 67(2588), 1-2.

MacCallum, G. A. (1926) Nematode eggs from the gill region of a shark, *Carcharhinus milberti*. Proceedings of the United States National Museum, 70 (2655), 1-2.

Macdonald, S. (1974) Host skin mucus as a hatching stimulant in *Acanthocotyle lobianchi*, a monogenean from the skin of *Raja* spp. Parasitology, 68, 331-338.

Macdonald, S. and Llewellyn, J. (1980) Reproduction in *Acanthocotyle greeni* n. sp. (Monogenea) from the skin of *Raja* spp. at Plymouth. Journal of the Marine Biological Association of the United Kingdom, 60, 81-88.

Machida, M. and Araki, J. (1992) Three aspidogastrean trematodes from marine fishes of Japan. Bulletin of the National Science Museum, Tokyo, series A, 18, 51-55.

MacKenzie, K. (1964) *Stichocotyle nephropis* Cunningham, 1887 (Trematoda) in Scottish waters. Annals and Magazine of Natural History, 6, 505-506.

Mann, K. H. (1962) Leeches (Hirudinea): their structure, physiology, ecology and embryology. Pergamon Press, New York, New York, 201 pp.

Manter, H. W. (1931) Some digenetic trematodes of marine fishes of Beaufort, North Carolina. Parasitology, 23, 396-411.

Manter, H. W. (1954) Some digenetic trematodes from fishes of New Zealand. Transactions of the Royal Society of New Zealand, 82, 475-568.

Manter, H. W. (1955) Two new monogenetic trematodes from elephant fishes (*Callorhynchus*) from South Africa and New Zealand. *In* Essays in the natural sciences in honor of Captain Alan Hancock. University of Southern California Press, Los Angeles, California, 211-220 pp.

Margolis, L. (1970) Nematode diseases of marine fishes. *In* A symposium on diseases of fishes and shellfishes. (Ed. Snieszko, S. F.) American Fisheries Society, Special Publication 5, Washington, D.C., 190-208 pp.

Marques, F. P. L. and Brooks, D. R. (2003) Taxonomic revision of *Rhinebothroides* (Eucestoda: Tetraphyllidea: Phyllobothriidae), parasites of neotropical freshwater stingrays (Rajiformes: Myliobatoidei: Potamotrygonidae). Journal of Parasitology, 89, 994-1017.

Marques, F. P. L., Brooks, D. R. and Lasso, C. A. (2001) *Anindobothrium* n. gen. (Eucestoda: Tetraphyllidea) inhabiting marine and freshwater potamotrygonid stingrays. Journal of Parasitology, 87, 666-672.

Martin, M. F. (1932) On the morphology and classification of *Argulus* (Crustacea). Proceedings of the Zoological Society, 50, 771-806.

Martini, F. H. and Flescher, D. (2002) Hagfishes. Family Myxinidae. *In* Fishes of the Gulf of Maine, third edition. (Eds. Collette, B. B. and Klein-MacPhee, G.) Smithsonian Institution Press, Washington, D.C., 9-16 pp.

Mattis, T. E. (1986) Development of two tetrarhynchidean cestodes from the northern Gulf of Mexico. Ph.D. Dissertation, The University of Southern Mississippi, Hattiesburg, Mississippi, 172 pp.

McAndrew, K. J., Sommerville, C., Wootten, R. and Bron J. E. (1998) The effects of hydrogen peroxide treatments on different life-cycle stages of the salmon louse, *Lepeophtheirus salmonis* (Krøyer, 1837). Journal of Fish Diseases, 21, 221-228.

McKenzie, V. J. and Caira, J. N. (1998) Three new genera and species of tapeworms from the longnose sawshark, *Pristiophorus cirratus*, with comments on their modes of attachment to the spiral intestine. Journal of Parasitology, 84, 409-421.

McVicar, A. H. and Fletcher, T. C. (1970) Serum factors in *Raja radiata* toxic to *Acanthobothrium quadripartitum* (Cestoda: Tetraphyllidea), a parasite specific to *R. naevus*. Parasitology, 61, 55-63.

Meehean, O. L. (1940) A review of the parasitic Crustacea of the genus *Argulus* in the collection of the United States National Museum. Proceedings of the United States National Museum, 88(3087), 459-522.

Minchin, D. and Jackson, D. (1993) *Udonella caligorum* Johnston, 1835 (Platyhelminthes: Udonellidae) associated with caligid copepods on farmed salmon. *In* Pathogens of wild and farmed fish: sea lice. (Eds. Boxshall, G. A. and Defaye, D.) Ellis Horwood, New York, New York, 346-355 pp.

Molnár, K., Baska, F. and Szekely, C. (1987) Fumagillin, an efficacious drug against renal sphaerosporosis of the common carp *Cyprinus carpio*. Diseases of Aquatic Organisms, 2, 187-190.

Molnar, K and Szekely, C. (1998) Occurrence of skrjabillanid nematodes in fishes of Hungary and in the intermediate host, *Argulus foliaceus*. Acta Veterinaria Hungarica, 46, 451-463.

Monod, T. (1926) Les Gnathiidae. Mémoires de la Société des Sciences Naturelles du Maroc, 13, 1-667.

Monod, T. (1928) Les argulidés du Musée do Congo. Revue de Zoologie et de Botanique Africaines, 16, 242-274.

Moravec, F. (1978) First record of *Molnaria erythrophthalmi* larvae in the intermediate host in Czechoslovakia. Folia Parasitologica, Praha, 25, 141-142.

Moravec, F. (1987) Revision of capillariid nematodes (subfamily Capillariinae) parasitic in fishes. Studije Ceskoslovenská Akademie Ved, 3, 1-141.

Moravec, F., Borucinska, J. D. and Frasca, S., Jr. (2001) *Pancreatonema americanum* sp. nov. (Nematoda, Habdochonidae) from the pancreatic duct of the dogfish shark, *Squalus acanthias*, from the coast of Massachusetts, USA. Acta Parasitologica, 46, 293-298.

Moravec, F. and Little, M. D. (1988) *Granulinema* gen. n. a new dracunculoid genus with two new species (*G. carcharhini* sp. n. and *G. simile* sp. n.) from the bull shark, *Carcharhinus leucas* (Valenciennes), from Louisiana, USA. Folia Parasitologica, Praha, 35, 113-120.

Moravec, F. and Nagasawa, K. (2000) Two remarkable nematodes from sharks in Japan. Journal of Natural History, 34, 1-13.

Moravec, F., Vidal-Martinez, V. and Aquirre-Macedo, L. (1999) Branchiurids (*Argulus*) as intermediate hosts of the daniconematid nematode *Mexiconema cichlasomae*. Folia Parasitologica, Praha, 46, 79.

Moreira, P. S. and Sadowsky, V. (1978) An annotated bibliography of parasitic isopoda (Crustacea) of chondrichthyes. Boletim do Instituto Oceanografico, Brazil, 27(2), 95-152.

Morris, P. A. (1966) A field guide to Pacific Coast shells: including shells of Hawaii and the Gulf of California. Houghton Mifflin Company, Boston, Massachusetts, 297 pp.

Moser, M. and Anderson, S. (1977) An intrauterine leech infection: *Branchellion lobata* Moore, 1952 (Piscicolidae) in the Pacific angel shark (*Squatina californica*) from California. Canadian Journal of Zoology, 55, 759-760.

Moser, M. and Sakanari, J. (1986) The effects of praziquantel on various larval and adult parasites from freshwater and

marine snails and fish. Journal of Parasitology, 72, 175-176.
Moyle, P. B. and Cech, J. J., Jr. (1996) Fishes: an introduction to ichthyology. Prentice Hall, Upper Saddle River, New Jersey, 590 pp.
Mudry, D. R. and Dailey, M. D. (1969) *Phlyctainophora squali* sp. nov. (Nematoda, Philometridae) from the spiny dogfish, *Squalis acanthias*. Proceedings of the Helminthological Society of Washington, 36, 280-284.
Mudry, D. R. and Dailey, M. D. (1971) Postembryonic development of certain tetraphyllidean and trypanorhynchan cestodes with a possible alternative life cycle for the order Trypanorhyncha. Canadian Journal of Zoology, 49, 1249-1253.
Mueller, K. W., Watanabe, W. O. and Head, W. D. (1992) Effect of salinity on hatching in *Neobenedenia melleni*, a monogenean ectoparasite of seawater-cultured tilapia. Journal of the World Aquaculture Society, 23, 199-204.
Mugridge, R. E. R. and Stallybrass, H. G. (1983) A mortality of eels, *Anguilla anguilla* L., attributed to Gnathiidae. Journal of Fish Diseases, 6, 81-82.
Nagasawa, K., Tanaka, S. and Benz, G. W. (1998) *Trebius shiinoi* n. sp. (Trebiidae: Siphonostomatoida: Copepoda) from uteri and embryos of the Japanese angelshark (*Squatina japonica*) and the clouded angelshark (*Squatina nebulosa*), and redescription of *T. longicaudatus*. Journal of Parasitology, 84, 1218-1230.
Nasin, C. S., Caira, J. N. and Euzet, L. (1997) Analysis of *Calliobothrium* (Tetraphyllidae: Onchobothriidae) with descriptions of three new species and erection of a new genus. Journal of Parasitology, 83, 714-733.
Nelson, J. S. (1994) Fishes of the world, third edition. John Wiley and Sons, New York, New York, 600 pp.
Newbound, D. R. and Knott, B. (1999) Parasitic copepods from pelagic sharks in western Australia. Bulletin of Marine Science, 65, 715-724.
Newman, W. A. and Foster, B. A. (1987) Southern hemisphere endemism among the barnacles: explained in part by extinction of northern members of amphitropical taxa? Bulletin of Marine Science, 41, 361-377.
Nichols, K. C. (1975) Observations on little-known flatworms: *Udonella*. International Journal for Parasitology, 5, 475-482.
Nickerson, W. S. (1895) On *Stichocotyle nephropis* Cunningham, a parasite of the American lobster. Zoologische Jahrbuecher Abteilung fuer Allgemeine Zoologie und Physiologie der Tiere, 8, 447-480.
Nickol, B. B. (1995) Phylum Acanthocephala. *In* Fish diseases and disorders, volume 1. Protozoan and metazoan infections. (Ed. Woo, P. T. K.) CAB International, Wallingford, United Kingdom, 447-473 pp.
Noble, E. R., Noble, G. A., Schad, G. A. and MacInnes, A. J. (1989) Parasitology: the biology of animal parasites, sixth edition. Lea and Febiger, Philadelphia, Pennsylvania, 574 pp.
Noga, E. J. (1996) Fish disease: diagnosis and treatment. Mosby, Saint Louis, Missouri, 367 pp.
Noga, E. J., Wright, J. F., Levine, J. F., Dykstra, M. J. and Hawkins, J. H. (1991) Dermatological diseases affecting fishes of the Tar-Pamlico Estuary, North Carolina. Diseases of Aquatic Organisms, 10, 87-92.
Odhner, T. (1910) *Stichocotyle nephropis* J. T. Cunningham ein aberranter Trematode der Digenenfamilie Aspidogastridae. Kungliga Svenska Vetenskapsakademiens Handlingar, 45, 3-16.
Olsen, L. S. (1952) Some nematodes parasitic in marine fishes. Publications of the Institute of Marine Science University of Texas, 11, 173-215.
Olson, A. C., Jr. and Jeffries, M. (1983) *Dendromonocotyle californica* sp. n. (Monogenea: Monocotylidae) from the bat ray, *Myliobatis californica*, with a key to species. Journal of Parasitology, 69, 602-605.
Olson, P. D., Cribb, T. H., Tkach, V. V., Bray, R. A. and Littlewood, D. T. J. (2003) Phylogeny and classification of the Digenea. International Journal for Parasitology, 33, 733-755.
Olson, R. E., Hanson, A. W. and Pratt, I. 1970. *Plectogtnathotrema hydrolagi* sp. n. (Trematoda: Cephaloporidae) from the ratfish (*Hydrolagus colliei*). Journal of Parasitology, 56, 724-726.
Olsson, P. J. (1869) *Nova genera parasitantia Copepodorum et Platyelminthium*. Acta Universitatis Lundensis, 6, 1-6.
O'Sullivan, J. B., McConnaughey, R. R. and Huber, M. E. (1987) A blood-sucking snail: the Cooper's nutmeg, *Cancellaria cooperi* Gabb, parasitizes the California electric ray, *Torpedo californica* Ayres. Biological Bulletin, 172, 362-366.
Overstreet, R. M. (1978) Marine maladies? Worms, germs and other symbionts from the northern Gulf of Mexico. Mississippi-Alabama Sea Grant Consortium, Ocean Springs, Mississippi, 140 pp.
Overstreet, R. M. (1983) Metazoan symbionts of crustaceans. *In* The biology of Crustacea, volume 6. Pathobiology. (Ed. Provenzano, A. J., Jr.) Academic Press, New York, New York, 156-251 pp.
Overstreet, R. M., Dyková, I. and Hawkins, W. E. (1992) Branchiura. *In* Microscopic anatomy of invertebrates, volume 9. Crustacea. (Eds. Harrison, F. W. and Humes, A. G.) Wiley-Liss, New York, New York, 385-413 pp.
Padrós, F., Palenzuela, O., Hispano, C., Tosas, O., Zarza, C., Bultó, P., Crespo, S. and Alvarez-Pellitero, P. (2001) *Myxidium leei* (Myxozoa) infections in aquarium-reared Mediterranean fish species. Diseases of Aquatic Organisms, 47, 57-62.
Palm, H. W. and Overstreet, R. M. (2000) *Otobothrium cysticum* (Cestoda: Trypanorhyncha) from the muscle of butterfishes (Stromateidae). Parasitology Research, 86, 41-53.
Palm, H. W. and Walter, T. (1999) *Nybelinia southwelli* sp. nov. (Cestoda, Trypanorhyncha) with the redescription of *N. perideraeus* (Shipley & Hornell, 1906) and synonymy of *N. herdmani* (Shipley & Hornell, 1906) with *Kotorella pronosoma* (Stossich, 1901). Bulletin of the Natural History Museum of London (Zoology), 65, 123-131.
Palmer, R., Rodger, H., Drinan, E., Dwyer, C. and Smith, P. R. (1987) Preliminary trials on the efficacy of ivermectin against parasitic copepods of Atlantic salmon. Bulletin of the European Association of Fish Pathologists, 7, 47-54.
Palombi, A. (1941) *Cercaria dentali* Pelseneer, forma larvale di *Ptychogonimus megastoma* (Rud.). Rivista di Parassitologia, 5, 127-128.
Palombi, A. (1942a) Il ciclo biologico di *Ptychogonimus megastoma* (Rud.). Osservazioni sulla morfologia e fisiologia delle forme larvali e considerazioni filogenetiche. Rivista di Parassitologia, 6, 117-172.
Palombi, A. (1942b) Notizie ed osservazioni ed accidentali ospitatori definitivi di *Ptychogonimus megastoma*. Annuario del Museo Zoologico della Regia Università di Napoli. Nuova Serie VII, 11, 1-3.
Paperna, I. and Por, F. D. (1977) Preliminary data on the Gnathiidae (Isopoda) of the northern Red Sea, the Bitter Lakes and the eastern Mediterranean and the biology of *Gnathia piscivora* n. sp. Rapports et Proces-Verbeaux des Reunions Commission Internationale pour l'Exploration Scientific de la Mer Mediterranee Monaco, 24(4), 195-197.
Parker, R. R., Kabata, Z., Margolis, L. and Dean, M. D. (1968) A review and description of *Caligus curtus* Müller, 1785 (Caligidae: Copepoda), type species of its genus. Journal of the Fisheries Research Board of Canada, 25, 1923-1969.

Parukhin, A. M. and Tkachuk, L. P. (1980) New species of trematode from fishes of the Indian Ocean. Biologicheskie Nauki, 6, 41-44. [In Russian]

Pascoe, D. and Cram, P. (1977) The effect of parasitism on the toxicity of cadmium to the three-spined stickleback, *Gasterosteus aculeatus* L. Journal of Fish Biology, 10, 467-472.

Pascual, S., Gonzalez, A., Arias, C. and Guerra, A. (1996) Biotic relationships of *Illex coindetii* and *Todaropsis eblanae* (Cephalopoda, Ommastrephidae) in the northeast Atlantic: evidence from parasites. Sarsia, 81, 265-274.

Perkins, P. S. (1983) The life history of *Cardiodectes medusaeus* (Wilson), a copepod parasite of lanternfishes (Myctophidae). Journal of Crustacean Biology, 3, 70-87.

Piasecki, W. (1989) Life cycle of *Tracheliastes maculatus* Kollar, 1835 (Copepoda: Siphonostomatoida: Lernaeopodidae). Wiadomosci Parazytologiczne, 35, 187-245.

Pike, C. S., III, Manire, C. A. and Gruber, S. H. (1993) Nutrition and nutritional diseases in sharks. *In* Fish medicine. (Ed. Stoskopf, M. K.) W. B. Saunders, Philadelphia, Pennsylvania, 763-769 pp.

Poulin, R. (1998) Evolutionary ecology of parasites: from individuals to communities. Chapman and Hall, London, United Kingdom, 212 pp.

Poulin, R. and FitzGerald, G. J. (1987) The potential of parasitism in the structuring of a salt marsh stickleback community. Canadian Journal of Zoology, 65, 2793-2798.

Poulin, R. and FitzGerald, G. J. (1988) Water temperature, vertical distribution, and risk of ectoparasitism in juvenile sticklebacks. Canadian Journal of Zoology, 66, 2002-2005.

Poulin, R. and FitzGerald, G. J. (1989a) A possible explanation for the aggregated distribution of *Argulus canadensis* Wilson, 1916 (Crustacea: Branchiura) on juvenile sticklebacks (Gasterosteidae). Journal of Parasitology, 75, 58-60.

Poulin, R. and FitzGerald, G. J. (1989b) Risk of parasitism and microhabitat selection in juvenile sticklebacks. Canadian Journal of Zoology, 67, 14-18.

Poulin, R. and FitzGerald, G. J. (1989c) Male-based sex ratio in *Argulus canadensis* Wilson, 1916 (Crustacea: Branchiura) ectoparasitic on sticklebacks. Canadian Journal of Zoology, 67, 2078-2080.

Poulin, R. and FitzGerald, G. J. (1989d) Shoaling as an anti-ectoparasite mechanism in juvenile sticklebacks (*Gasterosteus* spp.). Behavioral Ecology and Sociobiology, 24, 251-255.

Poynton, S. L., Campbell, T. W. and Palm, H. W. (1997) Skin lesions in captive lemon sharks *Negaprion brevirostris* (Carcharhinidae) associated with the monogenean *Neodermophthirius harkemai* Price 1963 (Microbothriidae). Diseases of Aquatic Organisms, 31, 29-33.

Presnell, J. K. and Schreibman, M. P. (1997) Humason's animal tissue techniques, fifth edition. The Johns Hopkins University Press, Baltimore, Maryland, 536 pp.

Price, E. W. (1937) North American monogenetic trematodes. I. The superfamily Gyrodactyloidea. Journal of the Washington Academy of Sciences 27(3&4), 124-164.

Price, E. W. (1938) North American monogenetic trematodes. II. The families Monocotylidae, Microbothriidae, Acanthocotylidae and Udonellidae (Capsaloidea). Journal of the Washington Academy of Sciences, 28, 110-198.

Price, E. W. (1942) North American monogenetic trematodes. V. The family Hexabothriidae, n. n. (Polystomatoidea). Proceedings of the Helminthological Society of Washington, 9, 39-56.

Price, E. W. (1963) A new genus and species of monogenetic trematode from a shark, with a review of the family Microbothriidae Price, 1936. Proceedings of the Helminthological Society of Washington, 30, 213-218.

Pritchard, M. H. and Kruse, G. O. W. (1982) The collection and preservation of animal parasites. University of Nebraska Press, Lincoln, Nebraska, 141 pp.

Rafi, F. (1988) Isopoda. *In* Guide to the parasites of fishes of Canada, part II. Crustacea. (Eds. Margolis, L. and Kabata, Z.) Department of Fisheries and Oceans, Ottawa, Canada, 129-148 pp.

Rand, T. G., Wiles, M. and Odense, P. (1986) Attachment of *Dermophthirius carcharhini* (Monogenea: Microbothriidae) to the Galapagos shark, *Carcharhinus galapagensis*. Transactions of the American Microscopical Society, 105, 158-169.

Read, C. P. (1948) A new capillarid nematode from the spiny dogfish, *Squalus acanthias*. Journal of Parasitology, 34, 163-164.

Redondo, M. J., Palenzuela, O., Riaza, A., Macías, Á. and Álvarez-Pellitero, P. (2002) Experimental transmission of *Enteromyxum scophthalmi* (Myxozoa), an enteric parasite of turbot *Scophthalmus maximus*. Journal of Parasitology, 88, 482-488.

Richardson, H. (1904) Contributions to the natural history of the Isopoda. Proceedings of the United States National Museum, 27, 1-89.

Richardson, H. (1905) A monograph on the isopods of North America. Bulletin of the United States National Museum, 54, 1-727.

Roberts, L. S. and Janovy, J., Jr. (2000) Foundations of parasitology, sixth edition. McGraw-Hill Companies, Dubuque, Iowa, 647 pp.

Roberts, R. J., Johnson, K. A. and Casten, M. T. (2004) Control of *Salmincola californiensis* (Copepoda: Lernaeapodidae) in rainbow trout, *Oncorhynchus mykiss* (Walbaum): a clinical and histopathological study. Journal of Fish Diseases, 27, 73-79.

Rohde, K. (1994) The minor groups of parasitic Platyhelminthes. Advances in Parasitology, 33, 146-234.

Rohde, K. (2001) The Aspidogastrea: an archaic group of Platyhelminthes. *In* Interrelationships of the Platyhelminthes. (Eds. Littlewood, D. T. J. and Bray, R. A.) Taylor and Francis, London, United Kingdom, 159-167 pp.

Rohde, K. (2002) Subclass Aspidogastrea Faust & Tang, 1936. *In* Keys to the Trematoda, volume 1. (Eds. Gibson, D. I., Jones, A. and Bray, R. A.) CAB International and The Natural History Museum, London, United Kingdom, 5-14 pp.

Rohde, K., Heap, M., Hayward, C. J. and Graham, K. J. (1992) *Calicotyle australiensis* n. sp. and *Caliocotyle* sp. (Monogenea, Monopisthocotylea) from the rectum and rectal glands, and *Rugogaster hydrolagi* Schell, 1973 (Trematoda, Aspidogastrea) from the rectal glands of holocephalans off the coast of southeastern Australia. Systematic Parasitology, 21, 69-79.

Rosa-Molinar, E., Williams, C. S. and Lichtenfels, J. R. (1983) Larval nematodes (Philometridae) in granulomas in ovaries of black-tip sharks, *Carcharhinus limbatus* (Valenciennes). Journal of Wildlife Diseases, 19, 275-277.

Ross, R. (1999) Freshwater stingrays from South America. *In* Aqualog special, 13. (Ed. Schäfer, F.) Aqualog Verlag, Rodgau, Germany, 64 pp.

Roth, M., Richards, R. H. and Sommerville, C. (1993a) Current practices in the chemotherapeutic control of sea lice infestations in aquaculture: a review. Journal of Fish Diseases, 16, 1-26.

Roth, M., Richards, R. H. and Sommerville, C. (1993b) Preliminary studies on the efficacy of two pyrethroid

compounds, resmethrin and lambda-cyhalothrin, for the treatment of sea lice (*Lepeophtheirus salmonis*) infestations of Atlantic salmon (*Salmo salar*). *In* Pathogens of wild and farmed fish: sea lice. (Eds. Boxshall, G. A. and Defaye, D.) Ellis Horwood, New York, New York, 275-289 pp.

Rudloe, J. (1971) The erotic ocean: a handbook for beachcombers. World Publishing, New York, New York, 448 pp.

Ruhnke, T. R. (1994a) *Paraorygmatobothrium barberi* n. g., n. sp. (Cestoda: Tetraphyllidae), with amended descriptions of two species transferred to the genus. Systematic Parasitology, 28, 65-79.

Ruhnke, T. R. (1994b) Resurrection of *Anthocephalum* Linton, 1890 (Cestoda: Tetraphyllidea) and taxonomic information on five proposed members. Systematic Parasitology, 29, 159-176.

Rushton-Mellor, S. K. (1992) Discovery of the fish louse, *Argulus japonicus* Thiele (Crustacea: Branchiura), in Britain. Aquaculture and Fisheries Management, 23, 269-271.

Rushton-Mellor, S. K. (1994) The genus *Argulus* (Crustacea: Branchiura) in Africa: identification keys. Systematic Parasitology, 28, 51-63.

Ruyck, R. de and Chabaud, A. G. (1960) Un cas de parasitisme attribuable a des larves de *Phlyctainophora lamnae* Steiner chez un sélacien, et cycle évolutif probable de ce nématode. Vie et Milieu, 11, 386-389.

Sakanari, J. A. and Moser, M. (1989) Complete life cycle of the elasmobranch cestode, *Lacistorhynchus dollfusi* Beveridge and Sakanari, 1987 (Trypanorhyncha). Journal of Parasitology, 75, 806-808.

Sangster, N. C. and Dobson, R. J. (2002) Anthelmintic resistance. *In* The biology of nematodes. (Ed. Lee, D. L.) Taylor and Francis, London, United Kingdom, 531-567 pp.

Sanjeeva Raj, P. J. (1974) A review of the fish-leeches of the Indian Ocean. Journal of the Marine Biological Association of India, 16, 381-397.

Santos, C. P., Lent, H. and Gibson, D. I. (2004) A new species of *Pseudanisakis* Layman & Borovkova, 1926 (Nematoda: Ascaridida) from *Rioraja agassizii* and *Psammobatis extenta* (Rajidae) in Brazilian southwestern Atlantic waters. Systematic Parasitology, 57, 229-235.

Sawyer, R. T. (1986) Leech biology and behavior, volume 2. Feeding biology, ecology, and systematics. Oxford Scientific Publications, Oxford, United Kingdom, 1065 pp.

Sawyer, R. T. and Hammond, D. L. (1973) Observations on the marine leech *Calliobdella carolinensis* (Hirudinea: Piscicolidae), epizootic on the Atlantic menhaden. Biological Bulletin, 145, 373-388.

Sawyer, R. T., Lawler, A. R. and Overstreet, R. M. (1975) Marine leeches of the eastern United States and the Gulf of Mexico with a key to the species. Journal of Natural History, 9, 633-667.

Schell, S. C. (1973) *Rugogaster hydrolagi* gen. et sp. n. (Trematoda: Aspidobothrea: Rugogastridae fam. n.) from the ratfish, *Hydrolagus colliei* (Lay and Bennett, 1839). Journal of Parasitology, 59, 803-805.

Schell, S. C. (1985) Handbook of trematodes of North America north of Mexico. University of Idaho Press, Moscow, Idaho, 263 pp.

Schlegel, M., Lom, J., Stechmann, A., Bernhard, D., Leipe, D., Dyková, I. and Sogin, M. L. (1996) Phylogenetic analysis of complete small subunit ribosomal RNA coding region of *Myxidium lieberkuehni*: evidence that myxozoa are metazoa and related to bilateria. Archiv für Protistenkunde, 147, 1-9.

Schmahl, G. and Mehlhorn, H. (1985) Treatment of fish parasites. 1. Praziquantel effective against Monogenea (*Dactylogyrus vastator*, *Dactylogyrus extensus*, *Diplozoon paradoxum*). Zeitschrift für Parasitenkunde, 71, 727-737.

Schram, F. R. (1986) Crustacea. Oxford University Press, New York, New York, 606 pp.

Schram, T. A. (1993) Supplementary descriptions of the developmental stages of *Lepeophtheirus salmonis* (Krøyer, 1837) (Copepoda: Caligidae). *In* Pathogens of wild and farmed fish: sea lice. (Eds. Boxshall, G. A. and Defaye, D.) Ellis Horwood, New York, New York, 30-47 pp.

Schultz, G. A. (1969) The marine isopod crustaceans. William C. Brown Publishers, Dubuque, Iowa, 359 pp.

Schuurmans-Stekhoven, J. H., Jr. and Botman, T. P. J. (1932) Zur Ernaehrungsbiologie von *Proleptus obtusus* Duj. und die von diesem Parasiten hervorgerufenen reaktiven Aenderungen des Wirtsgewebes. Zeitschrift für Parasitenkunde, 4, 220-239.

Shafir, A. and van As, J. G. (1985) The opportunistic nature of *Argulus japonicus* in maintaining host parasite relationships. South African Journal of Science, 81, 638.

Shafir, A. and van As, J. G. (1986) Laying, development and hatching of eggs of the fish ectoparasite *Argulus japonicus* (Crustacea: Branchiura). Journal of Zoology, London (A), 210, 401-414.

Shelton, R. G. J. (1978) On the feeding of the hagfish *Myxine glutinosa* in the North Sea. Journal of the Marine Biological Association of the United Kingdom, 58, 81-86.

Shimura, S. (1981) The larval development of *Argulus coregoni* Thorell (Crustacea: Branchiura). Journal of Natural History, 15, 331-348.

Shimura, S. and Asai, M. (1984) *Argulus americanus* (Crustacea: Branchiura) parasitic on the bowfin, *Amia calva*, imported from North America. Fish Pathology, 18, 199-203. [In Japanese, with English abstract]

Short, R. B. (1954) A new blood fluke, *Selachohemecus olsoni*, n. g., n. sp. (Aporocotylidae) from the sharp-nosed shark, *Scoliodon terra-novae*. Proceedings of the Helminthological Society of Washington, 21, 78-82.

Shvetsova, L. S. (1990) A new find of *Rugogaster hydrolagi* Schell, 1973 in *Hydrolagus ogibyi* from the southern Pacific. Parazitologiya, 24, 82-85. [In Russian, with English summary]

Siddall, M. E., Martin. D. S., Bridge, D., Desser, S. S. and Cone, D. K. (1995) The demise of a phylum of protists: phylogeny of Myxozoa and other parasitic Cnidaria. Journal of Parasitology, 81, 961-967.

Sindermann, C. J. (1990) Principal diseases of marine fish and shellfish, volume 1, second edition. Academic Press, San Diego, California, 521pp.

Smit, N. J. and Basson, L. (2002) *Gnathia pantherina* sp. n. (Crustacea: Isopoda: Gnathiidae), a temporary ectoparasite of some elasmobranch species from southern Africa. Folia Parasiologia, Praha, 49, 137-151.

Smit, N. J., Basson, L. and Van As, J. G. (2003) Life cycle of the temporary fish parasite, *Gnathia africana* (Crustacea: Isopoda: Gnathiidae). Folia Parasitologica, Praha, 50, 135-142.

Smith, B. E., Williams, E. H., Jr., Bunkley-Williams, L. and Benz, G. W. (in preparation) Isopods collected in association with elasmobranchs from the Gulf of California. Journal of Natural History.

Smith, D. G. (2001) Pennak's freshwater invertebrates of the United States: Porifera to Crustacea, fourth edition. John Wiley and Sons, New York, New York, 638 pp.

Smith, I. M., Smith, B. P. and Cook, D. R. (2001) Water mites (Hydrachnida) and other arachnids. *In* Ecology and classification of North American freshwater invertebrates, second edition. (Eds. Thorp, J. H. and Covich, A. P.) Academic Press, San Diego, California, 551-659 pp.

Smith, P. R., Moloney, M., McElligott, A., Clarke, S., Palmer, R., O'Kelly, J. and O'Brien, F. (1993) The efficiency of oral ivermectin in the control of sea lice infestations of farmed Atlantic salmon. *In* Pathogens of wild and farmed fish: sea lice. (Eds. Boxshall, G. A. and Defaye, D.) Ellis Horwood, New York, New York, 296-307 pp.

Soós, Á. (1965) Identification key to the leech (Hirudinoidea) genera of the world, with a catalogue of the species. I. Family: Piscicolidae. Acta Zoologica Academiae Scientiarum Hungaricae, 11, 417-463.

Southwell, T. (1913) Notes from the Bengal Fisheries Laboratory. Records of the Indian Museum, 9, 79-103.

Southwell, T. and Walker, A. J. (1936) Notes on a larval cestode from a fur seal. Annals of Tropical Medicine and Parasitology, 30, 91-100.

Specian, R. D., Ubelaker, J. E. and Dailey, M. D. (1975) *Neoleptus* gen n. and a revision of the genus *Prolepas* Dujardin, 1845. Proceedings of the Helminthological Society of Washington, 42, 14-21.

Sprent, J. F. A. (1983) Observations on the systematics of ascaridoid nematodes. *In* Concepts in nematode systematics. (Eds. Stone, A. R., Platt, H. M. and Khalil, L. F.) Academic Press, London, United Kingdom, 303-319 pp.

Staton, L., Erdahl, D. and El-Matbouli, M. (2002) Efficacy of Fumagillin and TNP-470 to prevent experimentally induced whirling disease in rainbow trout *Oncorhynchus mykiss*. *In* Whirling disease: reviews and current topics. (Eds. Bartholomew, J. L. and Wilson, J. C.) American Fisheries Society Symposium 29, Bethesda, Maryland, 239-247 pp.

Stoffregen, D. A. and Anderson, W. I. (1990) A myxosporidian parasite in the skeletal muscle of a black-tip reef shark, *Carcharhinus melanopterus* (Quoy and Gaimard, 1824). Journal of Fish Diseases, 13, 549-552.

Stoll, C. (1962) Cycle evolutif de *Paragnathia formica* (Hesse) (Isopoda-Gnathiidae). Cahiers de Biologie Marine, 3, 401-416.

Stoskopf, M. K. (1993a) Clinical pathology of sharks, skates, and rays. *In* Fish medicine. (Ed. Stoskopf, M. K.) W. B. Saunders, Philadelphia, Pennsylvania, 754-757 pp.

Stoskopf, M. K. (1993b) Environmental requirements and diseases of sharks. *In* Fish medicine. (Ed. Stoskopf, M. K.) W. B. Saunders, Philadelphia, Pennsylvania, 758-763 pp.

Stoskopf, M. K. (1993c) Shark pharmacology and toxicology. *In* Fish medicine. (Ed. Stoskopf, M. K.) W. B. Saunders, Philadelphia, Pennsylvania, 809-816 pp.

Stoskopf, M. K. (Ed.) (1993d) Fish medicine. W. B. Saunders, Philadelphia, Pennsylvania, 852 pp.

Strasburg, D. W. (1959) Notes on the diet and correlating structures of some central Pacific echeneid fishes. Copeia, 1959, 244-248.

Strasburg, D. W. (1963) The diet and dentition of *Isistius brasiliensis*, with remarks on tooth replacement in other sharks. Copeia, 1963, 33-40.

Stunkard, H. W. (1962) *Taeniocotyle* nom. nov. for *Macraspis* Olsson, 1869, preoccupied, and systematic position of the Aspidobothrea. Biological Bulletin, 122, 137-148.

Sutherland D. R. and Wittrock, D. D. (1986) Surface topography of the branchiuran *Argulus appendiculosus* Wilson, 1907 as revealed by scanning electron microscopy. Parasitology Research, 72, 405-415.

Swanepoel, J. H. and Avenant-Oldewage, A. (1992) Comments on the morphology of the pre-oral spine in *Argulus* (Crustacea: Branchiura). Journal of Morphology, 212, 155-162.

Székely, C. and Molnár, K. (1987) Mebendazole is an efficacious drug against pseudodactylogyrosis in the European eel (*Anguilla*). Journal of Applied Ichthyoloy, 3, 183-186.

Szidat, L. (1966) *Trigonostoma callorhynchi* n. g., n. sp. (Trematoda subclase Aspidogastrea) de los canales biliares de *Callorhynchus callorhynchus* L. del Atlantico sur. Neotropica, 12, 67-71.

Tandon, R. S. (1969) A new anaporrhuntine trematode (Fam. Gorgoderidae) *Nagmia yamagutia* n. sp. from the ovary of the ray, *Dasyatis uarnak*, from Bharat (India). Annotationes Zoologicae Japonenses, 42, 36-39.

Taylor, L. R., Compagno, L. J. V. and Struhsaker, P. J. (1983) Megamouth— a new species, genus, and family of lamnoid shark (*Megachasma pelagios*, family Megachasmidae) from the Hawaiian Islands. Proceedings of the California Academy of Science, series A, 43(8), 87-110.

Testa, J. and Dailey, M. D. (1977) Five new morphotypes of *Phyllobothrium delphini* (Cestoda: Tetraphyllidea), their relationship to existing morphotypes, and their zoogeography. Bulletin of the Southern Californian Academy of Sciences, 76, 99-110.

Thatcher, V. E. (1991) Amazon fish parasites. Amazoniana, 11, 263-572.

Thomassen, J. M. (1993) Hydrogen peroxide as a delousing agent for Atlantic salmon. *In* Pathogens of wild and farmed fish: sea lice. (Eds. Boxshall, G. A. and Defaye, D.) Ellis Horwood, New York, New York, 290-295 pp.

Thoney, D. A. (1990) The effects of trichlorfon, praziquantel and copper sulphate on various stages of the monogenean *Benedeniella posterocolpa*, a skin parasite of the cownose ray, *Rhinoptera bonasus* (Mitchill). Journal of Fish Diseases, 13, 385-389.

Thoney, D. A. and Burreson, E. M. (1986) Ecological aspects of *Multicalyx cristata* (Aspidocotylea) infections in northwest Atlantic elasmobranchs. Proceedings of the Helminthological Society of Washington, 53, 162-165.

Thoney, D. A. and Burreson, E. M. (1987) Morphology and development of the adult and cotylocidium of *Multicalyx cristata* (Aspidocotylea), a gall bladder parasite of elasmobranchs. Proceedings of the Helminthological Society of Washington, 54, 96-104.

Thoney, D. A. and Burreson, E. M. (1988) Revision of the Multicalycidae (Aspidocotylea) with comments on postlarval development. Proceedings of the Helminthological Society of Washington, 55, 62-67.

Thoney, D. A. and Hargis, W. J., Jr. (1991) Monogenea (Platyhelminthes) as hazards for fish in confinement. Annual Review of Fish Diseases, 1, 133-153.

Thwaite, J. W. (1927) On a collection of nematodes from Ceylon. Annals of Tropical Medicine and Parasitology, 21(2), 225-243.

Torres, P., Tort, L., Planas, J. and Flos, R. (1986) Effects of confinement stress and additional zinc treatment on some blood parameters in the dogfish *Scyliorhinus canicula*. Comparative Biochemistry and Physiology, 83C, 89-91.

Travassos, L., Teixeira de Freitas, J. F., Machado de Mendonça, J. and de Oliveira Rodrigues, H. (1963) Terceira excursão a Cabo Frio, Estado do Rio de Janeiro. Atas da Sociedade de Biologia do Rio de Janeiro, 7, 6-7.

Treasurer, J. W. (1993) Management of sea lice (Caligidae) with wrasse (Labridae) on Atlantic salmon (*Salmo salar* L.) farms. *In* Pathogens of wild and farmed fish: sea lice. (Eds. Boxshall, G. A. and Defaye, D.) Ellis Horwood, New York, New York, 335-345 pp.

Tsai, M.-L. and Dai, C.-F. (2003) Cannibalism within mating pairs of the parasitic isopod, *Ichthyoxenus fushanensis*. Journal of Crustacean Biology, 23, 662-668.

Tyler, G. A. (2001) Diphyllidean cestodes of the Gulf of California, México with descriptions of two new species of *Echinobothrium* (Cestoda: Diphyllidea). Journal of Parasitology, 87, 173-184.

Upton, N. P. D. (1987) Asynchronous male and female life cycles in the sexually dimorphic, harem-forming isopod *Paragnathia formica* (Crustacea: Isopoda). Journal of Zoology, London, 212, 677-690.

van der Land, J. and Dienske, H. (1968) Two new species of *Gyrocotyle* (Monogenea) from chimaerids (Holocephali). Zoologische Medelingen, Leiden, 43(8), 97-105.

Wägele, J.-W. (1987) Description of the postembryonal stages of the Antarctic fish parasite *Gnathia calva* Vanhoffen (Crustacea: Isopoda) and synonymy with *Heterognathia* Amar & Roman. Polar Biology, 7, 77-92.

Wägele, J.-W. (1988) Aspects of the life-cycle of the Antarctic fish parasite *Gnathia calva* Vanhoffen (Crustacea: Isopoda). Polar Biology, 8, 287-291.

Watson, D. E. and Thorson, T. B. (1976) Helminths from elasmobranchs in Central American fresh waters. *In* Investigations of the ichthyofauna of Nicaraguan lakes. (Ed. Thorson, T. B.) School of Life Sciences, University of Nebraska, Lincoln, Nebraska, 629-640 pp.

Wedemeyer, G. A. (1996) Physiology of fish in intensive culture systems. Chapman and Hall, New York, New York, 232 pp.

Wells, P. (1989) Health surveillance for Aquagard users. Fish Farmer, Sept./Oct. 1989.

Whittington, I. D. (1987a) Hatching in two monogenean parasites from the common dogfish (*Scyliorhinus canicula*): the polyopisthocotylean gill parasite, *Hexabothrium appendiculatum* and the microbothriid skin parasite*, Leptocotyle minor*. Journal of the Marine Biological Association of the United Kingdom, 67, 729-756.

Whittington, I. D. (1987b) A comparative study of the anatomy of the oncomiracidia of the hexabothriid monogeneans *Rajonchocotyle emarginata* and *Hexabothrium appendiculatum*. Journal of the Marine Biological Association of the United Kingdom, 67, 757-772.

Whittington, I. D. (1987c) Studies on the behaviour of the oncomiracidia of the monogenean parasites *Hexabothrium appendiculatum* and *Leptocotyle minor* from the common dogfish, *Scyliorhinus canicula*. Journal of the Marine Biological Association of the United Kingdom, 67, 773-784.

Whittington, I. D. (1990) The egg bundles of the monogenean *Dionchus remorae* and their attachment to the gills of the remora, *Echeneis naucrates*. International Journal for Parasitology, 20, 45-49.

Whittington, I. D. (1998) Diversity "down under:" monogeneans in the Antipodes (Australia) with a prediction of monogenean biodiversity worldwide. International Journal of Parasitology, 28, 1481-1493.

Whittington, I. D., Cribb, B. W., Hamwood, T. E. and Halliday, J. A. (2000) Host-specificity of monogenean (platyhelminth) parasites: a role for anterior adhesive areas? International Journal for Parasitology, 30, 305-320.

Whittington, I. D., Deveney, M. R. and Wyborn, S. F. (2001) A revision of *Benedenia* Diesing, 1858 including a redescription of *B. sciaenae* (van Beneden, 1856) Odhner, 1905 and recognition of *Menziesia* Gibson, 1976 (Monogenea: Capsalidae). Journal of Natural History, 35, 663-777.

Whittington, I. D. and Kearn, G. C. (1986) Rhythmical hatching and oncomiracidial behaviour in the hexabothriid monogenean *Rajonchocotyle emarginata* from the gills of *Raja* spp. Journal of the Marine Biological Association of the United Kingdom, 66, 93-111.

Whittington, I. D. and Kearn, G. C. (1988) Rapid hatching of mechanically-disturbed eggs of the monogenean gill parasite *Diclidophora luscae*, with observations on sedimentation of egg bundles. International Journal for Parasitology, 18, 847-852.

Whittington, I. D. and Kearn, G. C. (1990) Effects of urea analogs on egg hatching and movement of unhatched larvae of the monogenean parasite *Acanthocotyle lobianchi* from the skin of *Raja montagui*. Journal of Chemical Ecology, 16, 3523-3529.

Widder, E. A. (1998) A predatory use of counterillumination by the squaloid shark, *Isistius brasiliensis*. Environmental Biology of Fishes, 53, 267-273.

Williams, E. H., Jr. (1974) Treatments employed for control of parasites of selected fishes stocked in mariculture experiments (1969-1972). Proceedings of the World Mariculture Society, 5, 291-295.

Williams, E. H., Jr. (1978) *Conchoderma virgatum* (Spengler) (Cirripedia Thoracica) in association with *Dimemoura latifolia* (Steenstrup & Lutken) (Copepoda, Caligidae), a parasite of the shortfin mako, *Isurus oxyrhynchus* Rafinesque (Pisces, Chondrichthyes). Crustaceana, 34, 109-110.

Williams, E. H., Jr. and Bunkley-Williams, L. (1996) Parasites of offshore big game fishes of Puerto Rico and the western Atlantic. Puerto Rico Department of Natural and Environmental Resources, San Juan, Puerto Rico and the University of Puerto Rico, Mayaguez, Puerto Rico, 382 pp.

Williams, H. and Jones, A. (1994) Parasitic worms of fish. Taylor and Francis, London, United Kingdom, 593 pp.

Williams, H. H. (1960) The intestine in members of the genus *Raja* and host-specificity in the Tetraphyllidea. Nature, 188, 514-516.

Williams, H. H. (1966) The ecology, functional morphology and taxonomy of *Echeneibothrium* Beneden, 1850 (Cestoda: Tetraphyllidea), a revision of the genus and comments on *Discobothrium* Beneden, 1871, *Pseudanthobothrium* Baer, 1856, and *Phormobothrium* Alexander, 1963. Parasitology, 56, 227-285.

Williams, H. H. (1967) Helminth diseases of fish. Helminthological Abstracts, 36, 261-295.

Williams, H. H. (1968) The taxonomy, ecology and host-specificity of some Phyllobothriidae (Cestoda: Tetraphyllidea), a critical revision of *Phyllobothrium* Beneden, 1849 and comments on some allied genera. Philosophical Transactions of the Royal Society of London, series B, 253, 231-307.

Williams, H. H. and Bray, R. A. (1984) *Chimaerocestos prudhoei* gen. et sp. nov. representing a new family of tetraphyllideans and the first record of strobilate tapeworms from a holocephalan. Parasitology, 88, 105-116.

Williams, H. H., Colin, J. A. and Halvorsen, O. (1987) Biology of gyrocotylideans with emphasis on reproduction, population ecology and phylogeny. Parasitology, 95, 173-207.

Williams, H. H., McVicar, A. H. and Ralph, R. (1970) The alimentary canal of fish as an environment for helminth parasites. *In* Aspects of fish parasitology. (Eds. Taylor, A. E. R. and Muller, R.) Blackwell Scientific Publications, Oxford, United Kingdom, 43-77 pp.

Williams, H. H. and Richards, D. H. H. (1968) Observations on *Pseudanisakis rotundata* (Rudolphi, 1819) Mozgovoi, 1950, a common but little known nematode parasite of *Raia radiata* Donovan in the northern North Sea. Journal of Helminthology, 42, 199-200.

Williams, H. H. and Richards, D. H. H. (1978) *Proleptus mackenziei* sp. n. (Nematoda, Spirurida) from *Raja fyllae* in the Barents Sea. Zoologica Scripta, 7, 85-91.

Wilson, C. B. (1902) North American parasitic copepods of the family Argulidae, with a bibliography of the group and a systematic review of all known species. Proceedings of the United States National Museum, 25(1302), 635-742.

Wilson, C. B. (1904) The fish parasites of the genus *Argulus* found in the Woods Hole region. Bulletin of the Bureau of Fisheries, 24, 115-131.

Wilson, C. B. (1913) Crustacean parasites of West Indian fishes and land crabs, with descriptions of new genera and species. Proceedings of the United States National Museum, 44, 189-277.

Wishkovsky, A., Groff, J. M., Lauren, D. J., Toth, R. J. and Hedrick, R. P. (1990) Efficacy of fumagillin against proliferative kidney disease and its toxic side effects in rainbow trout (*Oncorhynchus mykiss*) fingerlings. Fish Pathology 25, 141-148.

Woo, P. T. K. (Ed.) (1995) Fish diseases and disorders, volume 1. Protozoan and metazoan infections. CAB International, Wallingford, United Kingdom, 808 pp.

Xylander, W. E. R. (2001) The Gyrocotylidea, Amphilinidea and the early evolution of Cestoda. *In* Interrelationships of the Platyhelminthes. (Eds. Littlewood, D. T. J. and Bray, R. A.) Taylor and Francis, London, United Kingdom, 103-111 pp.

Yamaguti, S. (1958) Systema helminthum, volume I. The digenetic trematodes of vertebrates, parts 1 and 2. Interscience Publishers, New York, New York, 1575 pp.

Yamaguti, S. (1959) Systema helminthum, volume II. The cestodes of vertebrates. Interscience Publishers, New York, New York, 860 pp.

Yamaguti, S. (1961) Systema helminthum, volume III. The nematodes of vertebrates, parts 1 and 2. Interscience Publishers, New York, New York, 1261 pp.

Yamaguti, S. (1963a) Systema helminthum, volume IV. Monogenea and Aspidocotylea. Interscience Publishers, New York, New York, 699 pp.

Yamaguti, S. (1963b) Parasitic Copepoda and Branchiura of fishes. Interscience Publishers, New York, New York, 1104 pp.

Yamaguti, S. (1963c) Systema helminthum, volume V. Acanthocephala. New York, New York, 423 pp.

Yamaguti, S. (1971) Synopsis of digenetic trematodes of vertebrates, volume I. Keigaku Publishing Company, Tokyo, Japan, 1074 pp.

Yamaguti, S. (1975) A synoptical review of the life histories of digenetic trematodes of vertebrates with special reference to the morphology of their larval forms. Keigaku Publishing Company, Tokyo, Japan, 590 pp.

Yano, K. and Musick, J. A. (2000) The effect of the mesoparasitic barnacle *Anelasma* on the development of reproductive organs of deep-sea squaloid sharks, *Centroscyllium* and *Etmopterus*. Environmental Biology of Fishes, 59, 329-339.

Yokoyama, H., Ogawa, K. and Wakabayashi, H. (1990) Chemotherapy with fumagillin and toltrazuril against kidney enlargement disease of goldfish caused by the myxosporean *Hoferellus carassii*. Fish Pathology, 25, 157-164.

Yorke, W. and Maplestone, P. A. (1962) The nematode parasites of vertebrates. Hafner Publishing Company, New York, New York, 536 pp.

Yoshinaga, T., Segawa, I., Kamaishi, T. and Sorimachi, M. (2000). Effects of temperature, salinity and chlorine treatment on egg hatching of the monogenean *Neoheterobothrium hirame* infecting Japanese flounder. Fish Pathology, 35, 85-88.

The Elasmobranch Husbandry Manual: Captive Care of Sharks, Rays and their Relatives, pages 417-426.
© 2004 Ohio Biological Survey

Chapter 25
Protozoal Diseases of Elasmobranchs

CAROLINE E. C. GOERTZ

Wise Laboratory of Environmental and Genetic Toxicology,
178 Science Bldg., 96 Falmouth St, PO Box 9300,
Portland, ME 04104-9300, USA.
E-mail: cgoertz@usm.maine.edu

Abstract: While more commonly described in teleosts, protozoal diseases have been found to affect both wild and captive elasmobranchs. Protozoa reported to parasitize elasmobranchs include flagellates (*Amyloodinium* spp. and trypanosome), amoebas, apicomplexans (haemogregarina and coccidia), microsporidia, and ciliates. As reports of treatments are limited to two case histories, studies are urgently needed to determine the pharmacodynamics, pharmacokinetics, safety, and efficacy of anti-protozoal drugs in elasmobranchs.

Most protozoa are microscopic unicellular organisms (eukaryotes) with one or more nuclei. Ciliates of the genera *Ichthyophthirius* and *Cryptocaryon* are detectable macroscopically as tiny white dots. Others, in the phyla Microsporidia and Apicomplexa, produce grossly visible whitish aggre-gations which, upon microscopic examination, are seen to consist of a mass of multiple small, uniform bodies that are spores or oocysts. At the ultrastructural and biochemical level protozoa share many similarities with cells from metazoans (multi-cellular organisms). Protozoa exemplify a multitude of different body plans, which is reflected in this diverse group of organisms being divided into multiple phyla.

Protozoa reported to parasitize elasmobranchs include representatives of the phyla Rhizopoda, Apicomplexa (including the class Coccidea), Micro-sporidia, Ciliophora, and the phyla commonly referred to as flagellates. Locomotion is typically achieved using one or more of three basic types of organelles (pseudopodia, flagella, and cilia), which help to identify the organism. A few of the apicomplexan life cycle stages use body flexion, gliding motion achieved by superficial binding sites shifted through internal contractile filament systems, undulating surface ridges, or slime production to accomplish locomotion.

Reproduction is either asexual or sexual and may alternate between the two. Asexual reproduction is usually by binary fission but may also occur through multiple fission or budding. Sexual reproduction is typically achieved by the union of haploid gametes formed from one or two gamonts. Another form of sexual reproduction, conjugation, or the union of only nuclei, is found in ciliates. These reproductive processes may result in the formation of cysts or spores that not only can lay dormant for prolonged periods of time, but also have additional protective mechanisms, which can resist the host's immune system and anti-protozoal treatments. Understanding the life cycle of pathogenic protozoa can aid in the development of effective treatment and control programs. Monoxenous parasites have a simple or direct life cycle. These parasites, such as some coccidian species, live within a single host throughout their life cycle; infective stages are transmitted by the host but little development occurs outside of the host. Indirect or heteroxenous life cycles are more complex involving two or more hosts. The different hosts of a heteroxenous parasite are the primary or definitive host in which the reproducing adult parasite is found, and the secondary or intermediate hosts which harbor other, immature life stages. Hemoparasites, such as trypano-somes and haemogregarines, have an indirect life cycle with leeches as the intermediate host. Controlling exposure to leeches would be an important part of control programs for these protozoa.

An excellent in-depth review of protozoa, their life cycles, and detailed identifying characteristics can

be found in *Protozoan Parasites of Fishes* (Lom and Dyková, 1992) and the *Illustrated Guide to the Protozoa* (Lee et al., 2002).

Reports of protozoal diseases in elasmobranchs appear to be relatively uncommon when compared to the frequency of these diseases in teleosts. However, since Cheung's (1993a) review the list of reported protozoal findings has expanded to include Microsporidia, amoebas, and additional ciliates. Several of these protozoal diseases were reported in captive animals via personal communication, emphasizing the need for publication of findings. Most reports in the literature refer to infections in wild-caught individuals and rarely indicated more than a light infection.

It is noteworthy that elasmobranchs can succumb to parasitic protozoa normally associated with teleosts, especially when elasmobranchs are exposed to an overwhelming challenge in closed systems. Such was the case in Lawler's (1980) report of Atlantic stingrays (*Dasyatis sabina*) succumbing to a massive infection of the dinoflagellate *Amyloodinium ocellatum*, after being exposed to teleosts carrying the parasite.

DETECTION AND DIAGNOSIS

Ante-mortem diagnosis

Evaluation of fresh samples and host tissues, including measurements and photo-micrographs, are indispensable for the identification of most groups of protozoa. The importance of quickly processing fresh diagnostic samples, from both ante-mortem and post-mortem examinations, cannot be overemphasized and should be done within minutes. Ante-mortem diagnosis is possible using traditional diagnostic methods employed for teleosts. Samples of mucus overlying suspect external lesions can be gently scraped away, smeared on a slide, and examined under a microscope. Gill surfaces may be examined in this fashion or, alternatively, small gill biopsies can be removed and examined as fresh mounts by placing a few small gill-filament tips, on a slide, with a drop of tank water, and sealing with a cover-slip. Examination should begin at low power and then progress to greater magnification.

Blood samples can be examined for hemoprotozoa, such as haemogregarines and trypanosomes, which can be seen in blood smears or within the buffy coat—the pale cream colored layer (consisting primarily of white blood cells) observed above packed red cells in centrifuged blood. Protozoa can be found during microscopic examination of diagnostic fluid obtained from the coelom and other aspirates. These fluids can be prepared for microscopic examination by smearing on a slide or, in the case of light infections, by concentration, through centrifugation, of potentially infected fluids and examination of the buffy coat (Woo, 1969). These and other techniques are discussed in greater detail in Lom and Dyková (1992).

Ante-mortem diagnostics can be used as part of the medical work-up of a diagnostic case but also as part of routine physical exams.

Post-mortem diagnosis

Necropsies should include the same diagnostic techniques described above. Since detection of ectoparasitic protozoa becomes difficult within ten minutes of death, skin scrapes from multiple areas and gill biopsies should be obtained and examined immediately, even in the absence of obvious lesions (Lom and Dyková, 1992). Prompt examination of fresh material is essential for diagnosis of delicate protozoa. Detection of protozoa in fresh tissues can be accomplished by making impression smears or wet mounts (i.e., taking a small piece of tissue and blotting the tissue several times onto a slide, or by gently squeezing the tissue between two slides).

SAMPLE PRESERVATION

While examination of diagnostic samples should routinely be performed within minutes of sampling, fresh material can be saved in a number of ways. Organisms may remain viable if held in a refrigerator. Slides can be placed in a Petri dish, covered, and sealed to prevent desiccation. Fluids can be stored in tightly sealed containers or syringes. If the volume of fluid is small, it can be placed in a small capillary tube sealed at both ends with clay or plasticine (Lom and Dyková, 1992). Cryopreservation techniques to preserve protozoa from teleosts should be valid for elasmobranch protozoa. Culture methods have been used successfully to maintain some teleost protozoa in vitro for further study and may be applicable to similar species of parasites found in elasmobranchs. *Trypanosoma scyllii* is the only protozoan parasite of elasmobranchs reported to have been successfully maintained in culture (Pulsford, 1984).

HISTOPATHOLOGY AND ELECTRON MICROSCOPY

Tissues obtained at necropsy can be prepared for histopathological examination using routine fixation and staining techniques. Clinical, ante-mortem biopsies can be processed in the same manner. Histopathological examination of a gill biopsy was important in the diagnosis of microsporidiosis in a leopard shark, *Triakis semifasciata*, (Garner et al., 1998). The most common fixation method used is placement of tissue samples (no more than 1.0 cm on a side) in a large volume of 10% buffered formalin; for adequate fixation tissues should take up less than 10% of the volume of fluid. Routine and special stains can be used to aid in the identification and classification of protozoa in tissue sections. Since most aquaria rely on outside histopathological services, discussion of these techniques and their use in fish medicine is left to the references (Lom and Dyková, 1992; Gardiner et al., 1988; Lee and Soldo, 1992). A few structures and identifying morphological features are not visible with light microscopy, so electron microscopy is essential for examination and proper identification of some protozoa. Again the reader is referred to the references for a more in-depth discussion of techniques (Poynton and Sterud, 2002).

PROTOZOA REPORTED IN ELASMOBRANCHS

Most reports in the literature deal with protozoa in wild-caught elasmobranchs; however, there are a growing number of reports dealing with captive animals. This increase may be due to increased numbers of animals held in captivity, a growing awareness of the possible presence of protozoa in elasmobranchs, and an improved ability to routinely screen for these parasites. Protozoan parasites reported in the literature, or by personal communication, comprise the flagellates (recently further divided into four phyla), amoebas, apicomplexans (which include *Coccidia*), microsporidia, and ciliates (Table 25.1).

Brief descriptions of the different groups of protozoa known to infest elasmobranchs are given below and include summaries of information presented in Lom and Dyková (1992), Roberts and Janovy (2000), and Cheung (1993a). The reader is referred to these and other references listed below for detailed information about protozoan life cycles, biology, and identification information.

Flagellates

Flagellates are microorganisms having one or more whip-like flagella (previously organized into the phylum Sarcomastigophora). While this group has now been divided into four phyla (Cavalier-Smith, 1993), the term “flagellates” is still commonly used. The flagella are used primarily for locomotion and may occur singly, in pairs, or large groups. Flagellates typically have one nucleus and reproduce primarily by longitudinal binary fission. Most species are free-living but there are many parasitic species (Lom and Dyková, 1992).

Amyloodinium ocellatum is an ectoparasitic dinoflagellate known to be indiscriminate in host selection in teleosts, infecting primarily the gills but also the skin, fins, eyes, branchial cavity surfaces, and even the gastrointestinal tract. Infested fish have a dull, velvety appearance, hence the common name “velvet disease.” The life cycle is complex and includes several morphologically distinct forms. The parasitic form, or trophont, is non-motile and pear-shaped, and attaches itself to surfaces using deeply penetrating rhizoids. As gill filaments become more densely infested with trophonts, host respiration becomes increasingly impaired. After a period of growth, during which adjacent host cells are being destroyed, the trophont detaches from the host and encysts, becoming a tomont. Successive reproductive divisions produce tomites and eventually result in the release of hundreds of flagellated, oval dinospores into the water. The free-swimming dinospore form is infective for over two weeks. Once a dinospore comes in contact with a susceptible host, it metamorphoses into the parasitic trophont, which does not have a flagellum (Lom and Dyková, 1992). While there are no reports of this parasite from wild-caught elasmobranchs, two captive Atlantic stingrays died from a massive infection within six days of being exposed to high concentrations of dinospores from teleost fish (Lawler, 1980). Although elasmobranchs do not appear to be as susceptible to protozoal infections as are teleosts, this case highlights the potential for elasmobranchs to succumb to protozoan parasites when exposed to heavy outbreaks affecting teleosts in the same water system.

Trypanosoma are long, spindle-shaped flagellates whose width is about that of a fish’s red blood cell. They have a single anterior flagellum, an undulating membrane, a nucleus, and a kinetoplast (a conspicuous part of the

Table 25.1. Protozoal parasites found in elasmobranchs, showing host species, site of infection, geographic locale where isolated, and source reference.

Protozoan species	Host species name	Host common name	Geographic locale	Site of infection	Reference
FLAGELLATES					
Family Blastodiniidae					
Amyloodinium ocellatum	*Dasyatis sabina*	Atlantic stingray	Aquarium (USA)	Gills	Lawler, 1980
Family Trypanosomatidae					
Trypanosoma carchariasi	*Carcharhinus* sp.	requiem sharks	Europe	Blood	Laveran, 1908
			Australia	Blood	Mackerras and Mackerras, 1961
Trypanosoma gargantua	*Hemiscyllium ocellatum*	epaulette shark	Australia	Blood	Mackerras and Mackerras, 1961
	Dipturus nasutus	rough skate	New Zealand	Blood	Laird, 1951
			Australia	Blood	Mackerras and Mackerras, 1961
Trypanosoma giganteum	*Dipturus oxyrinchus*	longnosed skate	Europe	Blood	Neumann, 1909
Trypanosoma humboldti	*Schroederichthys chilensis*	redspotted catshark	Chile	Blood	Morillas et al., 1987
Trypanosoma marplatensis	*Dasyatis microps*	smalleye stingray	Argentina	Blood	Bacigalupo and De la Plaza, 1948
Trypanosoma rajae	*Amblyraja radiata*	thorny skate	NW Atlantic	Blood	Laird and Bullock, 1969; So, 1972; Khan et al., 1980
	Centroscyllium fabricii	black dogfish	NW Atlantic	Blood	Khan et al., 1980
	Dipturus batis	skate	England	Blood	Coles, 1914
	Leucoraja erinacea	little skate	NW Atlantic	Blood	Bullock, 1958; Laird and Bullock, 1969
	Leucoraja ocellata	winter skate	NW Atlantic	Blood	Kudo, 1923
	Malacoraja senta	smooth skate	NW Atlantic	Blood	Khan et al., 1980
	Raja sp.	skates	Europe	Blood	Minchin and Woodcock, 1920
	Raja sp.	skates	England	Blood	Henry, 1913
	Raja asterias	starry ray	Europe	Blood	Laveran and Mesnil, 1902a; Neumann, 1909 (as *T. variable*)
	Raja clavata	thornback ray	Europe	Blood	Laveran and Mesnil, 1902a
	Raja undulata	undulate ray	Europe	Blood	Laveran and Mesnil, 1902a
Trypanosoma scyllii	*Scyliorhinus canicula*	smallspotted catshark	Europe	Blood	Pulsford, 1984
			England	Blood	Henry, 1913
	Scyliorhinus sp.	cat sharks	England	Blood	Coles, 1914
	Scyliorhinus stellaris	nursehound	Europe	Blood	Laveran and Mensil, 1902a
Trypanosoma sp.	*Raja clavata*	thornback ray	South Africa	Blood	Fantham, 1919
Family Bodonidae					
Ichthyobodo sp.	*Squalus acanthias*	spiny dogfish	Laboratory	Skin	Leibovitz and Lebovitz, 1985
	Mustelus canis	dusky smooth-hound	Laboratory	Skin	Leibovitz and Lebovitz, 1985
AMOEBA					
Unknown species	*Carcharhinus plumbeus*	sandbar shark	Aquarium (USA)	Liver	Mohan, pers. com.
Unknown species	*Stegostoma fasciatum*	zebra shark	Aquarium (USA)	Brain	St. Leger, pers. com.

Table 25.1 (continued). Protozoal parasites found in elasmobranchs, showing host species, site of infection, geographic locale where isolated, and source reference.

Protozoan species	Host species name	Host common name	Geographic locale	Site of infection	Reference
APICOMPLEXANS					
Family Haemogregarinidae					
Haemogregarina carchariasi	*Carcharhinus* sp.	requiem sharks	Europe	Blood cells	Laveran, 1908
			Australia	Blood cells	Mackerras and Mackerras, 1961
Haemagregarina dasyatis	*Dasyatis americana*	southern stingray	Bahamas	Blood cells	Saunders, 1958
Haemogregarina delagei	*Amblyraja radiata*	thorny skate	NW Atlantic	Blood cells	Laird and Bullock, 1969; So, 1972; Khan et al., 1980
	Leucoraja erinacea	little skate	NW Atlantic	Blood cells	Laird and Bullock, 1969
	Leucoraja ocellata	winter skate	NW Atlantic	Blood cells	Khan et al., 1980
	Malacoraja senta	smooth skate	NW Atlantic	Blood cells	So, 1972; Khan et al., 1980
	Raja asterias	starry ray	Europe	Blood cells	Laveran and Mesnil, 1902b
	Raja undulata	undulate ray	Europe	Blood cells	Laveran and Mesnil, 1902b
	Squalus acanthias	spiny dogfish	NW Atlantic	Blood cells	Laird and Bullock, 1969
Haemogregarina hemiscyllii	*Hemiscyllium ocellatum*	epaulette shark	Australia	Blood cells	Mackerras and Mackerras, 1961
Haemogregarina lobianci	*Torpedo marmorata*	marbled electric ray	Europe	Blood cells	Kohl-Yakimoff and Yakimoff, 1915
Haemogregarina torpedinis	*Torpedo torpedo*	common torpedo	Europe	Blood cells	Neumann, 1909
Haemogregarina sp.	*Dipturus batis*	skate	England	Blood cells	Coles, 1914
	Scyliorhinus sp.	cat sharks	England	Blood cells	Coles, 1914
Haemogregarina sp.	*Heterodontus portusjacksoni*	Port Jackson shark	Aquarium (specimen from Australia)	Blood cells	Pereira, pers. com.
Haemogregarina sp.	*Poroderma africanum*	striped catshark	Aquarium (specimen from Australia)	Blood cells	Pereira, pers. com.
Family Haemohormidiidae					
Haemohormidium sp.	*Centroscyllium fabricii*	black dogfish	NW Atlantic	Blood cells	Khan et al., 1980
Family Eimeriidae					
Eimeria euzeti	*Myliobatis aquila*	common eagle ray		Hepatic parenchyma	Daoudi et al., 1987
Eimeria gigantea	*Lamna nasus*	porbeagle shark		Spiral valve	Levine, 1985
Eimeria jiroveci	*Raja clavata*	thornback ray	Mediterranean	Intestine and spiral valve	Dykova and Lom, 1983
Eimeria lucida	*Mustelus canis*	dusky smooth-hound	Mediterranean	Spiral valve	Lom and Dyková, 1992 (as *Gloussia lucida*)
	Scyliorhinus canicula	smallspotted catshark	Mediterranean	Spiral valve	Lom and Dyková, 1992 (as *Gloussia lucida*)
	Squalus acanthias	spiny dogfish	Mediterranean	Spiral valve	Lom and Dyková, 1992 (as *Gloussia lucida*)
Eimeria quentini	*Aetobatus narinari*	spotted eagle ray	Malaysia	Nuclei of peritoneal cells	Boulard, 1977
Eimeria rajarum	*Dipturus batis*	skate	France	Posterior intestine	Van den Berghe, 1937
Eimeria scyllii	*Scyliorhinus stellaris*	nursehound	Mediterranean	Spiral valve	Levine, 1985

Table 25.1 (continued). Protozoal parasites found in elasmobranchs, showing host species, site of infection, geographic locale where isolated, and source reference.

Protozoan species	Host species name	Host common name	Geographic locale	Site of infection	Reference
Eimeria southwelli	*Aetobatus narinari*	spotted eagle ray	Indo-Pacific	Spiral valve of embryo	Halawani, 1930
	Rhinoptera bonasus	cownose ray	NW Atlantic	Serosa of viscera and uterus	Cheung, 1993b
			NW Atlantic and Aquaria	Coelomic cavity	Stamper et al., 1998
Eimeria squali	*Squalus acanthias*	spiny dogfish	NE Pacific	Spiral valve	Fitzgerald, 1975
Eimeria zygaenae	*Sphyrna zygaena*	smooth hammerhead	Indo-Pacific	Rectal contents	Mandal and Chakravarty, 1965
Eimeria sp. (?)	*Carcharias taurus*	sand tiger shark	Aquarium (South Africa)	Feces	Pereira, pers. com.
MICROSPORIDIA					
Unknown species	*Triakis semifasciata*	leopard shark	Aquarium (specimen from South Africa)	Gills, brain, pancreas, kidney, liver, and blood vessels	Garner et al., 1998
CILIATES					
Family Hartmannulidae					
Brooklynella sp.	*Heterodontus francisci*	horn shark	Aquarium (France)	Skin at the insertion of fins	Barthelemy, pers. com.
	Heterodontus galeatus	crested bullhead shark	Aquarium (France)	Skin at the insertion of fins	Barthelemy, pers. com.
Family Trichodinidae					
Trichodina oviducti	*Amblyraja radiata*	thorny skate	Newfoundland	Oviducts and claspers	Khan, 1972
	Leucoraja ocellata	winter skate	Newfoundland	Oviducts and claspers	Khan, 1972
	Raja sp.	skates	SW Atlantic	Oviducts	Evdokimova et al., 1969
Trichodina rajae	*Dasyatis sabina*	Atlantic stingray	SW Atlantic	Oviducts	Evdokimova et al., 1969
Family Scyphiidae					
Caliperia brevipes	*Leucoraja erinacea*	little skate	NW Atlantic	Gills	Laird, 1959
Family Uronematidae					
Cryptocaryon sp.	*Mustelus canis*	dusky smooth-hound	Laboratory	Skin	Leibovitz and Lebovitz, 1985
	Squalus acanthias	spiny dogfish	Laboratory	Skin	Leibovitz and Lebovitz, 1985
Uronema marinum (?)	*Dasyatis americana*	southern stingray	Captive born	Gills	Cheung, 1993a
Uronema sp.	*Dasyatis americana*	southern stingray	Aquarium (USA)		Stuart, pers. com.
			Aquarium (USA)	Kidneys	Terrell, pers. com.

mitochondria that stains similarly to the nucleus because of the presence of an abundant amount of DNA). Marine trypanosoma species exhibit polymorphism, or many morphological forms, which can make speciation challenging. Trypanosomes usually live in the plasma portion of blood, and hence are referred to as hemoflagellates; however, they may also live in other body fluids and may sometimes be found intracellularly. Trypanosomes are transmitted by leeches through direct inoculation of a host during bloodsucking activities or may be transmitted through the gastrointestinal system if the host ingests the invertebrate vector. In the vector, trypanosomes live in the intestines.

Trypanosomiasis in teleosts is associated with changes, including: blood values (decreased protein, red blood cell counts (RBC), and hemoglobin, and increased white blood cell counts (WBC) and globulin); generalized edema (fluid accumulation within tissues); weight loss; and, mortality. A short prepatent period (the time between infection of the host and the earliest time at which the parasitic organism can be recovered from the host) of 2-9 days is followed by days to weeks of increasing parasitemia (parasites in the blood) as the trypanosomes replicate. Heavy infections may result in death, especially in young fish, or a chronic phase with slowly diminishing numbers of parasites and an eventual absence from peripheral blood. Recovered fish have been known to suffer sudden, severe relapses, possibly related to stress. Trypanosomes have been reported in a number of skates and sharks with several species having the ability to infect multiple host species (Table 25.1). Those parasites listed as infecting only one host species may in fact be synonymous with other parasite species, due to the early practice of assigning species status to populations of trypanosomes found in different species of fishes. Recent studies in teleosts have indicated that strict host specificity is the exception.

Amoebas

Most amoebas are free-living species, while others are commensal and nonpathogenic and a few are parasitic. Free-living and commensal forms may become pathogenic if present in high concentrations on an immuno-compromised animal. Amoebas have an irregular, changing shape with one or more pseudopodia (temporary protrusions of a cell's cytoplasm) which are used for both locomotion and feeding.

Amoebiasis in teleosts occurs in gills and intestines, sometimes progressing to systemic disease. Gill infections can cause grossly visible changes and mortalities due to gill dysfunction. There are no reports in the literature of amoebiasis in elasmobranchs; however, two suspected cases of amoebas parasitizing the liver and the brain have been reported via personal communication (Table 25.1).

Apicomplexans

Apicomplexans are parasitic protozoa that possess a set of organelles, at the apex of the cell, used in the process of invading host cells. These organelles, called an apical complex, are visible only with a transmission electron microscope. Life cycles are variations of merogony, gametogony, and sporogony. Merogony is an asexual stage in which multiple fissions produce multiple, identical daughter merozoites. Gametogony is a developmental stage in which merozoites transform into "male" microgametocytes or "female" macrogametocytes; fusion or fertilization of these gametes produces a zygote, a process also termed syngamy. Sporogony is a second asexual stage involving multiple fission of the zygote and produces the sporozoite filled oocyst. All species are parasitic (Lom and Dyková, 1992).

Haemogregarina are primitive apicomplexan intracellular parasites (taxonomically within the class Coccidea) infecting red or white blood cells of vertebrate hosts, and the intestinal epithelium of invertebrate vectors such as leeches or hematophagous crustaceans. Like trypanosomes, haemogregarines are transmitted by direct inoculation to a host during bloodsucking activities, or may be transmitted through the gastrointestinal system if the host ingests the invertebrate vector. Developmental stages may escape from the disrupted membrane of host blood cells but then enter other blood cells. The parasite is typically adjacent to, and about the same size as, the nucleus but may attain a sufficient size to displace and disintegrate the nucleus and distort the cell's shape. In teleost fishes, most infections are slight and chronic, but some species of haemogregarines are known to be serious pathogens. The prevalence of hemogregarines is high in marine fish (Khan et al., 1980). *Haemogregarina delagei* has been reported in spiny dogfish, *Squalus acanthias*, and skates, *Raja* spp. (Laird and Bullock, 1969). A

number of other species of haemogregarines have been found in other elasmobranch species and have been named after their host (Table 25.1).

Eimeria spp. are apicomplexan intracellular parasites (taxonomically within the class Coccidea, and sometimes commonly referred to as coccidia with the infection being called coccidiosis), occurring in many different kinds of cells. In the invaded cell, merogony, or asexual multiple fission, occurs rapidly, thus increasing the number of organisms, called merozoites, which are released from one host cell and go on to infect more host cells. Some merozoites begin the sexual phase of the life cycle, developing into either macro- or microgametocytes (male and female, respectively), and are released from the host cell when fully developed. Fertilization occurs extracellularly and produces a zygote. The zygote, or oocyst, undergoes further development to form the infective stage, sporozoites, which are usually contained within sporocysts in the oocyst. Enzymatic action within the host's digestive tract breaks down ingested oocysts, releasing sporozoites which then go on to invade host cells in intestinal and/or extra-intestinal sites. Infected intestinal mucosa show different degrees of sloughing and necrosis due to the rapid multiplication of organisms, secondary bacterial infections, and hemorrhaging. In piscine coccidian infections, oocysts often sporulate (i.e., form sporozoites in sporocysts) within the host. Oocyst structure assists in the correct identification of protozoan genera and species.

Unlike infection in endothermic (heat generating) animals, piscine coccidia can be extra-intestinal and do not always show strict organ specificity. Several species of *Eimeria* spp. have been reported in skates, rays, and sharks, infecting many different tissues. *Eimeria southwelli* has been found to occur as an asymptomatic infection, at high incidence (i.e., 92%), in the coelomic cavities of wild-caught cownose rays (*Rhinoptera bonasus*), and has been associated with pathogenic outbreaks secondary to stress in captivity (Stamper et al., 1998).

Microsporidia

Microsporidia are intracellular parasites with unicellular spores and an elaborate hatching apparatus. Mitochondria are completely absent throughout its life cycle. Microsporidia interchange reproductive phases, alternating merogony, a proliferative phase producing a great number of parasites, with sporogony, which gives rise to mature spores. Microsporidia typically stimulate tremendous hypertrophy, or enlargement, of the infected host cell, forming structures called xenomas. Identification to genus is difficult and may require electron microscopy. Reports of microsporidiosis in elasmobranchs is limited to a single case in leopard sharks, which was diagnosed in a captive animal by histopathological examination of a gill biopsy after finding it in several tissues of deceased tank mates post-mortem (Garner et al., 1998).

Ciliates

The most conspicuous feature of ciliates (phylum Ciliophora) is the presence of simple cilia or ciliary organelles, which are used for several different functions including locomotion, adhesion to substrate, tactile perception, and movement of food particles. Cilia may cover the entire organism, be present on only some regions, or may be absent during certain phases of the life cycle. Ciliates have two or more nuclei, with at least one macronucleus and micronucleus. Division is usually by transverse binary fission (splitting in half), with occasional conjugation in which two cells form a temporary union to transfer genetic material. Numerous species are free-living but there are many commensal and parasitic species. The parasitic species lack host specificity, are ubiquitous, and can reach high densities in closed systems. In teleost fishes, light infections result in irritation of surface cells where the ciliates attach. If the infection is heavy or persists, destruction of gill and skin tissue leads to respiratory difficulties, secondary bacterial infections, and osmotic problems. Ciliates can penetrate into layers of deep tissues.

Ciliates are rarely reported in elasmobranchs. However, under unusual circumstances, elasmobranchs have been shown to be susceptible to this group of protozoans. Spiny dogfish and dusky smooth-hounds (*Mustelus canis*) have been infected with *Cryptocaryon* sp. under laboratory conditions (Leibovitz and Leibovitz, 1985). An unidentified ciliate (possibly *Uronema marinum*) invaded the gill tissues of a captive-born juvenile southern stingray (*Dasyatis americana*) (Cheung 1993a). Two unusual ciliates, *Trichodina oviducti* and *T. rajae*, have been found in the oviducts and seminal grooves of skates causing a mucopurulent discharge

(Evdokimova et al., 1969; Khan, 1972). *Caliperia brevipes* was found on the gills of a wild-caught little skate (*Leucoraja erinacea* = *Raja erinacea*) (Laird, 1959). Other infections have been reported as personal communications (Table 25.1).

TREATMENT

Only two reports of treatment regimes occur in the literature, and these as parts of case reports rather than rigorous pharmacological studies. Toltrazuril (e.g., Baycox®, Bayer Corporation, USA), a triazinetrione derivative used in domestic animals for the prevention and treatment of coccidiosis, was used to treat microsporidiosis in a leopard shark, which responded to treatment and was considered healthy at the time of the report (Garner et al., 1988). The same drug was used to treat a coccidial infection, *Eimeria* sp., in cownose rays and produced a resolution of clinical signs, but diagnostic samples taken from asymptomatic animals 11 months post-treatment were positive (Stamper et al., 1988). Additional studies are urgently needed to determine the pharmacodynamics, pharmacokinetics, safety, and efficacy of anti-protozoal drugs used with elasmobranchs.

REFERENCES

Bacigalupo, J. and N. De la Plaza. 1948. Presencia de tripanosomas en las rays de Mar del Plata. Revista de la Sociedad Argentina de Biologia 24: 269-274.

Boulard, Y. 1977. Description d'Eimeria quentini n. sp., parasite intranucleaire du peritoine doo la raie: *Aetobatis narinari* (Chondrichthyens, Myliobatidae) en malaise. Protistologica 13: 529-533.

Bullock, W. L. 1958. The blood protozoa of the marine fish of Southern New England. Journal of Parasitology 44: 24-25.

Cavalier-Smith, T. 1993. The kingdom Protozoa and its 18 phyla. Microbiology Review 57: 953-994.

Cheung, P. 1993a. Parasitic Diseases of Elasmobranchs. *In:* Fish Medicine, p. 782-807. M. K. Stoskopf (ed.). W. B. Saunders, Inc., Philadelphia, Pennsylvania, USA. 882 p.

Cheung, P. 1993b. *Eimeria southwelli* Halawani, 1930 (Apicomplexa: Coccidia) from a new host, the cownose ray, *Rhinoptera bonasus* Mitchill. Journal of Aquariculture and Aquatic Sciences 6: 68-75.

Coles, A. C. 1914. Blood parasites found in mammals, birds, and fishes in England. Parasitology 7: 17-61.

Daoudi, F., B. Radujkovic, A. Marques, and G. Bouix. 1987. Nouvelle espèces de Coccidies (Apicomplexa, Eimeriidae) des genres Eimeria Schneider, 1875, et Epieimeria Dyková et Lom, 1981, parasites de poisons marins de la baie de Kotor (Yougoslavie). Bulletin du Museum National d'Histoire Naturelle, Paris. 4 ser., 9: 321-332.

Dyková, I. and J. Lom. 1983. Fish coccidia: An annotated list of described species. Folia Parasitologica 30: 193-208.

Evdokimova, E. B., I. G. Kuznetzora, and G. A. Stein. 1969. Parasitic ciliates of the family Urceolariidae (Peritricha, Mobilia) from some fishes of the southwest Atlantic. Zoologicheskii Zhurnal 48: 1451-1455.

Fantham, H. B. 1919. Some parasitic protozoa found in South African fishes and amphibians. South African Journal of Science 15: 337-338.

Fitzgerald, P. R. 1975. New coccidian from the spiny dogfish shark (*Squalus acanthias*) and great sculpin (*Myoxocephalus polyacanthocephalus*). Journal of the Fisheries Research Board of Canada 32: 649-651.

Gardiner, C. H., R. Fayer, and J. P. Duber. 1988. An atlas of protozoan parasites in animal tissues. Agriculture Handbook, no 651, U.S. Department of Agriculture, Washington D.C., USA. 83 p.

Garner, M. M., T. J. Miller-Morgan, S. R. Brown, R. Reimschuessel, S. L. Poynton, T. I. Baldwin, and R. E. Olson. 1998. Microsporidiosis in Leopard Sharks. *In:* Proceedings of the Third International Symposium on Aquatic Animal Health. August 30-September 3, 1998. University of Maryland Aquatic Pathobiology Center, Baltimore, Maryland. Abstract. 145 p.

Halawani, A. 1930. On a new species of Eimeria (*E. southwelli*) from *Aetobatis narinari*. Annals of Tropical Medicine and Parasitology 24: 1-3.

Henry, H. 1913. A summary of the blood parasites of British sea fish. Journal of Pathological Bacteriology 18: 218-223.

Khan, R. A. 1972. Taxonomy, prevalence, and experimental transmission of a protozoan parasite, *Trichodina oviducti* Polyanskii (ciliata: Peritrichida) of the thorny skate, *Raja radiata* Donovan. Journal of Parasitology 58: 680-685.

Khan, R. A., M. Barrett, and J. Murphy. 1980. Blood parasites of fish from the northwestern Atlantic Ocean. Canadian Journal of Zoology 58: 770-781.

Kohl-Yakimoff, N. and W. L. Yakimoff. 1915. Hamogregarinen der Seefische. Centrablatt für Bakteriologie, Parasitenkunde und Infektionskrankheiten 76: 134-146.

Kudo, R. 1923. Skate trypanosome from Woods Hole. The Journal of Parasitology 9: 179-180.

Laird, M. 1951. Studies on the trypanosomes of New Zealand fish. Proceedings of the Zoological Society of London 1221: 285-309.

Laird, M. 1959. *Caliperia brevipes* n. sp. (Ciliata: Peritricha) epizoic on *Raja erinacea* Mitchell at Saint Andres, New Brunswick. Canadian Journal of Zoology 37: 283-288.

Laird, M. and W. L. Bullock. 1969. Marine fish haematozoa from New Brunswick and New England. Journal of the Fisheries Research Board of Canada 26: 1075-1102.

Laveran, A. 1908. Sur une hemogrégarine, un trypansome et un spirille, trouvés dans le sang d'un requin. Bulletin de la Société de Pathologie Exotique 1: 148-50.

Laveran, A. and F. Mensil. 1902a. Des Trypanosomes des Poissons. Archiv für Protistenkunde 1: 496-498.

Laveran, A. and F. Mesnil. 1902b. Sur les Hématozoaires des Poissons marins. Compté rendus hebdomadaires des séances d'Académie des Sciences, Paris 135: 567-570.

Lawler, A. R. 1980. Studies on *Amyloodinium ocellatum* (Dinoflagellata) in Mississippi Sound: Natural and experimental hosts. Gulf Research Reports 6: 403-413.

Lee, J. J., G. F. Leedale, and P. Bradbury. 2002. Illustrated Guide to the Protozoa (2nd Edition). Society of Protozoologists, Lawrence, Kansas, USA. 1432 p.

Lee, J. J. and A. T. Soldo. 1992. Protocols in Protozoology, Society of Protozoologists, Lawrence, Kansas, USA. 652 p.

Leibovitz, L. and S. S. Leibovitz. 1985. Skin diseases of sharks. *In:* Sharks: Recent Advances in Captive Biology, A Symposium on the Captive Maintenance of Elasmobranchs. An International Meeting. March 31-April 3, 1985. Organized by the National Aquarium in Baltimore and Department of Pathology, University of Maryland School of Medicine, Baltimore, Maryland, USA. Abstract. 6 p.

Levine, N. D. 1985. Erhardorina n.g., *Ascogregarina polynesiensis* n. sp., *Eimeria golemanskii* n. sp., *Isospora tamariscini* n. sp., *Gregarina kazumii* n. nom., New combinations and emendations in the names of Apicomplexan protozoa. Journal of Protozoology 32: 359-363.

Lom, J. and I. Dyková. 1992. Protozoan Parasites of Fishes. Elsevier Publishers, Amsterdam, Netherlands. 315 p.

Mackerras, M. J. and I. M. Mackerras. 1961. The haematozoa of Australian frogs and fish. Australian Journal of Zoology 9: 123-139.

Mandal, A. K. and M. M. Chakravarty. 1965. A new coccidium *Eimeria zygaena* from hammer headed shark, *Zygaena blochii*. Science and Culture 31: 381-382.

Minchin, E. A. and H. W. Woodcock. 1920. Observations on certain blood parasites of fishes occurring at Rovigno. Quarterly Journal of Microscopical Science 55: 113-154.

Morillas, J., M. George-Nascimento, H. Valeria, and R. A. Khan. 1987. *Trypanosoma humboldti* n. sp. from the Chilean catshark, *Schroederichthys chilensis* (Guichenot, 1848). Journal of Protozoology 34: 342-344.

Neumann, R. O. 1909. Studien uber protozoische parasiten im blut von meeresfischen. Zeitschrift fur Hygiene und Infektionskrankheiten 64: 1-112.

Poynton, S. L. and E. Sterud. 2002. Guidelines for species description of diplomond flagellates from fish. Journal of Fish Diseases 25: 15-31.

Pulsford, A. 1984. Preliminary studies on trypanosomes from the dogfish, *Scyliorhinus canicula* L. Journal of Fish Biology 24: 671-682.

Roberts, L.S. and J. Janovy. 2000. Gerald D. Schmidt & Larry S. Roberts' Foundations of Parasitology, 6th edition, McGraw Hill, Boston, Massachusetts, USA. 670 p.

Saunders, D. C. 1958. The occurrence of *Haemogregarina bigemina* Laveran and Mesnil and H. *Dasyatis* n. sp. in marine fish from Bimini, Bahamas, BWI. Transactions of the American Microscopical Society 77: 404-412.

So, B. K. F. 1972. Marine fish haematozoa from Newfoundland waters. Canadian Journal of Zoology 50: 543-554.

Stamper, M. A., G. A. Lewbart, P. R. Barrington, C. A. Harms, F. Geoly, and M. K. Stoskopf. 1998. *Eimeria southwelli* infection associated with high mortality of cownose rays. Journal of Aquatic Animal Health 10: 264-270.

Van den Berghe, L. 1937. On a new coccidian, *Eimeria rajarum* sp. n. with special reference to the biology of the oocysts. Parasitology 29: 7-11.

Woo, P. T. K. 1969. The haematocrit centrifuge for the detection of trypanosomes in blood. Canadian Journal of Zoology 47: 921-923.

PERSONAL COMMUNICATIONS

Barthelemy, D. 2001. Océanopolis, Brest, F-29275, France.

Mohan, P. 2001. 5802 Thorndale Dr., Kent, OH 44240, USA.

Pereira, N. 2001. Oceanário de Lisboa, 1990-005 Lisboa, Portugal.

St. Leger, J. 2001. Sea World, San Diego, CA 92109, USA.

Stuart, M. 2001. Oklahoma Aquarium, Jenks, Oklahoma 74037, USA.

Terrell, S. 2001. Disney's Animal Kingdom, Bay Lake, FL 32830, USA.

The Elasmobranch Husbandry Manual: Captive Care of Sharks, Rays and their Relatives, pages 427-431.
© 2004 Ohio Biological Survey

Chapter 26
An introduction to Viral, Bacterial, and Fungal Diseases of Elasmobranchs

SCOTT P. TERRELL

Veterinary Services - Disney's Animal Kingdom,
1200 N Savannah Circle,
Bay Lake, FL 32830, USA.
Email: Scott.P.Terrell@disney.com

Abstract: Compared to some other aspects of elasmobranch biology, the study of infectious diseases in this group is in its infancy. Diagnostic sampling of sick or dead animals should be considered an essential aspect of any husbandry program. Viral diseases are characterized by rapid onset, disease in multiple individuals, and often a stressful event that causes immunosuppression. Viral diseases are among the most difficult diseases to diagnose. There are currently no recognized treatment or control methods specific to viral diseases. General recommendations include supportive care and reducing stress. Outbreaks of bacterial disease are relatively uncommon in elasmobranchs. Reports of bacterial disease caused by *Vibrio* spp. are the most common. In contrast to viral diseases, most bacterial diseases can be diagnosed by bacterial culture of blood or diseased tissues such as the brain, liver, kidney, or spleen. Bacterial culture and antibiotic sensitivity testing may help guide antibiotic choice for elasmobranchs. Fungal diseases are typically characterized by disease in an individual animal rather than explosive outbreaks affecting multiple animals. Fungal agents can cause disease in all age groups of animals (in contrast to bacteria and viruses that commonly infect young or old individuals). Treatment of fungi is difficult, and prevention is the best tool for management. The occurrence of fungal disease may be reduced by maintaining susceptible sharks in appropriate environments (i.e., appropriate water quality and temperature regimes) and by reducing stress associated with capture or handling. Skin wounds are a potential portal of entry for fungi, so skin wounds should be avoided or closely monitored when they do occur.

Compared to some other aspects of elasmobranch biology, the study of infectious diseases in this group is in its infancy. Reports of viral, bacterial, and fungal diseases are few in number and often involve case reports of disease in single animals. It is doubtful that this lack of data on infectious diseases of elasmobranchs reflects the true situation, but rather suggests a lack of data collection and diagnostic sampling by people studying the animals, and institutions holding or displaying them. Much of the information in this chapter is taken from the author's personal experience, communication with colleagues, and most importantly from the book *Fish Medicine* by Stoskopf (1993). In addition, an attempt has been made by the author to sample the scientific literature available on this topic for background on various organisms and the diseases they cause.

This chapter will provide information on basic disease biology, brief descriptions of various organisms reported from elasmobranch species, descriptions of disease syndromes, and methods of diagnosis and sampling for animals suspected to have viral, bacterial, or fungal disease.

Diagnostic sampling of sick or dead animals should be considered an essential aspect of any husbandry program. It is only by attempts to cultivate and identify microorganisms that medical and biology professionals can gather baseline data. Furthermore, I encourage professionals studying elasmobranchs to publish findings concerning infectious disease in peer reviewed scientific journals so that information can be disseminated. There exists an abundance of experience and knowledge that cannot be shared without publication.

A more complete discussion of medications successfully used with elasmobranchs, and the diseases treated, is provided in Chapter 29 of this manual.

VIRUSES AND VIRAL DISEASES

Viruses are among the smallest infectious agents and often are responsible for explosive outbreaks of disease in both human and animal populations, including fishes. Several viral diseases of teleosts are well characterized as causes of significant disease outbreaks. In general, most viral diseases are characterized by rapid onset, disease in multiple individuals, and often a stressful event that causes immunosuppression. Viral diseases are among the most difficult diseases to diagnose. Cultivation and identification of viruses require specialized techniques and equipment. In general, viral diseases are not treatable with conventional medications or antibiotics and it is up to the immune system of the infected individual to "fight" the virus so that healing and recovery may begin.

Viral diseases

Two different viral diseases have been reported in elasmobranchs, with most reports coming from the dusky smooth-hound (*Mustelus canis*). These diseases, although uncommon to rare and poorly studied, exemplify some of the common characteristics of viral disease.

A viral skin disease has been reported in the dusky smooth-hound, affecting a small percentage of animals. The disease is characterized by areas (~1-10 mm diameter) of white to gray skin discoloration (McAllister and Stoskopf, 1993; Leibovitz and Leibovitz, 1985). There are no recognized systemic effects in infected animals. A herpes virus has been identified in association with this disease (Leibovitz and Leibovitz, 1985). The disease typically manifests itself following a stressful event and poses no significant risk to the victim. The disease will spontaneously resolve itself after a variable period of time. Diagnosis of this disease was made by microscopic and ultra-structural (electron microscope) exams of abnormal skin tissue (Leibovitz and Leibovitz, 1985).

The other viral disease reported from elasmobranchs is a disease reported in dusky smooth-hound and leopard sharks (*Triakis semifasciata*) (Johnston, 1975; Kahn and Newman, 1982). The disease is called viral erthryocytic necrosis; it is a viral infection of red blood cells, the oxygen carrying cells of the blood. Animals infected with this virus may become ill or die, with evidence of destroyed red blood cells (hemolysis) and post-mortem evidence of pale organs (McAllister and Stoskopf, 1993). Viral erthryocytic necrosis typically affects young animals with no immunity to the virus. The disease is caused by an iridovirus. The virus specifically targets and attacks red blood cells and can be diagnosed by finding characteristic cellular changes in a blood smear (McAllister and Stoskopf, 1993). Infected cells often contain large intra-cytoplasmic (within the cytoplasm, not the nucleus) "inclusions" in routinely stained blood smears.

Husbandry recommendations

It is easily appreciated that the extent of knowledge concerning viral diseases of elasmobranchs is extremely limited. The study and diagnosis of viral diseases is difficult. However, this fact should not discourage people and organizations housing elasmobranchs from attempting diagnosis of these diseases.

Certain characteristics of a disease or disease outbreak may suggest a viral cause:

1. Disease or death occurs in a young immunologically naive animal or animals.
2. Disease or death occurs in multiple animals over a short period of time.
3. Disease or death occurs shortly after introducing a new specimen or species to the collection.
4. Disease or death occurs following a stressful event (e.g., capture and transport, environmental or water quality changes, unusual social interactions, etc.).
5. Outbreak of disease fails to respond to an appropriate antibiotic therapy or treatment.

If you suspect an outbreak of viral disease in an elasmobranch collection, certain practices may help improve your chances of diagnosis. Clinical samples such as blood or tissue biopsies should be taken from sick animals and sent to a laboratory that specializes in diseases of fishes. Some state or commercial diagnostic laboratories may be comfortable with fish submissions. Collect and freeze tissue, whole blood, and serum samples at -70 °C for viral culture attempts.

A complete post-mortem examination should be performed on animals that die. Tissues such as brain, liver, kidney, heart, or any diseased tissue should be frozen at -70 °C for viral culture attempts. In addition, tissues should be collected into 10% neutral buffered formalin for histopathology. Samples can be small (<1 cm^3) and still be valuable.

Treatment and control

There are currently no recognized treatment or control methods specific to viral diseases. General recommendations include supportive care and minimizing stress.

BACTERIA AND BACTERIAL DISEASES

Bacteria are single-celled organisms that are responsible for disease in a variety of animal species including teleosts and elasmobranchs. Bacterial diseases of elasmobranchs are the most thoroughly studied of the diseases discussed in this chapter. In contrast to other species, elasmobranchs (sharks specifically) may have a unique relationship with bacteria. While it is quite common in all species to find "commensal" bacteria living on the skin surface or within the gastrointestinal tract, sharks are unique in that bacteria are commonly identified within internal organs such as liver and muscle with no evidence of disease (Knight et al., 1987). In other words, bacteria can grow on tissues such as the livers, muscles, and kidneys of absolutely healthy animals. It is theorized that many of these bacteria use or aid in metabolism of urea produced as a normal by-product of shark metabolism.

Another unique aspect of bacteria and elasmobranchs is the relationship between shark bites and bacterial infection in humans. Bacterial infection commonly follows shark bite injury in humans and this secondary infection can often be quite serious. All shark bites should be considered contaminated wounds with high potential for "infection." These wounds, regardless of severity, should be treated by appropriate medical personnel.

Bacterial disease and elasmobranchs

Outbreaks of bacterial disease are relatively uncommon in elasmobranchs. Reports of bacterial disease caused by *Vibrio* spp. are the most common.

Aeromonas salmonicida, a common teleost pathogen, has been documented as a cause of disease in a blacktip reef shark (*Carcharhinus melanopterus*) (Briones et al., 1998).

Flavobacterium sp. was recently isolated by the author from bonnethead shark (*Sphyrna tiburo*) pups showing evidence of neurologic disease. Disease caused by *Flavobacterium* spp. in teleosts results from a toxin produced by the bacteria (Stoskopf, 1993).

Several species of *Vibrio* spp. have been implicated as disease agents in sharks. The most common *Vibrio* sp. isolated from sharks is *Vibrio carchariae* (Grimes et al., 1984; Stoskopf, 1993). *Vibrio carchariae* has been repeatedly implicated as the cause of meningitis (inflammation of the outer covering of the brain) in sand tiger (*Carcharias taurus*), lemon (*Negaprion brevirostris*), and sandbar (*Carcharhinus plumbeus*) sharks, and the spiny dogfish (*Squalus acanthias*) (Stoskopf, 1993). The trematode, *Dermophthirius* sp., has been implicated as a vector of *Vibrio carchariae*, transmitting the disease from shark to shark (Grimes et al., 1984). *Vibrio carchariae* has been isolated from sandbar sharks in association with chronic skin ulcers. A variety of *Vibrio* spp. was isolated from Port Jackson (*Heterodontus portusjacksoni*) and epaulette (*Hemiscyllium ocellatum*) sharks, and southern fiddler rays (*Trygonorrhina fasciata*), that died following a change in salinity (Callinan, 1988).

Husbandry recommendations

In contrast to viral diseases, most bacterial diseases can be diagnosed by bacterial culture of blood or diseased tissues such as the brain, liver, kidney, or spleen. In the author's experience, bacterial culture of cerebrospinal fluid has been a useful diagnostic tool in sharks showing evidence of neurologic disease. *Vibrio* spp. require specialized techniques for isolation, so a diagnostic lab should be contacted prior to submission of samples.

It is important to remember the unique relationship between elasmobranchs and bacteria when interpreting bacterial culture results from sick fishes (i.e., it is always possible to isolate certain species of bacteria from the tissues of healthy sharks). Culture results should be interpreted in combination with a clinical history of illness or death, and evidence of disease in ante- or post-mortem tissues.

Certain characteristics of a disease or disease outbreak may suggest a bacterial cause:

1. Bacterial diseases can be sudden in onset.
2. Disease may be seen in individual animals or whole populations.
3. Disease may be initiated by a stressful event (e.g., an environment or water quality change, movement to a new environment, other diseases, etc.).
4. Lesions, such as "boils", abscesses, or skin hemorrhages, may be observed.
5. Sick animals may respond to appropriate antibiotic treatment.

If you suspect an outbreak of bacterial disease in an elasmobranch collection, certain practices may help improve your chances of diagnosis. Clinical samples, such as blood or biopsies of skin lesions should be taken from sick animals and sent to a laboratory that specializes in diseases of fishes. Contact the diagnostic lab prior to submission of samples to be sure it is equipped with specialized media and techniques to isolate *Vibrio* spp. Culture of blood or abnormal tissues (e.g., abscesses, "boils," ulcers, etc.) should be made. Blood collected ante- or post-mortem can be submitted for bacterial culture.

A complete post-mortem examination should be performed on animals that die. Small samples of tissues should be collected aseptically during the post-mortem exam for bacterial culture. Additional tissue samples should be fixed in 10% neutral buffered formalin for histopathology. Isolation of the brain and culture of the cerebrospinal fluid may be valuable. This practice is essential if the shark had evidence of neurologic disease prior to death.

Treatment and control

Few people have experience with treatment of bacterial diseases in elasmobranchs. Some pharmacokinetic studies have been done with antibiotics and sharks to determine appropriate drug dosing and treatment intervals (Stoskopf *et al.*, 1986). Bacterial culture and antibiotic sensitivity testing may help guide antibiotic choice in elasmobranchs.

The reader is directed to Chapter 29 of this manual for more information about the treatment of bacterial diseases.

FUNGI AND FUNGAL DISEASES

Fungi are common pathogens of a wide variety of species including fishes. Fungal diseases are typically characterized by disease in an individual animal rather than explosive outbreaks affecting multiple animals. Fungi are transmitted via environmental exposure (i.e., contact with contaminated soil in the case of mammals or contaminated water in the case of fishes). The most common fungal pathogen of teleost fishes is *Saprolegnia* spp., an opportunistic environmental pathogen that typically colonizes damaged skin, gill, or fin tissue. However, these oomycetes cannot tolerate saltwater. Fungal agents can cause disease in all age groups of animals (in contrast to bacteria and viruses that commonly infect young or old individuals).

Fungal diseases of elasmobranchs

A specific fungal disease is well characterized in the bonnethead shark. Known as "bonnethead shark disease" this syndrome is the result of infection by the fungus *Fusarium solani*. In addition to bonnethead sharks, this disease has been described in scalloped hammerhead sharks (*Sphyrna lewini*). Affected sharks develop white pustules (or pimples) along their lateral line as well as other sites on the skin and cephalofoil ("bonnet"). In some cases skin erosions or ulcers may be seen. There may be hemorrhages or swelling of the skin as well as hemorrhage into deep muscle or cartilage. Disease in sharks typically follows some environmental change or stressor. In the author's experience, disease caused by *Fusarium* spp. is seen when shallow, warm-water sharks have been kept in deeper, cold water aquarium systems. The disease is progressive, and attempts to treat affected sharks with anti-fungal medication have been unsuccessful to date. Affected sharks die with evidence of deep invasion of the fungus into skin, muscle, cartilage and, occasionally, internal organs.

Once a fungal disease is suspected in an animal, the diagnosis can be made by examination of fluid collected from pustules or from dermal pores adjacent to affected areas of skin. In the author's experience, a bloody red fluid can often be squeezed, with a light pressure, from dermal pores around the head or cephalofoil. This fluid contains fungal elements that are visible by light microscopy. The fluid may be submitted for fungal culture at an appropriate laboratory.

Husbandry recommendations

Certain characteristics of a disease or disease outbreak may suggest a fungal cause:

1. Disease occurs in an animal at any age.
2. Disease occurs in a single animal.
3. White pustules, pimples, or ulcers are seen on the skin.
4. Disease occurs following handling or other stressful event (e.g., reduction in water temperature, injury to the skin, movement to new environment, etc.)
5. Disease fails to respond to appropriate antibiotic therapy or treatment.

If you suspect an outbreak of fungal disease in an elasmobranch collection, certain practices may help improve your chances of diagnosis. Clinical samples, such as fluids from pustules and pimples, or tissue biopsies, should be taken from sick animals and sent to a laboratory that specializes in diseases of fishes. These samples should be cultured for fungi. Some labs may have to send fungal cultures to specialty labs for identification. A rapid initial diagnosis may be achieved by examining fluid collected from pustules or pimples under a microscope.

Treatment and control

As mentioned previously, treatment of fungi is difficult. Some anecdotal success has been reported for *Fusarium* sp. infections in bonnethead sharks by increasing water temperatures. Because of the difficulty of treatment of this disease, prevention is the best tool for management. The occurrence of this disease may be reduced by maintaining susceptible sharks in appropriate environments (i.e., appropriate water quality and temperature regimes) and by reducing stress associated with capture or handling. Skin wounds are a potential portal of entry for fungi, so skin wounds should be avoided or closely monitored when they do occur.

The reader is directed to Chapter 29 of this manual for more information about the treatment of fungal diseases.

QUARANTINE

Quarantine is an important tool for disease prevention when dealing with infectious viral, bacterial, fungal, or parasitic diseases. Quarantine should be imposed any time a new animal is transferred from the wild, another institution, or one independent system to another in the same institution.

A quarantine period allows for detection of disease agents possibly introduced from a newly acquired animal to animals within the existing collection. Quarantine promotes a recovery period for animals following transport, acclimatization of animals into a new environment, and recovery of the immune system, thus increasing resistance to novel pathogens. A minimum 30-day quarantine period is recommended for elasmobranchs. The extent of diagnostic testing (e.g., blood sampling, bacterial culture, fecal examination, etc.) performed during quarantine should be determined at each individual institution. The reader is directed to Chapter 10 of this manual for more information about elasmobranch quarantine regimes.

REFERENCES

Briones, V., A. Fernandez, M. Blanco, F. Ramiro, J. Garcia, J. L. Mendez, J. Goyache, and M. L. De Vincente. 1998. Hemorrhagic septicemia by *Aeromonas salmonicida salmonicida* in a black-tip reef shark (*Carcharhinus melanopterus*). Journal of Veterinary Medicine 45(7): 443-445.

Callinan, R. B. 1988. Diseases of Australian native fishes. *In:* Fish Diseases: Refresher Course for Veterinarians, Proceeding 106, p. 459-472. Post Graduate Committee of Veterinary Science, University of Sydney, Australia.

Grimes, D. J., J. Stemmler, H. Hada, D. Maneval, F. M. Hetrick, E. B. May, R. T. Jones, M. K. Stoskopf, and R. R. Colwell. 1984. Vibrio species associated with mortality of sharks held in captivity. Microbial Ecology 10: 271-282.

Johnston, M. R. L. 1975. Distribution of *Pirhemocyton:* Chatton and Blanc, and other, possibly related, infections of poikilotherms. Journal of Protozoology 22: 529-535.

Kahn, R. A. and M. W. Newman. 1982. Blood parasites from fish of the Gulf of Maine to Cape Hatteras, Northwest Atlantic Ocean, with notes on the distribution of fish hematozoa. Canadian Journal of Zoology 60: 396-402.

Knight, I. I., D. J. Grimes, and R. R. Colwell. 1987. Bacterial hydrolysis of urea in the tissues of carcharhinid sharks. Canadian Journal of Fisheries and Aquatic Sciences 45: 357-360.

Leibovitz, L. and S. S. Leibovitz. 1985. A viral dermatitis of the smooth dogfish, *Mustelus canis*. Journal of Fish Diseases 8: 273-279.

McAllister, P. E. and M. K. Stoskopf. 1993. Shark viruses. *In:* Fish Medicine, p. 780-782. M. K. Stoskopf (ed.). W. B. Saunders Company, Philadelphia, Pennsylvania, USA. 882 p.

Stoskopf, M. K., S. Kennedy-Stoskopf, J. Arnold, J. Andrews, and M. T. Perlstein. 1986. Therapeutic aminoglycoside antibiotic levels in brown sharks, *Carcharhinus plumbeus* (Nardo). Journal of Fish Diseases 9(4): 303-311.

Stoskopf, M. K. 1993. Fish Medicine. W. B. Saunders Company, Philadelphia, Pennsylvania, USA. 882 p.

The Elasmobranch Husbandry Manual: Captive Care of Sharks, Rays and their Relatives, pages 433-439.
© 2004 Ohio Biological Survey

Chapter 27
Histological and Histopathological Examination of Elasmobranchs: Emphasis on the Collection and Preparation of Tissues

JOSEPH M. GROFF

Department of Pathology, Microbiology, and Immunology,
School of Veterinary Medicine, University of California,
Davis, California 95616, USA.
E-mail: josephvmd@aol.com

Abstract: An essential component of the husbandry and management of any captive population, including elasmobranchs, is an active program of disease surveillance and prevention. The foundation of the latter activity is the proper diagnosis of disease conditions including a postmortem (necropsy) examination of affected animals, incorporating the histological examination of a complete set of tissues. The necropsy examination should ideally be performed immediately following death or euthanasia, to prevent rapid autolysis due to a prolonged postmortem interval. Alternatively, the carcass may be refrigerated at temperatures of 4°C if the necropsy cannot be performed immediately following death of the animal, although a refrigerated carcass should be examined as soon as possible. Freezing is discouraged as a method of preservation of the carcass. A complete set of tissues should be collected for histological examination prior to a detailed gross examination of the tissues to prevent excessive handling that can result in artifactual changes of the tissue. Likewise, the tissues should be handled with care to avoid artifactual changes and dissected using forceps and a razor blade, scalpel blade, or sharp scissors prior to immediate placement in tissue fixative. A maximum thickness of 1.0 cm or a volume of 1.0 cm^3 is preferred for the best fixation. A final volume of one part tissue to a minimum of 10 parts fixative (1:10) is highly recommended for the best results. A solution of 10% neutral-buffered formalin is the standard fixative for histopathological examination of animal tissues and may be used for the routine examination of elasmobranch tissues. However, acid-fixatives, such as Bouin's and Davidson's solutions, result in superior tissue fixation and cytological detail, and are often the preferred fixatives for elasmobranch tissues or piscine tissues in general. The histological examination of biopsy tissue, especially biopsies of the integument and gills, often provides valuable information and is recommended as a diagnostic procedure in elasmobranchs. The collection of tissues for electron-microscopic examination is not a routine procedure, although electron-microscopic examination is often required for a definitive or more complete pathological diagnosis. The most common fixatives for electron-microscopic examination are a 2% glutaraldehyde solution in a 0.1 M phosphate or cacodylate buffer, Trump's 4F:1G solution in a 0.1 M phosphate buffer, and 10% neutral-buffered formalin. The fixative of choice is Trump's 4F:1G solution, since it provides excellent fixation and the tissues can be held in the fixative for extended periods at 4°C. Similar to tissues collected for histological examination, a general guideline is fixation of one part tissue in a minimum of 10 parts fixative for a maximum of 1-2 hours at room temperature. In contrast to tissues collected for histological examination, tissues for electron-microscopic examination should not exceed a volume of 1.0 mm^3. Confidence and accuracy of the pathological diagnosis not only depends on the proper collection and preparation of tissues, but is also dependent on the expertise of the pathologist. In this context, the pathologist should be selected based on a familiarity and expertise with the normal anatomy and physiology, husbandry, medicine, and pathology/diseases of elasmobranchs.

Although the surveillance and prevention of disease are essential components of the husbandry and management of captive populations, these activities are often absent or otherwise not properly performed or applied to aquatic animal populations, including elasmobranchs. The foundation of any disease surveillance and prevention program is the proper diagnosis of disease conditions, including a complete evaluation of the history and husbandry of the captive population, analysis of the water quality parameters, and an antemortem physical examination or a complete postmortem (necropsy) examination of the affected animals. Components of the antemortem and postmortem examination include a detailed gross examination; cytological examination of tissue scrapings and/or imprints of the external (gills and integument) and internal tissues; and the collection of tissues for hematological, chemical, toxicological, microbiological (viral, bacterial, parasitic, and mycotic), and histological evaluation.

In the event of mortality or euthanasia, a complete necropsy examination is always recommended, since the failure to perform a complete necropsy often results in an incorrect diagnosis of the primary disease condition or the cause of the disease. An example of an improper postmortem diagnostic effort is the evaluation of cutaneous and branchial wet-mount preparations without the benefit of a complete necropsy examination or the histological evaluation of a limited number of tissues. This limited examination may reveal an external parasitic infection that may not be the primary cause of the disease, since the majority of infectious disease conditions in captive populations are secondary to poor husbandry, stress, or other primary disease conditions. The consequences of an incorrect diagnosis are obvious and may result in an avoidable loss of animals due to additional morbidity and mortality in the population. Likewise, treatment of the secondary infectious disease will generally not prevent recurrence of the primary disease condition. Therefore, this discussion will focus on the proper collection and preparation of tissue samples for histological examination, increasing the accuracy, and consequently the confidence, of the pathological diagnosis.

BASIC CONCEPTS OF DISEASE

Prior to any discussion of pathology and disease in an individual organism or population, a conceptual framework of disease needs to be established by definition of the pertinent terminology. Therefore, disease can be defined as any definitive morbid condition or process that has a characteristic set of symptoms or qualities, whereas pathology is the study of disease. The various aspects of pathology include the cause or etiology, the developmental process or pathogenesis, the biochemical and morphological alterations or lesions of the cells and tissues, and the functional significance or clinical consequence of these alterations. The etiology of disease may be intrinsic (genetic) or extrinsic (acquired); the latter includes diseases due to infectious, environmental, toxic, physical or traumatic, metabolic, and/or nutritional etiologies.

Neoplastic disease may have an extrinsic and/or intrinsic component, whereas a disease with an uncertain or unknown etiology is referred to as an idiopathic disease. The cause of any particular disease may be due to a single etiology, such as a highly virulent infectious agent, or multiple etiologies. For example, fish exposed to poor water quality or low concentrations of a toxin are generally more susceptible to a primary and/or secondary infectious disease. Disease can be further classified according to the progression and severity of the condition. Acute disease has a rapid onset and progression, whereas chronic disease has a slow progression and long duration. Disease that is neither acute nor chronic may be classified as subacute or subchronic, whereas disease that has an extremely rapid progression can be considered peracute. Clinical disease is grossly perceptible or conspicuously apparent and characterized by observations and/or the results of tests, whereas subclinical disease is not apparent or does not result in clinical manifestations and is difficult to characterize. Subclinical disease may progress to clinical disease.

Infection is often used synonymously with disease but is more correctly defined as the invasion and colonization of the tissues by microbial pathogens and the consequent response of the host to this event. A pathogen is any organism capable of causing disease, whereas pathogenicity is the ability of an organism to produce disease. Pathogenicity of an infectious agent is dependent on the contagious and invasive properties of the pathogen, and the ability of the pathogen to resist defense mechanisms of the host that will vary with a particular strain. Infection that results in apparent symptoms, i.e., disease, is often referred to as clinical infection but is more correctly characterized as a clinical disease due to an infectious etiology. In contrast, a subclinical infection is synonymous with asymptomatic infection that is generally not grossly perceptible or conspicuous and does not result in disease. Therefore, the detection or

presence of any infectious agent does not imply the presence of disease. Asymptomatic infections may progress to clinical infections or may remain subclinical, although the host may function as a reservoir of infection to other members of the population, referred to as the carrier state. Furthermore, exposure to infectious agents is a normal and continual event that does not necessarily result in infection or clinical disease during the lifespan of any individual organism. The manifestation of clinical disease in a population or epizootiology is dependent on a complex interaction between the host organism, the environment, and the pathogen. For example, the ability of a viral pathogen to cause disease is dependent on the status of the host, including species, age, and life stage; water quality parameters; and the pathogenicity of the viral strain.

Pathogens are normal components of the aquatic ecosystem that have coexisted and evolved with the host in the natural environment, and generally do not result in serious disease within the wild population. However, captivity often provides conditions that affect the complex interaction of the host and pathogen. These conditions often exacerbate the manifestation of disease in a captive population, but do not create the host-pathogen interaction.

There are several classifications of the clinical or pathological diagnosis. A definitive diagnosis of vibriosis would be an appropriate diagnosis in a shark with a primary disease condition due to a *Vibrio* sp. infection. In contrast, a morphological diagnosis only characterizes the lesions of the specific tissues that are affected by the disease, whereas an etiological diagnosis associates the morphological diagnosis with the causative or etiological agent of the lesions. For example, an ulcerative dermatitis would be a correct morphological diagnosis if the disease condition resulted in cutaneous inflammation with the development of cutaneous ulcers. If the cutaneous inflammation and subsequent ulceration were due to a *Vibrio* sp. infection, the correct etiological diagnosis would be a bacterial ulcerative dermatitis due to a *Vibrio* sp., although the definitive diagnosis for this disease condition would be a cutaneous vibriosis. The distinction is that the etiological agent characterizes or qualifies the morphological lesion in the etiological diagnosis, whereas the affected tissue or organ characterizes or identifies the location of the disease condition in the definitive diagnosis.

A morphological diagnosis can generally be obtained by the gross and histological examination of tissues if there are structural alterations to the tissues, but cannot be obtained if cellular lesions are limited to functional (i.e., subcellular or biochemical) alterations. The latter effect may occur with an acute environmental or toxic insult. In this context, the histological examination of tissues may not result in an etiological diagnosis or definitive diagnosis, since the latter diagnoses often require ancillary tests in addition to the histological examination.

The accuracy and confidence of any pathological diagnosis is dependent on the proper collection and preparation of tissue samples.

COLLECTION AND PREPARATION OF TISSUES

Those that are not familiar with the normal anatomy of elasmobranchs should consult the manuals by Ashley and Chiasson (1988), Gans and Parsons (1964), and Harrison (1949), prior to the necropsy examination. Hamlett (1999) has summarized the biology of elasmobranchs, including a review of the various organ systems.

Necropsy

A necropsy examination should ideally be performed immediately following death or euthanasia, to prevent rapid autolysis due to a prolonged postmortem interval. Autolysis will occur in all tissues but is especially rapid in the gills, gastrointestinal tract, brain, and spinal cord. For example, structural alterations of the gills can occur within five minutes of death (Ferguson, 1989). Alternatively, the carcass should be removed from the water without further delay and refrigerated at temperatures of 4°C if the necropsy cannot be performed immediately. However, a refrigerated carcass should be examined as soon as possible, since refrigeration of poikilotherms (i.e., cold-blooded animals) only decreases the rate of autolysis, rather than preventing it altogether. Animals that have been dead for several hours (e.g., overnight) should be necropsied without further delay. Freezing is discouraged as a method of carcass preservation since freezing disrupts the normal structural integrity of cells and tissues (freezing artifact), although a frozen carcass can often be used for microbiological and toxicological analyses.

Tissue collection

It is recommended that a complete set of tissues (i.e., representative tissues from all of the organ systems) be collected for histological examination, prior to a detailed gross examination of the tissues, to prevent excessive handling that can result in artifactual changes of the tissue. For example, a cursory examination of the gills should be followed by collection of gill tissue for histological examination, prior to further gross examination and wet-mount or cytological examination of the gills. Additional sections of any tissue may be taken or substituted for the original sample(s), as necessary, during the gross examination. The complete set of tissues should always include any gross lesions and the adjacent normal tissue. For example, sections of a gross lesion from the liver, such as a focus of capsular and subcapsular hepatic hemorrhage and inflammation, should include the lesion, border of the lesion, and ~1.0 cm section of the grossly normal adjacent tissue. An additional section of normal liver that is not adjacent to the gross lesion should also be collected and submitted for histological examination. Care should be exercised during the necropsy examination to avoid contact of the tissues with water, saline solutions, other chemicals, or other tissues from the same animal or different animals, including gastrointestinal contents. The tissues should be handled with care to avoid artifactual changes and dissected using forceps and a razor blade, scalpel blade, or sharp scissors, prior to immediate placement in tissue fixative. Razor blades and scalpel blades should ideally be replaced after two or three uses to avoid artifactual changes. A maximum tissue thickness of 1.0 cm, or a volume of 1.0 cm^3, is preferred for the best fixation.

The pathologist often prefers the identification of tissues prior to submission for histological examination. This procedure will prevent confusion concerning origination of the tissue, especially if a lesion obscures the normal microscopic anatomy of the tissue or if multiple sections of a single organ are procured for examination. Tissues can be placed in tissue cassettes or tissue bags labeled with pencil prior to fixation. Alternatively, separate vials or containers can be used for larger tissues such as the entire heart or brain. For juvenile fishes, the entire carcass can be immersed in tissue fixative following exposure of the internal viscera by a ventral midline incision. Containers should have a wide opening to facilitate placement of the tissues into the container without excess manipulation or handling. A final volume of one part tissue to a minimum of 10 parts fixative (1:10) is highly recommended for the best results. Tissues preserved in fixative can be held at room temperature prior to shipment or submission to the laboratory.

Tissue fixation

The various fixatives and formulations that are used for the histological examination of tissues have previously been discussed in detail and should be consulted as necessary (Bancroft and Cook, 1994; Hopwood, 1990; Presnell and Schreibman, 1997). A solution of 10% neutral-buffered formalin is the standard fixative for histopathological examination of animal tissues and may be used for the routine examination of elasmobranch tissues, but is not always the preferred fixative. The advantages of formalin are related to convenience, since it is commercially available and is generally the fixative of choice for various histochemical stains. Tissues fixed in formalin can be held in the fixative, pending submission to the diagnostic laboratory, and can be used for electron-microscopic (ultrastructural) examination if necessary. However, formalin often results in less than ideal tissue fixation and cytological detail, sometimes compromising the accuracy of the histological examination and, therefore, the confidence of the pathological diagnosis.

In contrast to formalin, the acid-fixatives, such as Bouin's and Davidson's solutions, result in superior tissue fixation and cytological detail and are often the preferred fixatives for elasmobranch tissues and piscine tissues in general. More specifically, Bouin's solution is the preferred fixative for the histological examination of the eyes, brain and spinal cord, and whole juvenile fishes. Since Bouin's solution results in demineralization of tissues, it is the fixative of choice for mineralized tissues such as cartilage and integument. However, tissues fixed in acid-fixatives need to be placed in 70% ethanol after a maximum fixation of 12-48 hours to prevent over-fixation of the tissues. A maximum fixation of 24 hours is recommended for small sections of tissue. Acid-fixatives preclude the use of various histochemical stains and the electron-microscopic examination of tissues, due to the harsh nature of the fixatives. Bouin's solution contains picric acid, which is explosive when dry. Therefore, picric acid should be purchased as an aqueous solution and picric acid and Bouin's solution should be properly stored and discarded to prevent drying. Adjustments to the final pH and osmolarity of the fixative, to coincide with the pH and osmolarity of the extracellular fluid of the species to be examined, have often been recommended by histologists. However, this is not

necessary for routine diagnostic procedures, regardless of the fixative, since the cytological changes that may occur are minimal or insignificant and do not affect examination of the tissues.

Due to the advantages and disadvantages of the various fixatives, collection of a duplicate set of tissues in formalin and an acid-fixative (preferably Bouin's solution) is recommended, especially for large animals such as elasmobranchs. One or both sets of tissues may be submitted to the diagnostic laboratory with the understanding that only one set of tissues (preferably the tissues fixed in Bouin's solution) will be processed for histological examination, whereas the additional set will be saved for future examination or staining as necessary. Tissues may be placed in a smaller container with a small amount of fixative (such as formalin, for tissues initially fixed in formalin, or 70% ethanol, for tissues initially fixed in Bouin's solution), following adequate fixation for shipment to the diagnostic laboratory. The container should permit easy placement and removal of the tissues. Sealing the opening of the container and the lid with Parafilm (American National Can Company, Chicago, Illinois) and placing the container(s) in a well-sealed plastic bag will prevent leakage during shipment.

Tissue fixation for electron microscopy

The various fixatives and formulations used for electron-microscopic examination of tissues have previously been discussed in detail and should be consulted as necessary (Dykstra, 1993; Hayat, 2000; Hopwood and Milne, 1991). The collection of tissues for electron-microscopic examination is not a routine procedure, although electron-microscopic examination is often required for a definitive or more complete pathological diagnosis. Ideally, tissues that exhibit unusual gross lesions should be collected for electron-microscopic examination pending the results of the histological examination.

The most common fixatives for electron-microscopic examination are a 2% glutaraldehyde solution in a 0.1 M phosphate or cacodylate buffer, Trump's 4F:1G solution in a 0.1 M phosphate buffer, and 10% neutral-buffered formalin (Dykstra, 1993). The fixative of choice is Trump's 4F:1G solution, since it provides excellent fixation and the tissues can be held in the fixative for extended periods at 4°C (Dykstra, 1993). Phosphate buffers are generally preferred over cacodylate buffers, since the latter contain arsenic and are therefore toxic. Similar to tissues collected for histological examination, a general guideline is the fixation of one part tissue in a minimum of 10 parts fixative for a maximum of 1-2 hours at room temperature. If glutaraldehyde was used as the fixative, tissues should then be placed in a 0.1 M buffer solution (using the same buffer that was used for fixation) for washing or storage prior to submission. The tissues should be collected using a sharp razor blade to avoid artifactual changes. In contrast to tissues collected for histological examination, tissues for electron-microscopic examination should not exceed a volume of 1.0 mm^3. It should be emphasized that electron-microscopic examination is more expensive and less rapid than a routine histological examination.

Biopsy

A tissue sample taken from a living animal is referred to as a biopsy. The histological examination of biopsy samples, especially biopsies of the integument and gills, can provide valuable information and is therefore recommended as a diagnostic procedure in elasmobranchs. As the term implies, an excisional biopsy is simply the procurement of tissue by excision, using appropriate surgical instruments such as a scalpel blade. The collection of a small, circular section of integument is referred to as a punch biopsy. The punch biopsy is performed using a tissue punch, which is a specialized instrument for cutting and removing a section of the integument. Finally, the collection of a tissue core using a needle is referred to as a needle biopsy. Needle biopsies are generally used for the collection of tissue from the internal viscera, but are not the preferred procedure for the collection of integument and gill samples.

Cutaneous neoplasms are often raised lesions that can simply be excised at the base of the lesion. Cutaneous biopsy sites can be closed with an appropriate absorbable suture material, although this is generally not necessary and is often difficult due to the relative lack of cutaneous elasticity, especially of the dorso-lateral integument. The presence of dermal scales further complicates this procedure. Application of a topical antibiotic ointment to the biopsy site and/or a prophylactic injection of antibiotic can often follow a biopsy procedure, but is generally not necessary nor indicated. However, the systemic administration of antibiotics to elasmobranchs with ulcerative lesions, due to a primary or secondary bacterial etiology, is recommended.

The procurement of cutaneous scrapings is considered a biopsy procedure. Scrapings may be collected and placed in tissue fixative for histological

examination. Finally, gill biopsies are simple to perform and provide highly diagnostic information. The collection of branchial tissue should be limited to excision of the proximal aspects (or tips) of the primary filaments, using sharp scissors.

The collection of needle or wedge biopsies, from the internal viscera, is more difficult and generally provides less diagnostic information than cutaneous or gill biopsies, but can be performed if other diagnostic techniques (e.g., an evaluation of the history, water quality, cutaneous or gill biopsies, wet-mount preparations, etc.) do not provide adequate diagnostic information. The collection of internal biopsies is dependent on a knowledge of elasmobranch anatomy and/or the use of imaging techniques such as radiography or ultrasonography to locate the organ(s). However, the collection of biopsy samples using endoscopy, to evaluate the internal organs, is generally more successful and provides the best results.

The collection and preparation of biopsy samples is similar to the collection of tissues during the necropsy examination, although the small amount of tissue that is obtained generally precludes the collection of a duplicate set of tissues. Therefore, one fixative should be selected and formalin is generally the preferred fixative. Biopsy procedures that result in the procurement of small tissue samples, such as samples obtained using a needle biopsy, endoscopy, or cutaneous scraping, should be placed in a labeled tissue cassette to prevent loss of samples.

SELECTION OF THE PATHOLOGIST

Confidence and accuracy of the pathological diagnosis not only depend on the proper collection and preparation of tissues, but are also dependent on the expertise of the pathologist. In this context, the pathologist should be selected based on a familiarity and expertise with the normal anatomy and physiology, husbandry, medicine, and pathology of elasmobranchs. Additional factors to evaluate during the selection of a diagnostic laboratory or pathologist include the availability of the pathologist, enthusiasm of the pathologist to work closely with the client, quality of the report, time required for reporting the results, cost of the examination, and ability to recommend and perform additional diagnostic procedures as necessary. In general, quality should not be sacrificed for convenience.

The pathologist should be considered a member of the husbandry team and a client-pathologist relationship should be developed and fostered for the best results. Both the pathologist and the client should be readily available to discuss the results of the pathological examination.

A detailed pathological report, describing the morphological lesions and the clinical significance of those lesions, is not an unreasonable request. The report should include recommendations on management and/or treatment strategies, if possible, or the need for additional diagnostic procedures. However, it must be understood that the histological examination of tissues may not result in a definitive diagnosis or even a morphological diagnosis in all cases requiring additional diagnostic procedures or ancillary tests.

The time required for completion of the pathological report should be discussed with the pathologist, although requests by the client should be reasonable for routine cases. In contrast, a more rapid report is not an unreasonable request in the event of a progressive severe morbidity and/or mortality in the population. A reasonable cost for the examination is dependent on the various diagnostic procedures that are requested and performed, but should be similar to the cost of the same procedures in other veterinary species.

The pathologist or diagnostic laboratory should have the capability to perform additional diagnostic procedures including additional histochemical staining; electron-microscopic examination of tissues; and the hematological, chemical (clinical chemistry), toxicological, and microbiological analyses of samples.

REFERENCES

Ashley, L. M. and R. B. Chiasson. 1988. Laboratory Anatomy of the Shark, 5th edition. William C. Brown, Dubuque, Iowa, USA. 84 p.

Bancroft, J. D. and H. C. Cook. 1994. Manual of Histological Techniques and their Diagnostic Applications. Churchill Livingstone, Edinburgh, UK. 457 p.

Dykstra, M. J. 1993. Specimen preparation. *In:* A Manual of Applied Techniques for Biological Electron Microscopy, p. 1-77. M. J. Dykstra (ed.). Plenum Press, New York, USA. 272 p.

Ferguson, H. W. 1989. Introduction. *In:* Systemic Pathology of Fish, p. 3-10. H. W. Ferguson (ed.). Iowa State University Press, Ames, Iowa, USA. 240 p.

Gans, C. and T. S. Parsons. 1964. A Photographic Atlas of Shark Anatomy: The Gross Morphology of *Squalus acanthias.* Academic Press, New York, USA. 106 p.

Hamlett, W. C. 1999. Sharks, Skates, and Rays: The Biology of Elasmobranch Fishes. Johns Hopkins University Press, Baltimore, Maryland, USA. 544 p.

Harrison, B. M. 1949. The Dissection of the Shark (the Dogfish; *Squalus acanthias*): A Laboratory Manual. Burgess Publishing Company, Minneapolis, Minnesota, USA. 54 p.

Hayat, M. A. 2000. Principles and Techniques of Electron Microscopy: Biological Applications, 4th edition, Cambridge University Press, Cambridge, England. 543 p.

Hopwood, D. 1990. Fixation and fixatives. *In:* Theory and Practice of Histological Techniques, 3rd edition, p. 21-42. J. D. Bancroft and A. Stevens (eds.). Churchill Livingstone, Edinburgh, UK. 800 p.

Hopwood, D. and G. Milne. 1991. Fixation. *In:* Electron Microscopy in Biology: A Practical Approach, p. 1-15. R. Harris (ed.). IRL Press, Oxford, UK. 327 p.

Presnell, J. K. and M. P. Schreibman. 1997. Humason's Animal Tissue Techniques, 5th edition. Johns Hopkins University Press, Baltimore, Maryland, USA. 572 p.

The Elasmobranch Husbandry Manual: Captive Care of Sharks, Rays and their Relatives, pages 441-446.
© 2004 Ohio Biological Survey

Chapter 28
Goiter in Elasmobranchs

GERALD L. CROW

Waikiki Aquarium, University of Hawaii,
2777 Kalakaua Ave.,
Honolulu, HI 96815, USA.
E-mail: crow@waquarium.org

Abstract: Goiters are commonly observed in elasmobranch specimens from aquariums around the world. Swelling as the result of goiter can become expansive and may result in death if left untreated. Several types of goiters (i.e., diffuse hyperplastic, diffuse colloid, and multinodular colloid) can be differentiated, all of which are unlikely to result from a simple iodide deficiency. Maintaining iodide concentrations close to natural seawater levels (i.e., 0.06 mg l^{-1}), and nitrate concentrations <10 mg l^{-1}, appears to reduce the incidence of goiter. In seawater systems where iodide and nitrate concentrations cannot be controlled, an iodine derivative supplementation dosage of 10-30 mg kg body $weight^{-1}$ $week^{-1}$ PO is recommended.

Enlargement of the thyroid gland is commonly referred to as goiter. As early as the 1900's goiter has been observed in wild and captive fishes. Although goiter is often reported, the actual etiology of the condition is poorly understood. This paper reviews the current status of goiter and goiter treatment in captive elasmobranch fishes.

Goiter has been observed in both free-swimming and captive elasmobranchs since its first observation by Cameron and Vincent (1915) off Nanaimo, British Columbia. Goiter has been observed in 18 species of captive elasmobranchs (Table 28.1). Goiter is considered widespread throughout captive facilities and is particularly common in closed-system, ozonated water systems. Goiter is typically observed as a progressive swelling of the thyroid gland, which can expand to as much as 300 times its normal size. This condition, if left untreated, can result in difficulty swallowing, causing decreased food intake, starvation, and eventually, death.

The basic structure of the thyroid gland is common throughout jawed vertebrates. The thyroid gland in elasmobranchs is an encapsulated organ located in loose connective tissue between the ventral side of the coracohyal and the medial side of the coracomandibular muscles (Honma et al., 1987); i.e., the thyroid gland is essentially located in the middle of the lower jaw muscles. The tissue of the thyroid gland is comprised of follicles with a highly vascular blood capillary system (Ferguson, 1911; Norris, 1985). Each follicle is formed of epithelial cells surrounding a fluid-filled lumen. The lumen contains a colloid suspension of an iodide-rich protein called thyroglobulin, which is engulfed by follicle cells under stimulation by the thyroid-stimulating hormone (TSH) and converted by hydrolysis into T4 (thyroxine) before being secreted into the blood stream. Studies on the release of thyroid hormone in elasmobranchs are currently being conducted at the University of Manitoba, Canada (Eales, pers. com.).

In teleost fishes only T4 is released by the thyroid gland, while in mammals both T4 and T3 (Triiodothyronine) are released. The thyroid hormones T4 and T3 are present in both the bound (total) and unbound (free) state in circulating blood; however, thyroid hormone-sensitive tissues have only T3 receptors. Thus, only T3 has biological activity and T4 acts as a prohormone available for enzymatic conversion into T3 (Leary et al., 1999). In the spiny dogfish (*Squalus acanthias*), the liver has been observed

Table 28.1. Published reports of goiter in captive elasmobranchs, showing species and reporting institution.

Species name	Common name	Institution name	Reference
Carcharhinus galapagensis	Galapagos shark	Ueno Zoo	Uchida and Abe, 1987
Carcharhinus melanopterus	blacktip reef shark	Sea Life Park Hawaii	Crow et al., 1998
Carcharhinus obscurus	dusky shark	Ueno Zoo	Masahito et al., 1982
Carcharhinus plumbeus	sandbar shark	Ueno Zoo	Masahito et al., 1982
Carcharias taurus	sand tiger shark	New England Aquarium	Crow et al., 1998
Chiloscyllium punctatum	brownbanded bambooshark	Steinhart Aquarium	Crow et al., 1998
Dasyatis akajei	red stingray	Ueno Zoo	Masahito et al., 1982
Dasyatis lata	brown stingray	Sea Life Park Hawaii	Crow et al,. 1998
Ginglymostoma cirratum	nurse shark	Shedd Aquarium	Nigrelli and Ruggieri, 1974
Hemiscyllium ocellatum	epaulette shark	Steinhart Aquarium	Crow et al., 1998
Heterodontus francisci	horn shark	Shedd Aquarium	Nigrelli and Ruggieri, 1974
Heterodontus japonicus	Japanese bullhead shark	Ueno Zoo	Uchida and Abe, 1987
Negaprion brevirostris	lemon shark	Shedd Aquarium	Nigrelli and Ruggieri, 1974
Raja eglanteria	clearnose skate	Mote Marine Laboratory	Crow et al,. 1998
Scyliorhinus canicula	smallspotted catshark	Basel Zoo	Straub, 1995
Triaenodon obesus	whitetip reef shark	Sea Life Park Hawaii	Crow et al., 1998
Triakis scyllium	banded houndshark	Ueno Zoo	Masahito et al., 1982
Triakis semifasciata	leopard shark	Shedd Aquarium	Nigrelli and Ruggieri, 1974

as a site for peripheral production of T3 (Leary et al., 1999). The kidney and perhaps other organs may also produce T3. Excess circulating T4 can be excreted without producing the active hormone T3.

Some preliminary information on T4 and T3 concentrations in elasmobranchs is available. Immature sharks have lower serum T4 and T3 concentrations than ovulating and pregnant females (Volkoff et al., 1999). Immature captive whitetip reef sharks (*Triaenodon obesus*) showed no significant sexual differences in serum T4 and T3 (Crow et al., 1999). Serum T4 had a significant increase during winter with a mean concentration of 6.58 ng ml^{-1}, compared to a summer mean concentration of 3.62 ng ml^{-1} (Crow et al., 1999). Whitetip reef sharks with goiters had T4 concentrations of 0.93-0.99 ng ml^{-1} and T3 concentrations of 0.22-0.33 ng ml^{-1}, while non-goitered whitetip reef sharks had T4 concentrations of 3.1-7.9 ng ml^{-1} and T3 concentrations of 0.89-1.1 ng ml^{-1} (Crow et al., 1998).

Iodine is an essential nutrient for all animal species. Although iodine occurs globally, its geographic distribution is variable. Iodine is found in organic deposits and in sedimentary phosphate rock. Iodine occurs in plant tissue and seawater, predominately as inorganic iodide, and is readily absorbed in the intestinal tract (Miller and Ammerman, 1995; Wong, 1991). The surface waters of the ocean typically contain the highest concentration of iodine (Wong, 1991).

Seawater contains two species of dissolved inorganic iodine (iodide and iodate) (Wong, 1991). Artificial, coastal, and well seawater can have variable elemental compositions and need to be monitored carefully (Atkinson and Bingham, 1997; Crow et al., 1998). Iodide is thought to be the most biologically active form of iodine and diffusion uptake of iodide occurs across the gills and stomach, with excretion primarily at the kidney and rectal gland (Shuttleworth, 1988). Water chemistry can vary between aquariums and iodine speciation needs to be monitored carefully. Facilities using saltwater wells may have different iodide and iodate speciation. Thus, total iodine alone does not give a full picture of the iodide available to elasmobranchs (Crow et al., 1998). In addition, ozone alters the speciation of iodine by reducing iodide (and dissolved organic iodide) to iodate (Sherrill et al., 2000).

The diets of captive elasmobranchs typically rely on herring (*Clupea harengus*) and smelt (*Osmerus* spp.) which are relatively low (i.e., 5-10 mg kg^{-1}) in iodine (Lall, 1989). Malnutrition can increase the likelihood and severity of goiter (Gaitan, 1990) and ascorbic acid deficiency can reduce iodide uptake (Agrawal and Mahajan, 1981). Hunt and Eales (1979) found that iodide uptake was at least 84% from surrounding water and 16% from diet in the rainbow trout. The percentage of iodide uptake in elasmobranchs is unknown.

GOITROGENIC AGENTS

A goitrogen is a chemical that interferes with the function of the thyroid gland. These chemicals cause thyroid enlargement by acting directly on the thyroid gland, altering the regulatory mechanism, affecting peripheral metabolism, or causing the excretion of thyroid hormones (Gaitan, 1990). Excess nitrogen (in the form of nitrate) may be a goitrogen. Bromide, fluoride, calcium, cobalt, manganese, and sulfides can all inhibit normal iodine uptake. Excess iodine can inhibit thyroid activity (see Miller and Ammerman, 1995).

HISTOPATHOLOGY

Enlargement of the thyroid gland can result from the following conditions (Robbins, 1994): (1) hyperthyroidism (thyrotoxicosis—elevated circulating T4 and T3 concentrations); (2) hypothyroidism (reduced concentrations of circulating T4 and T3); (3) thyroiditis (swelling caused by interstitial and infectious processes); (4) tumor (nodular or cyst formation); and (5) congenital anomalies. All of these conditions must be considered and may affect thyroid hormone concentrations. Typically, enlargement of the thyroid gland in captive elasmobranchs results in both hypertrophy (increase in size) and hyperplasia (increase in cell number) of the follicles (Crow et al., 2001). The shape of the follicles and amount of colloid present within the follicle vary widely.

Crow et al. (2001) examined goiters of captive elasmobranchs and reported the following types of goiters:

1. Diffuse hyperplastic goiter: the thyroid gland consisted of small to medium-sized follicles with little to no colloid. Follicular cells tended to be columnar.

2. Diffuse colloid goiter: the thyroid gland consisted of large rounded follicles, containing colloid, with some scattered small follicles. Follicle cell shape varied from cuboidal to columnar. A few papillary projections were present.

3. Multinodular colloid goiter: follicles varied in size from large to small, mostly with colloid. Follicular cells ranged from flattened, to cuboidal, to columnar in shape. Fibrous bands divided the thyroid gland into nodules and fibrous scarring, with areas of hemosiderin, indicating a previous hemorrhage.

Diffuse hyperplastic goiter results from a reduction of circulating T4 and T3 with an elevation of TSH. This goiter is characterized by a loss of colloid, papillary infolding of the follicular epithelium, and prominent cellular hyperplasia and hypertrophy (Greer et al., 1967). These goiters are characteristic of iodine deficiency or goitrogenic agents blocking the uptake of iodine. If an elasmobranch has a strict iodine deficiency (i.e., insufficient iodine available in the water and food) this is the type of goiter you would expect. Low iodine in the thyroid gland results in thyroid stimulation, in an attempt to produce more circulating T4 and T3, and eventually, the thyroid gland becomes depleted of colloid as it attempts to supply this increased demand.

Diffuse colloid goiter is thought to derive from: (1) diffuse hyperplastic thyroid glands that begin to receive sufficient iodine and produce normal concentrations of thyroid hormones, resulting in iodine storage in the already enlarged follicles (Marine and Lenhart, 1909); and/or (2) slowly growing goiters in areas of moderate or intermittent iodine deficiency that may already be colloid-rich at the time of the goiter's first appearance (Gerber et al., 1981). This condition could occur if the thyroid gland becomes less responsive to TSH stimulation and the supply of iodine fluctuates (Gerber et al., 1981). Nearly all long-standing colloid goiters are transformed into multinodular colloid goiters (Robbins, 1994).

The Ueno Zoo (Tokyo, Japan) has been the most active in thyroid assessment. Interestingly, all three types of goiter have been found at this facility, suggesting that a strict iodine deficiency alone did not account for all of these goiters. It is possible that iodide deficiency and a goitrogenic agent produced a synergistic response, exacerbating the development of goiter. It is equally possible that the thyroid gland compensates for low level iodine concentrations and attempts to create some sort of equilibrium, resulting in a colloid goiter.

To sum up the challenge of goiter determination, Robbins (1994) states "…that there is no simple correlation between morphologic lesions and resultant clinical manifestations. A multinodular goiter, for example, in one instance may be associated with normal thyroid function, in another with hyperfuction, and yet another with hypofunction…". Stoskopf (1993) stated that

sharks with goiters are hypothyroid, have low circulating levels of T4, and have hyperplastic non-colloid goiters. Crow et al. (2001) found hypothyroid animals, having low circulating T4 and T3, with both hyperplastic and colloid goiters.

Available data is fragmentary and the exact etiology for the development of goiter is uncertain. A case can be made for iodine deficiency within a typical closed-system aquarium, where levels of iodide and dissolved organic iodine are nearly undetectable (Sherrill et al., 2000). Nitrate rises rapidly in closed systems (Spotte, 1992) and is purported to reduce the absorption and retention of iodide from the thyroid gland, leading to iodine deficiency and diffuse hyperplastic goiter. Mechanisms that lead to other types of goiter in elasmobranchs are unknown. Recent studies in humans found that the thyroid gland attempts to compensate for iodine deficiency by increasing the uptake of iodine and increasing the fraction of circulating T3 (Dumont et al., 1995). After a prolonged period, large goiters have a reduced efficiency for the synthesis and secretion of thyroid hormones (Dumont et al., 1995).

PROPHYLAXIS

In the early years of captive elasmobranch husbandry, goiter was thought to be related to thyroid tumors. It was believed that standard diets were naturally high in iodine, precluding the need to supplement. However, trout (*Salmo* spp.) and salmon (*Oncorhynchus* spp.) culture revealed cases of goiter that responded to treatments of iodide added to the water or food. Thereafter, algal or iodide supplements have typically been used to treat thyroid enlargements in captive fishes. A wide variety of treatments have been used for goiter in elasmobranchs (Table 28.2). Stoskopf (1990) noted that the typical level of iodide provided to elasmobranchs is more than required to balance an iodine-deficient diet.

Dietary uptake of iodide in elasmobranchs requires detailed study. In terrestrial animals, bioavailable iodine is absorbed in the gastrointestinal tract and is usually provided as a supplement in the form of potassium iodide, sodium iodide, or calcium iodate (Miller and Ammerman, 1995). These oral supplements, at a threshold level, have reduced goiters. Synthyroid (synthetic T4) has had variable results in therapy with skates and rays, requiring lower doses than carcharhinid sharks (Stoskopf, 1990). At the Ueno Aquarium, the iodine level of aquarium water was adjusted to 0.2 mg l^{-1} (where natural seawater = 0.06 mg l^{-1}), resulting in existing goiter regression and the development of no new cases (Uchida and Abe, 1987). In another example, well-developed goiters regressed rapidly when sharks were placed in a natural seawater lagoon, where diet remained unchanged (Crow et al., 1998), suggesting that low iodide availability in seawater (and water chemistry) played a key role in goiter development. In mammals, the level of supplemented iodide is typically <0.5 mg kg body weight^{-1} week^{-1} (Miller and Ammerman, 1995).

In closed system aquariums it is likely that goiters will develop and supplementation will be

Table 28.2. Treatments for goiter in elasmobranchs, showing compound, dosages, and reporting institution. Both Mazuri Vita-ZU shark/ray and Sea Tabs refer to commercial supplements.

Compound	Dosage	Institution name	Reference
Calcium iodate	1087 mg kg of food^{-1} week^{-1}	Mazuri Vita-ZU shark/ray	As recommended
Calcium iodine	0 03-0 05 mg kg body weight^{-1} week^{-1}	Burger's Zoo	Janse, pers. com.
CLM01	1.5 ml week^{-1} (each specimen)	Basel Zoo	Straub, 1995
Potassium iodide	0.2 mg l^{-1} (constant immersion)	Ueno Zoo	Uchida and Abe, 1987
Potassium iodide	1.2 mg kg body weight^{-1} week^{-1}	Blackpool Sea Life Centre	Lloyd, 1995
Potassium iodide	10 mg kg body weight^{-1} week^{-1}	Acquario di Genova	Gili, pers. com.
Potassium iodide	10 mg kg body weight^{-1} week^{-1}	Virginia Aquarium and Marine Science Center	Firchau, pers. com.
Potassium iodide	10-21.6 mg kg body weight^{-1} week^{-1}	Aquarium of the Americas	Hewitt, pers. com.
Potassium iodide	0.89 µg kg body weight^{-1} week^{-1}	Sea Tabs	As recommended
Potassium iodide	20 mg kg body weight^{-1} week^{-1}	Oceanario de Lisboa	Correia, pers. com.
Thyro-block	32.5 mg kg body weight^{-1} week^{-1}	Sea World Adventure Park Orlando	Davis, pers. com.
Yodolactina (iodine)	420 mg kg of food^{-1} week^{-1}	Acuario de Veracruz	Marín-Osorno, pers. com.

necessary. There is no exact formula for iodide supplementation in elasmobranchs. Water chemistry, elasmobranch species, species composition, species density, age, reproductive condition, and diet (i.e., food type, fresh or frozen, etc.) may all affect thyroid health and goiter development. In facilities where goiters are expected to develop, an iodine derivative should be supplied prior to the onset of goiter and a safe dose of 10-30 mg kg body weight^{-1} week^{-1} is recommended. As stated by Stoskopf (1990), this dosage is more than a dietary supplement and may be high for some species. However, without thyroid assessments, hormone concentrations at known supplementation levels, and knowledge about uptake kinetics, it is best to err slightly on the high end of supplementation.

CONCLUSIONS

Although goiters are commonly observed in captive elasmobranchs around the world, few detailed studies have been conducted. A wide range of treatments has been attempted with variable results. Studies on the development of goiter and the factors that promote this enlargement are critical to successful treatment. Goiter appears to be a reaction to iodide deficiency and an attempt by the thyroid gland to compensate for prolonged deficiencies, with some goitrogenic interaction. Studies of thyroid hormone utilization and processing, and controlled experimental iodide therapy, are much needed.

ACKNOWLEDGEMENTS

I would like to thank the many facilities that contributed information for this paper. I would like to thank my previous coauthors for their contributions to make this review paper possible. Thanks to Ilze Berzins for her review of this article.

REFERENCES

Agrawal, N. K. and C. L. Mahajan. 1981. Effect of ascorbic acid deficiency on the uptake of iodine by thyroid and non-thyroid tissues of an air breathing freshwater fish, *Channa punctatus* Bloch. Journal of Fish Biology 18: 411-416.

Atkinson, M. J. and C. Bingham. 1997. Elemental composition of commercial sea salts. Journal of Aquariculture and Aquatic Sciences 8: 39-43.

Cameron, A. T. and S. Vincent. 1915. Note on an enlarged thyroid occurring in an elasmobranch fish (*Squalus sucklii*). Journal of Medical Research 37: 251-256.

Crow, G. L., M. J. Atkinson, B. Ron, S. Atkinson, A. D. K. Skillman, and G. T. F. Wong. 1998. Relationship of water chemistry to serum thyroid hormones in captive sharks with goiters. Aquatic Geochemistry 4: 469- 480.

Crow, G. L., B. Ron, S. Atkinson, and L. E. L. Rasmussen. 1999. Serum T4 and serum T3 concentrations in immature captive whitetip reef sharks, *Triaenodon obesus*. Journal of Experimental Zoology 284: 500-504.

Crow, G. L., W. H. Luer, and J. C. Harshbarger. 2001. Histological assessment of goiters in elasmobranch fishes. Journal of Aquatic Animal Health 13: 1-7.

Dumont, J. E., A. M. Ermans, C. Maenhaut, F. Coppee, and J. B. Stanbury. 1995. Large goiter as a maladaptation to iodine deficiency. Clinical Endocrinology 43: 1-10.

Ferguson, J. S. 1911. The anatomy of the thyroid gland of elasmobranchs, with remarks on hypobranchial circulation in these fishes. American Journal of Anatomy 11: 151-210.

Gaitan, E. 1990. Goitrogens in food and water. Annual Review of Nutrition 10: 21-39.

Gerber, H., H. Studer, A. Conti, H. Engler, H. Kohler, and A. Haeberelli. 1981. Re-accumulation of thyroglobulin and colloid in rat and mouse thyroid follicles during intense thyrotropin stimulation: A clue to the pathogenesis of colloid goiters. Journal of Clinical Investigations 68: 1338-1347.

Greer, M. A., H. Studer, and J. W. Kendall. 1967. Studies on the pathogenesis of colloid goiter. Endocrinology 81: 623-632.

Honma, Y., Y. Iwata, and A. Chiba. 1987. Comparative histology of the thyroid gland in some elasmobranchs. Report of the Sado Marine Biological Station, Niigata University 17: 1-12.

Hunt, D. W. C. and J. G. Eales. 1979. Iodine balance in rainbow trout (*Salmo gairdneri*) and effects of testosterone propionate. Journal Fish Research Board Canada 36: 282-285.

Lall, S. P. 1989. The minerals. *In:* Fish Nutrition, p. 219-257. J. E. Halver (ed.). Academic Press Inc., San Diego, California, USA. 500 p.

Leary, S. C., J. S. Ballantyne, and J. F. Leatherland. 1999. Evaluation of thyroid economy in elasmobranch fishes, with measurements of hepatic 5-monodeiodinase activity in wild dogfish. Journal of Experimental Zoology 284: 492-499.

Lloyd, N. 1995. Treatment of goiter in Atlantic nurse sharks *Ginglymostoma cirratum* at the Blackpool Sea Life Centre. International Zoo Yearbook 34:95-98.

Marine, D. and C. H. Lenhart. 1909. Colloid glands (goiters): their etiology and physiological significance. Bulletin of the Johns Hopkins Hospital 20: 131-139.

Masahito, P., T. Ishikawa, and S. Takayama. 1982. Neoplastic lesions in fish thyroid. Igaku No Ayumi 120: 399-406.

Miller, E. R. and C. B. Ammerman. 1995. Iodine bioavailability. *In:* Bioavailability of Nutrients for Animals: Amino Acids, Minerals and Vitamins, p. 157-167. C. B. Ammerman, D. H. Baker, and A. J. Lewis (eds.). Academic Press, San Diego, California, USA. 436 p.

Nigrelli, R. F. and G. D. Ruggieri. 1974. Hyperplasia and neoplasia of the thyroid in marine fishes. Mount Sinai Journal of Medicine 41: 283-293.

Norris, D. O. 1985. Vertebrate Endocrinology. Lea and Febiger, Philadelphia, Pennsylvania, USA. 634 p.

Robbins, S. L. 1994. Thyroid gland. *In:* Pathologic Basis of Disease, p. 1121-1133. R. S. Cotran, V. L. Kumar, and J. Robbins (eds.). W. B. Saunders, Philadelphia, Pennsylvania, USA. 1472 p.

Sherrill, S., B. Whitaker, and G. T. F. Wong. 2000. Ozonation effects on the speciation of dissolved iodine in artificial seawater at the National Aquarium in Baltimore. *In:* Proceedings of the American

Association of Zoo Veterinarians and International Association for Aquatic Animal Medicine Joint Conference, September 17-21, 2000, New Orleans, Louisiana, USA, p. 181-183.
Shuttleworth, T. J. 1988. Salt and water balance: Extrarenal mechanisms. *In:* Physiology of Elasmobranch Fishes, p. 171-199. Springer-Verlag, New York, USA. 324 p.
Spotte, S. 1992. Captive Seawater Fishes. John Wiley and Sons Inc., New York, USA. 942 p.
Stoskopf, M. K. 1990. Shark diagnostics and therapeutics: A short review. Journal of Aquariculture and Aquatic Sciences 5: 33-43.
Stoskopf, M. K. 1993. Fish Medicine. W. B. Saunders, Philadelphia, Pennsylvania, USA. 882 p.
Straub, J. O. 1995. First results of goiter treatment in aquarium-kept sharks using an algae extract. *In:* Proceedings of the Third International Aquarium Congress, April 25-29, 1993, Boston, Massachusetts. p. 322-333. New England Aquarium, Boston, USA.
Uchida, H. and Y. Abe. 1987. The prevention of goiter in captive sharks. International Zoo Yearbook 26: 59-61.
Volkoff, H., J. P. Wourms, E. Amesbury, and F. Snelson 1999. Structure of the thyroid gland, serum thyroid hormones, and the reproductive cycle of the Atlantic stingray, *Dasyatis sabina*. Journal of Experimental Zoology 284: 505-516.
Wong, G. T. F. 1991. The marine geochemistry of iodine. Reviews of Aquatic Science 4: 45-73.

PERSONAL COMMUNICATIONS

Correia, J. 2002. Oceanário de Lisboa, 1990-005 Lisboa, Portugal.
Davis, R. 2002. SeaWorld Florida, Orlando, FL 32821, USA.
Eales, J. G. 2000. University of Manitoba, Winnipeg, R3T 2N2, Canada.
Firchau, B. 2002. Virginia Aquarium and Marine Science Center, Virginia Beach, VA 23451, USA.
Gili, C. 2002. Acquario di Genova, 16128 Genova, Italy.
Hewitt, J. 2000. Aquarium of the Americas, New Orleans, LA 70130, USA.
Janse, M. 2001. Burger's Ocean, Arnhem, 6816 SH, Netherlands.
Marín-Osorno, R. 2001. Acuario de Veracruz, Veracruz, CP 91170, Mexico.

The Elasmobranch Husbandry Manual: Captive Care of Sharks, Rays and their Relatives, pages 447-466.
© 2004 Ohio Biological Survey

Chapter 29
Pharmacology in Elasmobranchs

M. Andrew Stamper

The Living Seas
Walt Disney World Resorts,
2020 North Avenue of the Stars,
Lake Buena Vista, FL 32830, USA.
E-mail: andy.stamper@disney.com

Stephen M. Miller

Aquarium of the Americas
Canal Street,
New Orleans, LA 70130, USA.
E-mail: smiller@audubоninstitute.org

Ilze K. Berzins

The Florida Aquarium,
701 Channelside Dr.,
Tampa, FL 33602, USA.
E-mail: iberzins@flaquarium.org

Abstract: Elasmobranchs held in the care of humans occasionally require medical and chemotherapeutic intervention. When applying chemotherapeutics it is critical to understand any physiological, anatomical, and environmental influences, as well as the property of the drugs intended for use. The way in which a drug is administered to an animal is influenced by several factors: (1) the pharmaceutical form of the drug available; (2) the physical or chemical properties of the drug; (3) how quickly the onset of action should occur; (4) restraint of the animal; (5) behavioral characteristics of the animal; and (6) the nature of the condition of concern. Routes of chemotherapeutic administration include bath or immersion, topical, oral, and injectable (i.e., IV, IM, or IP). There is a wide range of drugs that may be used with elasmobranchs and these may be broadly categorized as antibacterial, antiviral, antifungal, antiparasitic, anesthetics, anti-inflammatory, hormones and steroids, nutritional and mineral supplements, and fluid support. Use of any of these drugs is considered “off-label”, meaning that the U.S. Food and Drug Administration (FDA) has not specifically cleared these drugs for use in elasmobranchs but allowed provision for them to be employed under the direction of properly licensed personnel. A literature search for the period between 1971 and 2001 yielded a total of 14 peer-reviewed publications relating to the clinical treatment of elasmobranchs, only two of which were pharmacokinetic studies. The pharmacology of drugs used for elasmobranchs is a new and developing area of study, requiring a great deal of rigorous research to advance the field.

The ultimate goal of this chapter is to provide a useful, fact-based, formulary for elasmobranchs. However, as in most areas of aquatic medicine, much of the available information is anecdotal. Although useful as a starting point, there are few guidelines that have proven steadfast. What we will attempt to provide for the reader is a summary of what is available in the scientific literature, a presentation of facts on commonly used drugs, and a discussion of dosing challenges. The number of variables affecting drug absorption and efficacy are great and will only be better understood through carefully designed studies. Therefore we will conclude this chapter with a discussion on how to conduct an appropriate drug trial.

LITERATURE SEARCH

In conducting multiple literature searches, the following key words were used: shark, stingray, ray, elasmobranch, medical, drug(s), therapy, therapeutic, and pharmacology. Searches for 1971-2001 yielded a total of only 14 peer-reviewed publications and only two of these were actual pharmacokinetic (drug trial) studies (Stoskopf et al., 1986; Willens et al., 1999).

There are a number of general fish formularies available in the literature (Stoskopf, 1993a; Carpenter, et al., 2001; Noga, 1996); however, almost all of the drugs listed have not been rigorously evaluated for use in fishes. Extrapolation of drug dosages from other types of exotic animals (e.g., reptiles, birds, etc.) can be done, but again, efficacy in fishes is basically unknown.

SPECIES DIFFERENCES AND EFFECT ON DOSAGES

There are a number of factors that can alter or mediate the efficacy of drugs used in elasmobranchs. These factors can be divided into two general categories, biological and environmental. Biological factors to consider when developing a therapeutic plan include species variation, physiological and anatomical variation (e.g., poikilotherms vs. other species that may produce internal heat), renal portal systems, vascular flow, high glomerular filtration rates, osmotic homeostasis, overall size/weight, sexual maturity, body condition, stress level, disease/immune status, seasonal patterns, nutritional status, and lipid content of the liver (Evans, 1997). Species variation more specifically refers to body design, gill area to body-weight ratio, and benthic versus pelagic (Stoskopf, 1993b). Environmental considerations address water quality parameters including, but not limited to, temperature, pH, salinity, and mineral content of the water.

Drug absorption, distribution, metabolism, and mode of excretion (i.e., hepatic, gill, and/or renal) will affect each drug differently. Although it is useful to be aware of potential factors that may influence a response to a chemotherapeutic agent, it is not always possible to predict clinical effects. Individual variation can create response variations within a species and may have a dramatic effect on the outcome.

In addition to trying to understand physiological, anatomical, and environmental influences, the properties of drugs themselves need to be considered. Characteristics such as lipophilic (fat soluble), hydrophilic (water soluble), protein binding, binding to other chemicals, binding sites (i.e., where the drug interacts with the body to produce the effect), size of molecules, etc., will all influence the drug reaction.

ROUTES OF CHEMOTHERAPEUTIC ADMINISTRATION

Basically, all routes of drug administration in domestic animals may be used for elasmobranchs. There are preferred methods depending on the particular clinical situation. A drug is of no use unless it can be delivered to the patient in a form and at a site that is appropriate. The way in which a drug is administered to an animal is influenced by several factors (Carpenter et al., 2001): (1) the pharmaceutical form of the drug available; (2) the physical or chemical properties of the drug; (3) how quickly the onset of action should occur; (4) restraint of the animal; (5) behavioral characteristics of the animal; and (6) the nature of the particular condition of concern.

Bath / prolonged immersion

Use of a medicated bath is often dependent on the volume of water in which the animals are being housed, the sensitivity of the animal, and the cost of the drug. Immersion drugs are used primarily for the control of ectoparasites and minor dermatological conditions. Internal absorption and efficacy is dependent on gill surface area and the disease status of gills. Drugs used can be affected by temperature, salinity, mineral content, and pH

of the water. Sunlight and ozone can affect, and in some cases destroy, some immersion drugs.

Topical

When applying medications topically, you need to consider contact times. In mammals, many topical drugs require at least 10 minutes to be effective. Limited use of these drugs may be possible for elasmobranchs if the animal can be restrained out of the water for extended periods of time. Caution needs to be used when applying drugs with alcohol as the carrier medium, since alcohol is irritating to elasmobranch skin. Theoretically some products, such as DMSO (dimethyl sulfoxide), can aid the absorption of medications through the skin and gills, but the efficacy and safety of these "transport" drugs have not been fully investigated.

Oral (Per Os, PO)

Oral medication effectiveness is complicated by absorption challenges. Many factors affect drug absorption, including food type (e.g., drugs such as tetracycline may bind with salts incidentally ingested while feeding, affecting drug absorption), the drug's characteristics (described previously), and species variation in gastric properties (e.g., gastric pH, gastric enzymes, and gastrointestinal transit times). These factors will all impact the absorption and efficacy of the drug.

Injectable routes

Drugs that are given by injection are called parenteral drugs. The three routes used in elasmobranchs are intramuscular (IM), intravascular (IV), and intracoelomic or intraperitoneal (IP). Intracardiac (IC) is rarely used in a therapeutic context but can be an option during euthanasia or for the emergency administration of drugs such as epinephrine.

Intramuscular (IM)

Intramuscular administration is commonly used in situations where physical restraint of the animal is difficult or impossible. The drug can be given to unrestrained animals using a pole syringe or directly with a needle and syringe under minimal restraint. The ideal area for drug administration is the epaxial muscle area surrounding the dorsal fin, avoiding the more extensively vascularized red muscle area found in the lateral region. In order to avoid the accidental IV administration of IM drugs, the administrator can pull back on the syringe plunger prior to injection. If blood is observed, the needle should be redirected and the area sampled again prior to injection. This precautionary measure is not possible when using a pole syringe. Theoretically, the distribution of drugs is assisted by locomotory movements of the animal which, in turn, facilitates vascular and lymphatic pumping (Stoskopf, 1993a).

There has been no demonstrated benefit to using disinfectant scrubs prior to giving an injection. The experiences of many people discourage the use of alcohol because it has been demonstrated to cause irritation to the skin. Caution must be taken to minimize leakage of drugs since shark skin is less elastic than that of higher vertebrates and needles can leave relatively large exit wounds. In addition, shark muscle is at a higher resting potential (i.e., muscle bundles are tightened) resulting in less flexibility for drug absorption. This combination of skin and muscle characteristics increases the chance of drugs leaking out into the environment. Movements of the animal and associated flexing of the muscles exacerbates drug leakage, as do larger needles and larger volumes of drugs. One possible solution to this problem is the use of a pneumatic dart to deliver the drug, as the needle stays in the animal and prevents drug leakage. The dart is removed after a period of time, thus allowing the drug to be absorbed before it can leak out. As a final cautionary note, be familiar with the carrier medium of the drug. Some carrier forms, such as those found in long-acting oxytetracycline and high concentration injectable enrofloxacin, have been demonstrated to be caustic to muscle and connective tissue in other animals, and cause a sterile abscess at the site of injection. Please read the drug insert prior to giving a new drug.

Intravascular (IV)

The intravascular route is the most useful route of drug administration for rapid onset of action. There are three commonly used sites for IV administration: (1) the caudal tail vein, (2) the dorsal cutaneous sinus, and (3) the lateral cutaneous vein (Ashley and Ciasson, 1988) (Figure 29.1).

The problem with many drugs is that they are rapidly metabolized, resulting in a short half-life.

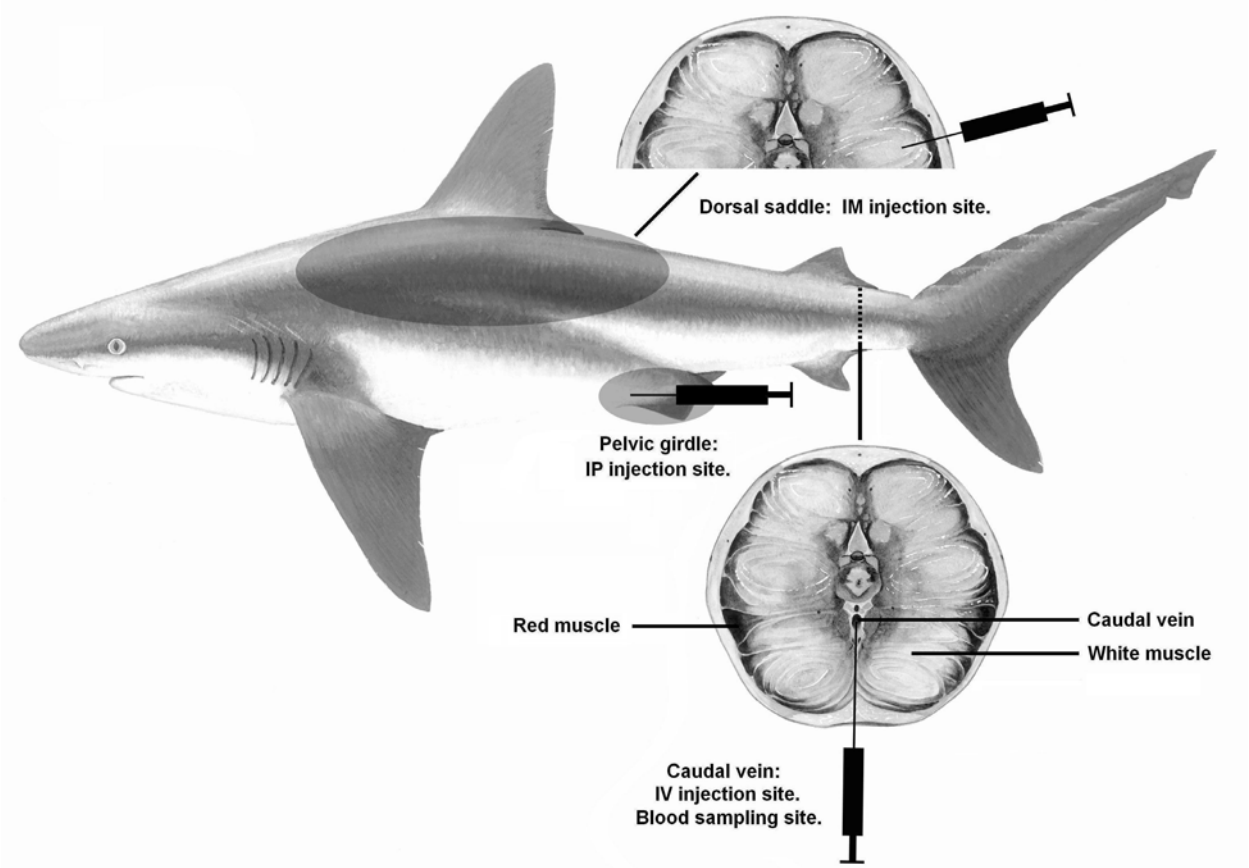


Figure 29.1. Preferred sites for the intravenous (IV), intraperitoneal (IP), and intramuscular (IM) injection of elasmobranchs. Blood may be sampled from the caudal vein (i.e., the IV injection site).

This process can necessitate administration of the drug several times a day, or continuously, to be effective. IV catheters have been employed for continuous drug administration, but experience in this area is limited.

Table 29.1. Medical abbreviations used to represent the frequency of delivery for therapeutic agents.

Abbreviation		Frequency of delivery
SID, q 24 hours	:	Once a day
BID, q 12 hours	:	Twice a day
TID, q 8 hours	:	Three times daily
QID, q 6 hours	:	Four times daily
EOD, q 48 hours	:	Every other day
ETD, q 72 hours	:	Every third day

Many drugs that are caustic when administered by other routes may be safe when administered intravenously.

Intracoelomic or Intraperitoneal (IP)

Absorption through an intracoelomic site is thought to be similar to IV administration, although overall true efficacy is still unknown. Since the liver occupies the majority of the coelom, liver laceration is a concern when introducing a needle into the cavity. Liver laceration can be avoided in most species of sharks and rays by giving injections into the region where the spiral colon contacts the coelomic wall. This area is preferred as a site for administering fluids because intestinal tissue is more resistant to trauma. Found on the right side, just anterior to the pelvic girdle of the animal when it is lying on its ventrum, the size and shape of this region will depend on the species, as well as the nutritional and reproductive status of the animal.

FREQUENCY OF ADMINISTRATION

Table 29.1 summarizes agreed medical abbreviations representing the frequency of delivery for therapeutic agents.

Table 29.2. Categories of chemotherapeutic agents used in aquatic animal medicine showing examples.

Categories	Example chemotherapeutic agents
Antibacterial	aminoglycosides, cephalosporins, quinolones, tetracyclines, chloramphenicol, sulfonamides, metronidazole, and iodine.
Antiviral	idouridine, vidaribine, and trifluridine.
Antifungal	itraconazole, fluconazole, ketoconazole, and amphotericin B.
Antiparasitic	copper, metronidazole, chloroquine, primaquine, diclazuril, toltrazuril, salinity changes, organophosphates, levamasole, praziquantel, fenbendazole, lufeneron, and dimilin.
Anesthetics	ethanol, benzocaine, etomidate, metomidate, halothane-oxygen-nitrous oxide, quinaldine, tricaine methanesulfonate, alphaxalone-alphadonlone, azaperone, carfentanil citrate, detomidine HCl, ethanol, ketamine HCl, medetomidine, propofol, teletamine/ zolazepam, xylazine HCl, sodium pentobarbital, pentobarbitone, eugenol, metomidate HCl, diazepam, and medazolam HCl.
Anti-inflammatory	flunixin meglumine.
Hormones and steroids	methylprednisolone, dexamethasone, stanozolol, thyroxine-Na levothyroxine, vasotocin, oxytocin, gonadotropin, and GnRH.
Nutritional / mineral supplements	vitamins A, D, K, E, C and B; potassium iodide and iodate.
Fluid support	elasmobranch balanced salt solution.

DRUG CATEGORIES AND APPLICATION

The number of different chemotherapeutic agents is enormous and continually expanding. During the course of a clinical evaluation there are usually multiple problems identified; problems that must be prioritized as to their effect on the animal's morbidity (i.e., disease status) and/or mortality (i.e., is the drug potentially life threatening?). Prior to administering medications a full evaluation of the drug should be undertaken (i.e., will it potentially assist the patient, have no effect at all, or result in potentially detrimental effects?). Treatments should be accompanied by the correction of deviant environmental parameters, as these can critically impact the effectiveness of drug therapy. Other factors to consider include, the route of delivery, interactions with other drugs, risk of under-dosing, potential resistance of micro-organisms, and drug toxicity. Correct diagnosis, followed by careful, controlled, and appropriate application of medications, as well as meticulous documentation of all known factors, are essential to progress the field of chemotherapeutics and provide effective treatment.

The different categories of agents used in aquatic medicine are listed in Table 29.2. The following discussion highlights key considerations for select categories. Table 29.3 provides dosage rates and administration routes for some commonly used drugs. It is important to remember that efficacy of these drugs is unknown in most cases. The authors do not take responsibility for information provided in Table 29.3. For more information about specific drugs, readers are directed to individual clinicians cited within the table. Drug dose experiences will be added periodically to the web-based version of this manual and such contributions, which should conform to the format established within the manual, are encouraged.

Antibacterial agents

One of the key principals of appropriate anti-bacterial application is targeting the right bacteria. Culture, identification, and sensitivity of the isolated organism to antimicrobials are critical, but so is determining whether the isolated bacterium

Table 29.3. Elasmobranch drug formulary providing anecdotal information for drug use in various species of elasmobranch. Authors do not take responsibility for doses or protocols presented.

Drug Name	Species name	Common name	Body weight (kg)	Body length (cm)	Problem
Antimicrobials					
Amikacin sulfate	General		-	-	Bacteria (G-)
	Carcharhinus melanopterus	blacktip reef shark	1.20	-	Bacteria (G-)
			-	-	Bacteria (G-)
	Orectolobus japonicus	Japanese wobbegong	12.85	127	Bacteria (G-)
	Rhinoptera bonasus	cownose ray	6.64	-	Bacteria (G-)
	Stegostoma fasciatum	zebra shark	0.80	-	Bacteria (G-)
	Triaenodon obesus	whitetip reef shark	20.65	-	Bacteria (G-)
Ampicillin	General		-	-	Bacteria
Aztreonam	*Carcharhinus melanopterus*	blacktip reef shark	1.20	-	Bacteria (G-)
	Chiloscyllium plagiosum	whitespotted bambooshark	0.70	-	
			0.87	-	
Ceftazadime	General		-	-	Bacteria (G-)
	Carcharhinus melanopterus	blacktip reef shark	1.20	-	Bacteria (G-)
			-	-	Bacteria (G-)
	Chiloscyllium plagiosum	whitespotted bambooshark	0.70	-	Bacteria (G-)
			0.87	-	Bacteria (G-)
	Sphyrna tiburo	bonnethead shark	-	-	Bacteria (G-)
	Taeniura lymma	bluespotted ribbontail ray	0.20	-	Bacteria (G-)
	Triaenodon obesus	whitetip reef shark	20.65	-	Bacteria (G-)
	Triakis semifasciata	leopard shark	6.00	-	
Cephalexin	*Pristis zijsron*	longcomb sawfish	16.40	193	
Chloramphenicol	General		-	-	Bacteria
			-	-	Bacteria
			-	-	Bacteria
	Rhinoptera bonasus	cownose ray	1.24	-	Bacteria
			6.20	-	Bacteria
	Taeniura lymma	bluespotted ribbontail ray	0.20	-	Bacteria
Chloramphenicol succinate	General		-	-	Bacteria
Chlortetracycline			-	-	Bacteria
Ciprofloxacin	*Carcharhinus melanopterus*	blacktip reef shark	2.90	90	Bacteria (G- and G+)
Clindamycin phosphate	*Taeniura lymma*	bluespotted ribbontail ray	0.95	-	
Dihydrostreptomycin	General		-	-	Bacteria
Enrofloxacin	General		-	-	Bacteria (G- and G+)
			-	-	Bacteria (G- and G+)
			-	-	Bacteria (G- and G+)
	Aetobatus narinari	spotted eagle ray	1.5-5.0	-	Skin inflammation
	Carcharhinus melanopterus	blacktip reef shark	2.55	77	Bacteria (G- and G+)
			4.25	89	Bacteria (G- and G+)
			2.90	90	Bacteria (G- and G+)
			5.59	98	Bacteria (G- and G+)
			5.59	98	Bacteria (G- and G+)
			6.44	100	Bacteria (G- and G+)
			1.00	-	Bacteria (G- and G+)
			1.20	-	Bacteria (G- and G+)
			1.20	-	Bacteria (G- and G+)
			1.20	-	Bacteria (G- and G+)
			5.30	-	Bacteria (G- and G+)
			5.40	-	Bacteria (G- and G+)
			5.40	-	Bacteria (G- and G+)
			5.50	-	Bacteria (G- and G+)
			5.50	-	Bacteria (G- and G+)
			-	-	Bacteria (G- and G+)
			-	-	Bacteria (G- and G+)
			-	-	Bacteria (G- and G+)
			-	-	Bacteria (G- and G+)
			-	-	Bacteria (G- and G+)
			-	-	Bacteria (G- and G+)
			-	-	Bacteria (G- and G+)
			-	-	Bacteria (G- and G+)
	Carcharhinus plumbeus	sandbar shark	9.50	120	Bacteria (G- and G+)
	Chiloscyllium plagiosum	whitespotted bambooshark	0.70	-	Bacteria (G- and G+)
			0.70	-	Bacteria (G- and G+)
	Orectolobus japonicus	Japanese wobbegong	12.85	127	Bacteria (G- and G+)
	Paratrygon aiereba	ceja stingray	-	-	Bacteria (G- and G+)

* denotes pharmacokinetics have been preliminarily studied. † denotes mortality.

Dose	Route	Duration	Effectiveness / Comments	Reference
2.5 mg kg^{-1}	IM	q48h x5	May be nephrotoxic	Mylniczenko, pers. com.
3.0 mg kg^{-1}	IM	q48h x6	May be nephrotoxic	Mylniczenko, pers. com.
5.0 mg kg^{-1}	IM	q12h	May be nephrotoxic	Miller, pers. com.
3.0 mg kg^{-1}	IM	q48h x3	May be nephrotoxic	Mylniczenko, pers. com.
3.0 mg kg^{-1}	IM	q72h x3	May be nephrotoxic	Mylniczenko, pers. com.
3.0 mg kg^{-1}	IM	q72h x3	May be nephrotoxic	Mylniczenko, pers. com.
4.0 mg kg^{-1}	IM	q48h x7	May be nephrotoxic	Mylniczenko, pers. com.
10.0 mg kg^{-1}	PO, IM	q24 h		Stoskopf, 1993.
100.0 mg kg^{-1}	IP	q48h x5		Mylniczenko, pers. com.
100.0 mg kg^{-1}	IP	q48h x5		Mylniczenko, pers. com.
100.0 mg kg^{-1}	IP	q48h x5		Mylniczenko, pers. com.
20.0 mg kg^{-1}	IM, IP	q72h x10-15d		Miller, pers. com.
30.0 mg kg^{-1}	IM	q48h x14		Mylniczenko, pers. com.
30.0 mg kg^{-1}		q48h x5		Mylniczenko, pers. com.
20.0 mg kg^{-1}	IM	q72h x5		Mylniczenko, pers. com.
20.0 mg kg^{-1}	IM	q72h x5		Mylniczenko, pers. com.
30.0 mg kg^{-1}	IP	q48h x?		Mylniczenko, pers. com.
20.0 mg kg^{-1}	IM	q48h x5		Mylniczenko, pers. com.
30.0 mg kg^{-1}	IM	q48h x6		Mylniczenko, pers. com.
20.0 mg kg^{-1}	IM	q48h x5		Mylniczenko, pers. com.
30.0 mg kg^{-1}	PO (in food)	q12h x10		Mylniczenko, pers. com.
50.0 mg kg^{-1}	PO, IM	Once (then 25 mg kg^{-1} q24h)		Miller, pers. com.
20-40.0 mg kg^{-1}	IM, IP	q48h x15d		Miller, pers. com.
20-50.0 mg kg^{-1}	IP	q7d x2		Miller, pers. com.
50.0 mg kg^{-1}	PO (in food)	q48h x10		Mylniczenko, pers. com.
25.0 mg kg^{-1}	PO (in food)	q24h x14		Mylniczenko, pers. com.
20.0 mg kg^{-1}	IM	q48h x5		Mylniczenko, pers. com.
40.0 mg kg^{-1}	PO, IM	q24h		Stoskopf, 1993.
10-20 mg kg^{-1}	PO	q24h		Stoskopf, 1993.
10.0 mg kg^{-1}	PO (in food)	q24h x15		Mylniczenko, pers. com.
20.0 mg kg^{-1}	IP	q24h x21		Mylniczenko, pers. com.
10.0 mg kg^{-1}	PO, IM	q24h		Stoskopf, 1993.
7.5 mg kg^{-1}	PO, IM, IP	q24h		Miller, pers. com.
5-10.0 mg kg^{-1}	PO	q24h x10-14d	Injectable form	Miller, pers. com.
5-10.0 mg kg^{-1}	IM, IP	q48h x10-15d		Miller, pers. com.
10.0 mg kg^{-1}	PO	3d	Successful (n=5)	Janse, pers. com.
10.0 mg kg^{-1}	PO (in food)	q48h x17		Mylniczenko, pers. com.
12.0 mg kg^{-1}		q24h x14		Mylniczenko, pers. com.
10.0 mg kg^{-1}	IM	q48 x5		Mylniczenko, pers. com.
10.0 mg kg^{-1}	PO (in food)	q48h x5		Mylniczenko, pers. com.
10.0 mg kg^{-1}		q24h x14		Mylniczenko, pers. com.
11.0 mg kg^{-1}	PO (in food)	q24h x14		Mylniczenko, pers. com.
10.0 mg kg^{-1}	PO (in food)	q48h		Mylniczenko, pers. com.
10.0 mg kg^{-1}	PO (in food)	q48h x10		Mylniczenko, pers. com.
10.0 mg kg^{-1}	PO (in food)	q24h		Mylniczenko, pers. com.
10.0 mg kg^{-1}	PO (in food)	q48h x10		Mylniczenko, pers. com.
9.0 mg kg^{-1}	PO (in food)	q48d		Mylniczenko, pers. com.
10.0 mg kg^{-1}	PO (in food)	q24h		Mylniczenko, pers. com.
10.0 mg kg^{-1}	PO (in food)	q48h		Mylniczenko, pers. com.
10.0 mg kg^{-1}	PO (in food)	q24h		Mylniczenko, pers. com.
10.0 mg kg^{-1}	PO (in food)	q48h		Mylniczenko, pers. com.
10.0 mg kg^{-1}	PO (in food)	q48h		Mylniczenko, pers. com.
10.0 mg kg^{-1}	PO (in food)	q48h x15		Mylniczenko, pers. com.
2.5 mg kg^{-1}	Subconjuctival	q48h x5		Mylniczenko, pers. com.
10.0 mg kg^{-1}	PO (in food)	q24h		Mylniczenko, pers. com.
10.0 mg kg^{-1}	PO (in food)	q24h		Mylniczenko, pers. com.
10.0 mg kg^{-1}	PO (in food)	q48h		Mylniczenko, pers. com.
10.0 mg kg^{-1}	PO (in food)	q48h		Mylniczenko, pers. com.
10.0 mg kg^{-1}	PO (in food)	q24h x15		Mylniczenko, pers. com.
10.0 mg kg^{-1}	PO (in food)	q48h x5		Mylniczenko, pers. com.
10.0 mg kg^{-1}	PO (in food)	q24h		Mylniczenko, pers. com.
10.0 mg kg^{-1}	PO (in food)	q48h x3		Mylniczenko, pers. com.
10.0 mg kg^{-1}		q48h x5		Mylniczenko, pers. com.
10.0 mg kg^{-1}	PO (in food)	q24h x8		Mylniczenko, pers. com.

Table 29.3 (continued). Elasmobranch drug formulary providing anecdotal information for drug use in various species of elasmobranch. Authors do not take responsibility for doses or protocols presented.

Drug Name	Species name	Common name	Body weight (kg)	Body length (cm)	Problem
	Pristis zijsron	longcomb sawfish	16.40	193	Bacteria (G- and G+)
	Rhinoptera bonasus	cownose ray	1.00	-	Skin inflammation
			1.24	-	Bacteria (G- and G+)
			6.20	-	Bacteria (G- and G+)
			-	-	Bacteria (G- and G+)
			-	-	Bacteria (G- and G+)
			-	-	Bacteria (G- and G+)
	Stegostoma fasciatum	zebra shark	7.38	145	Bacteria (G- and G+)
			2.24	-	Bacteria (G- and G+)
			2.25	-	Bacteria (G- and G+)
			6.81	-	Bacteria (G- and G+)
			6.81	-	Bacteria (G- and G+)
	Taeniura lymma	bluespotted ribbontail ray	0.50	-	Skin inflammation
			0.20	-	Bacteria (G- and G+)
			0.27	-	Bacteria (G- and G+
			0.28	-	Bacteria (G- and G+)
			0.95	-	Bacteria (G- and G+)
	Triaenodon obesus	whitetip reef shark	5.90	105	Bacteria (G- and G+)
			7.25	117	Bacteria (G- and G+)
			9.60	132	Bacteria (G- and G+)
			2.10	-	Bacteria (G- and G+)
			2.56	-	Bacteria (G- and G+)
			4.55	-	Bacteria (G- and G+)
			5.36	-	Bacteria (G- and G+)
			20.65	-	Bacteria (G- and G+)
	Triakis semifasciata	leopard shark	6.00	-	Bacteria (G- and G+)
	Rhina ancylostoma	bowmouth guitarfish	65.00	-	Dermal wound
Erythromycin	General		-	-	
Florfenicol	General		-	-	
Furazolidone	General		-	-	
			-	-	
Gentamycin	*Carcharhinus plumbeus*	sandbar shark	-	-	Experimental
			-	-	Bacteria (G-)
K permanganate	General		-	-	
Kanamycin sulfate	General		-	-	
			-	-	
			-	-	
Metronidazole	*Paratrygon aiereba*	ceja stingray	1.05	-	
			1.15	-	
Naladixic acid	General		-	-	
			-	-	
Neomycin	General		-	-	
			-	-	
Nitrofurazone	*Carcharhinus melanopterus*	blacktip reef shark	-	-	
	General		-	-	
			-	-	
			-	-	
	Paratrygon aiereba	ceja stingray	1.05	-	
			1.15	-	
Oxolinic acid	General		-	-	
Oxytetracycline	General		-	-	
			-	-	Bacteria (surface infection)
			-	-	
			-	-	
			-	-	
			-	-	
	Rhina ancylostoma	bowmouth guitarfish	65.00	-	
	Taeniura lymma	bluespotted ribbontail ray	-	-	
Sarafloxacin	General		-	-	
Sulfadimethoxine	General		-	-	

* denotes pharmacokinetics have been preliminarily studied. † denotes mortality.

Dose	Route	Duration	Effectiveness / Comments	Reference
10.0 mg kg^{-1}	PO (in food)	q24h x10		Mylniczenko, pers. com.
10.0 mg kg^{-1}	PO	5d	Successful (n=2)	Janse, pers. com.
5.0 mg kg^{-1}	PO (in food)	q24h x14		Mylniczenko, pers. com.
10.0 mg kg^{-1}	PO (in food)	q48h x14		Mylniczenko, pers. com.
10.0 mg kg^{-1}	PO (in food)	q48h x14		Mylniczenko, pers. com.
10.0 mg kg^{-1}	PO (in food)	q48h x14		Mylniczenko, pers. com.
10.0 mg kg^{-1}	PO (in food)	q48h x14		Mylniczenko, pers. com.
10.0 mg kg^{-1}	PO (in food)	q48h x5		Mylniczenko, pers. com.
10.0 mg kg^{-1}	IM	q72h x3		Mylniczenko, pers. com.
10.0 mg kg^{-1}	PO (in food)	q48h x3		Mylniczenko, pers. com.
10.0 mg kg^{-1}	PO (in food)	q48h x3		Mylniczenko, pers. com.
10.0 mg kg^{-1}	PO (in food)	q48h x3		Mylniczenko, pers. com.
6.5 mg kg^{-1}	IM	3d	Successful (n=1)	Janse, pers. com.
10.0 mg kg^{-1}	PO (in food)	q24h x10		Mylniczenko, pers. com.
10.0 mg kg^{-1}	PO (in food)	q24h x7		Mylniczenko, pers. com.
10.0 mg kg^{-1}	PO (in food)	q24h x7		Mylniczenko, pers. com.
10.0 mg kg^{-1}	IP	q24h x21		Mylniczenko, pers. com.
10.0 mg kg^{-1}	IM	q24h x14		Mylniczenko, pers. com.
10.0 mg kg^{-1}	PO (in food)	q48h x60		Mylniczenko, pers. com.
10.0 mg kg^{-1}	PO (in food)	q24h x10		Mylniczenko, pers. com.
10.0 mg kg^{-1}	PO (in food)	q24h x30		Mylniczenko, pers. com.
10.0 mg kg^{-1}	PO (in food)	q48h x30		Mylniczenko, pers. com.
10.0 mg kg^{-1}	IM	q48h x5		Mylniczenko, pers. com.
10.0 mg kg^{-1}	PO (in food)	q24h x7		Mylniczenko, pers. com.
10.0 mg kg^{-1}	PO (in food)	q24h		Mylniczenko, pers. com.
10.0 mg kg^{-1}	PO (in food)	q48h x5		Mylniczenko, pers. com.
5.0 mg kg^{-1}	IM	3d	No effect.	McEwan, pers. com.
100.0 mg kg^{-1}	PO, IM	q24h x7 - 10d		Miller, pers. com.
40-50.0 mg kg^{-1}	PO, IM, IP,	q12-24h		Miller, pers. com.
1-10.0 mg l^{-1}	Immersion	1d	Possibly carcinogenic.	Miller, pers. com.
50-100.0 mg kg^{-1}	PO (in food)	q24h x10-15d	Inactivated by light.	Miller, pers. com.
2 mg kg^{-1} loading dose, 1 mg kg^{-1} subsequently	IM	8h and 72h	*This regime was designed to prevent blood levels from becoming greater than the theoretical level which might cause nephrotoxicity.	Stoskopf, 1986.
6.0 mg kg^{-1}	IM	q6d	Blood levels are theoretically effective to sensitive organisms, nephrotoxic.	Stoskopf, 1993.
5.0 mg l^{-1}	Immersion	30-60min		Miller, pers. com.
20.0 mg kg^{-1}	IP	q3d x14d		Miller, pers. com.
50-100.0 mg l^{-1}	Immersion	5h q72h x3		Miller, pers. com.
50.0 mg kg^{-1}	PO (in food)	q24h		Miller, pers. com.
50.0 mg kg^{-1}	PO (in food)	q48h x3		Mylniczenko, pers. com.
50.0 mg kg^{-1}	PO (in food)	q48h x3		Mylniczenko, pers. com.
75-100.0 mg l^{-1}	Immersion	1-2h (repeat)	Quinolone, Bacteria (Gram negative).	Miller, pers. com.
0.0-30.0 mg l^{-1}	Immersion	Continuous		Miller, pers. com.
20.0 mg kg^{-1}	PO	q24h		Stoskopf, 1993.
66.0 mg l^{-1}	Immersion	q3d up to x3		Miller, pers. com.
4.0 mg l^{-1}	Immersion	1d (continuous)		Mylniczenko, pers. com.
20-60.0 mg l^{-1}	Immersion	Continuous Change 50-75 % water daily.	Carcinogen, may be inactivated by light.	Miller, pers. com.
50.0 mg l^{-1}	Immersion	q24h		Stoskopf, 1993.
100.0 mg l^{-1}	Immersion	30min Change 50-75 % water daily.	Carcinogen, may be inactivated by light.	Miller, pers. com.
20.0 mg l^{-1}	Immersion	q72h x3 (water change q3d)		Mylniczenko, pers. com.
20.0 mg l^{-1}	Immersion	Water change q3d		Mylniczenko, pers. com.
5-25.0 mg kg^{-1}	PO	q24h	Quinolone, Bacteria (Gram negative).	Miller, pers. com.
50.0 mg kg^{-1}	PO	q24h x10d		Miller, pers. com.
400.0 mg l^{-1}	Immersion	1h	Yellow brown foam may develop.	Miller, pers. com.
10-100.0 mg l^{-1}	Immersion	Continuous		Miller, pers. com.
20.0 mg kg^{-1}	PO	q8h		Miller, pers. com.
10.0 mg kg^{-1}	IM	q24h	50% water changes between treatments.	Miller, pers. com.
25-50.0 mg kg^{-1}	IM, IP			Miller, pers. com.
75.0 mg kg^{-1}	PO	10d	Wound healed.	McEwan, pers. com.
6.5 mg l^{-1}	Immersion			Janse, pers. com.
10-14.0 mg kg^{-1}	PO	q24h x10d	Fluoroquinolone	Miller, pers. com.
50.0 mg kg^{-1}	PO (in food)	5d	Available as powder, ormetoprim, coccidiostat.	Miller, pers. com.

Table 29.3 (continued). Elasmobranch drug formulary providing anecdotal information for drug use in various species of elasmobranch. Authors do not take responsibility for doses or protocols presented.

Drug Name	Species name	Common name	Body weight (kg)	Body length (cm)	Problem
Tobramycin	*Carcharhinus plumbeus*	sandbar shark	-	-	Bacteria (G-)
			-	-	Bacteria (G-)
Trimethoprim/sulfa	General		-	-	Bacteria
			-	-	Bacteria
	Carcharhinus melanopterus	blacktip reef shark	6.44	100	Bacteria
	Taeniura lymma	bluespotted ribbontail ray	0.20	-	Bacteria
			0.95	-	Bacteria
	Triaenodon obesus	whitetip reef shark	8.00	-	Bacteria
Triple antibiotic ointment	General		-	-	Bacteria
Trovafloxacin	*Leucoraja erinacea*	little skate	-	-	Bacteria
Antiparasitics					
Diflubenzuron	General		-	-	Chiton-containing parasites
Fenbendazole	General		-	-	Nematodes
	Carcharhinus melanopterus	blacktip reef shark	1.20	-	Nematodes
	Paratrygon aiereba	ceja stingray	1.05	-	Nematodes
			1.15	-	Nematodes
	Paratrygon leopoldi	white-blotched stingray	0.23	-	Nematodes
	Stegostoma fasciatum	zebra shark	1.20	-	Nematodes
			1.30	-	Nematodes
			1.30	-	Nematodes
			1.40	-	Nematodes
Flumequine	General		-	-	
			-	-	
Formalin	*Urobatis jamaicensis*	yellow stingray	-	-	
Freshwater Immersion	*Stegostoma fasciatum*	zebra shark	10.0-30.0	-	Trematodes (Skin)
	Taeniura lymma	bluespotted ribbontail ray	-	-	Trematodes (Skin)
Praziquantel	*Aetobatus narinari*	spotted eagle ray	1.5-5.0	-	Trematodes (Skin and gills)
	Carcharhinus limbatus	blacktip shark	3.00	-	Trematodes (Skin)
	Rhina ancylostoma	bowmouth guitarfish	65.00	-	Trematodes
	Stegostoma fasciatum	zebra shark	1.20	-	Trematodes
			1.30	-	Trematodes
			1.30	-	Trematodes
			1.40	-	Trematodes
Praziquantel (no alcohol)	General		-	-	Trematodes
	Carcharhinus melanopterus	blacktip reef shark	1.40	61	Trematodes
			2.55	77	Trematodes
			1.20	-	Trematodes
	Carcharhinus plumbeus	sandbar shark	-	-	Trematodes
	Cephaloscyllium ventriosum	swellshark	4.27	85	Trematodes
	Paratrygon aiereba	ceja stingray	1.05	-	Trematodes
			1.15	-	Trematodes
	Paratrygon leopoldi	white-blotched stingray	0.23	-	Trematodes
	Triaenodon obesus	whitetip reef shark	5.90	105	Trematodes
Sodium chloride	*Paratrygon aiereba*	ceja stingray	1.05	-	
			1.15	-	
	Paratrygon leopoldi	white-blotched stingray	0.23	-	
Trichlorofon (Neguvon 75%, Bayer)	*Carcharias taurus*	sand tiger shark	-	-	Trematode (Benedinia)
Trichlorofon (Tugon 80, Bayer)	*Aetobatus narinari*	spotted eagle ray	2.00	-	Trematodes (Skin)

* denotes pharmacokinetics have been preliminarily studied. † denotes mortality.

Dose	Route	Duration	Effectiveness / Comments	Reference
2.5 mg kg^{-1} loading dose, 1 mg kg^{-1} 4h later	IM	q24h	*This regime was designed to prevent blood levels from become greater than the theoretical level which might cause nephrotoxicity (n=3).	Stoskopf, 1986.
6.0 mg kg^{-1}	IM	q6d	Experimental, blood levels are theoretically effective to sensitive organisms, nephrotoxic (n=3).	Stoskopf, 1993.
2.0 mg l^{-1}	Immersion	5-12h q24h x5-7d	50 % water change between treatments.	Miller, pers. com.
30.0 mg kg^{-1}	PO	q24h x10-14d		Miller, pers. com.
30.0 mg kg^{-1}	PO (in food)	q24h x14		Mylniczenko, pers. com.
30.0 mg kg^{-1}	PO (in food)	q24h x10		Mylniczenko, pers. com.
30.0 mg kg^{-1}	PO (in food)	q24h x10		Mylniczenko, pers. com.
30.0 mg kg^{-1}	PO (in food)	q72h x5		Mylniczenko, pers. com.
Ad Lib	Topical	q12h		Miller, pers. com.
10-100 mg kg^{-1}	Oral	144 hours +	n=30 (refer also body text).	Willens et al., 1999.
0.026 ml l^{-1}	Immersion	q7-14d x3 (continuous)		Mylniczenko, pers. com.
10.0 mg kg^{-1}	IM; PO, (in food)	q24h x10d		Miller, pers. com.
50.0 mg kg^{-1}	PO (in food)	q14d x2		Mylniczenko, pers. com.
50.0 mg kg^{-1}	PO (in food)	q14d x2		Mylniczenko, pers. com.
50.0 mg kg^{-1}	PO (in food)	q14d x2		Mylniczenko, pers. com.
50.0 mg kg^{-1}	PO (in food)	q14d x2		Mylniczenko, pers. com.
100.0 mg kg^{-1}	PO (in food)	Once	†Death	Mylniczenko, pers. com.
100.0 mg kg^{-1}	PO (in food)	Once	†Death	Mylniczenko, pers. com.
100.0 mg kg^{-1}	PO (in food)	Once	†Death	Mylniczenko, pers. com.
100.0 mg kg^{-1}	PO (in food)	Once	†Death	Mylniczenko, pers. com.
30.0 mg kg^{-1}	IM, IP		High antibiotic levels.	Miller, pers. com.
50-100.0 mg l^{-1}	Immersion	3h		Miller, pers. com.
25.0 mg l^{-1}	Immersion		†Death (immediate).	Mylniczenko, pers. com.
	Immersion	10min	Successful (n=2)	Janse, pers. com.
	Immersion	1.5min	Successful (n=1)	Janse, pers. com.
20-30.0 mg l^{-1}	Immersion	45-90min	Successful (n=5)	Janse, pers. com.
50.0 mg kg^{-1}	PO		Successful (n=4)	Janse, pers. com.
5.0 mg kg^{-1}	PO	Once		McEwan, pers. com.
8.0 mg kg^{-1}	PO (in food)	Once	†Death	Mylniczenko, pers. com.
8.0 mg kg^{-1}	PO (in food)	Once	†Death	Mylniczenko, pers. com.
8.0 mg kg^{-1}	PO (in food)	Once	†Death	Mylniczenko, pers. com.
8.0 mg kg^{-1}	PO (in food)	Once	†Death	Mylniczenko, pers. com.
2.0 mg l^{-1}	Immersion	Continuous (water change in 7d)	Urticaria 48h post addition in *Carcharhinus melanopterus*, *Carcharhinus plumbeus*, *Pristis* spp.; resolved in few days with water change.	Mylniczenko, pers. com.
10.0 mg l^{-1}	Immersion	1d		Mylniczenko, pers. com.
5.0 mg l^{-1}	Immersion	1d		Mylniczenko, pers. com.
10.0 mg l^{-1}	Immersion	3h		Mylniczenko, pers. com.
10.0 mg l^{-1}	Immersion	3h	*Stegostoma fasciatum* showed ataxia and vomition within an hour at this dose, resolved when placed in fresh saltwater.	Mylniczenko, pers. com.
10.0 mg l^{-1}	Immersion	3h (once)		Mylniczenko, pers. com.
2.0 mg l^{-1}	Immersion	1d		Mylniczenko, pers. com.
2.0 mg l^{-1}	Immersion	SID, 14d		Mylniczenko, pers. com.
2.0 mg l^{-1}	Immersion	1d		Mylniczenko, pers. com.
2.0 mg l^{-1}	Immersion	1d		Mylniczenko, pers. com.
2000.0 mg l^{-1}	Immersion	14d (continuous)		Mylniczenko, pers. com.
2000.0 mg l^{-1}	Immersion	q72h x3 (continuous)		Mylniczenko, pers. com.
2000.0 mg l^{-1}	Immersion	14d (continuous)		Mylniczenko, pers. com.
0.5 mg l^{-1}	Immersion	q12hrs (7d apart)	Treated overnight without lights. Ozone turned off on morning of treatment as ozone breaks down the drug. *Rhina ancylostoma* within same exhibit almost died.	McEwan, pers. com.
0.2 mg l^{-1}	Immersion		†Death (n=1)	Janse, pers. com.

Table 29.3 (continued). Elasmobranch drug formulary providing anecdotal information for drug use in various species of elasmobranch. Authors do not take responsibility for doses or protocols presented.

Drug Name	Species name	Common name	Body weight (kg)	Body length (cm)	Problem
	Carcharhinus amblyrhynchos	grey reef shark	-	-	Trematodes (Skin)
	Carcharhinus limbatus	blacktip shark	-	-	Trematodes (Skin)
	Rhinobatos typus	giant shovelnose ray	-	100-120	Trematodes (Skin)
	Rhinoptera bonasus	cownose ray	1.50	-	Trematodes (Skin)
Antifungals					
Itraconazole	General		-	-	Fungus
Ketoconazole	General		-	-	Fungus
Miconazole	General		-	-	Fungus
Emergency					
Dexamethasone	General		-	-	Shock / adrenal exhaustion
Dexamethasone sodium phosphate	*Rhinoptera bonasus*	cownose ray	6.20	-	
	Stegostoma fasciatum	zebra shark	1.30	-	
	Triaenodon obesus	whitetip reef shark	20.65	-	
Doxapram	General		-	-	Respiratory depression
	Carcharhinus melanopterus	blacktip reef shark	5.25	96	Respiratory depression
			5.50	-	Respiratory depression
	Triaenodon obesus	whitetip reef shark	4.55	-	Respiratory depression
Prednisolone	General		-	-	Shock
Prednisone	*Chiloscyllium plagiosum*	whitespotted bambooshark	0.70	-	
	Stegostoma fasciatum	zebra shark	6.81	-	
Nutritional / Fluid support					
Dextrose (5%)	General		-	-	Dehydration
	Rhina ancylostoma	bowmouth guitarfish	65.00	-	Dehydration
Hormone Drugs					
Iodine (Betadine solution)	General		-	-	Bacteria (superficial infection)
Thyroxine	General		-	-	Hypothyroidism / goiter
Miscellaneous Drugs					
Flunixin meglumine	General		-	-	Hyponatremia / adrenal exhaustion
Furosemide	General		-	-	Fluid build-up in coelom or generalized edema
Ketoprofen	*Taeniura lymma*	bluespotted ribbontail ray	0.20	-	Anti-inflammatory
			0.95	-	Anti-inflammatory
Meloxicam	*Taeniura lymma*	bluespotted ribbontail ray	0.95	-	Anti-inflammatory
Ranitidine	*Carcharhinus melanopterus*	blacktip reef shark	2.90	90	
Ranitidine (injectable)	*Triaenodon obesus*	whitetip reef shark	5.90	105	
Stanozolol	*Atelomycterus marmoratus*	coral catshark	0.22	25	
	Carcharhinus melanopterus	blacktip reef shark	1.20	-	
	Paratrygon aiereba	ceja stingray	1.05	-	
			1.15	-	
Sucralfate	*Carcharhinus melanopterus*	blacktip reef shark	2.90	90	
			5.59	98	
	Triaenodon obesus	whitetip reef shark	5.90	105	
Trinsicon	*Carcharhinus melanopterus*	blacktip reef shark	6.17	95	
			6.44	100	
Vibrio vaccine	*Taeniura lymma*	bluespotted ribbontail ray	0.20	-	Vaccination

* denotes pharmacokinetics have been preliminarily studied. † denotes mortality.

Dose	Route	Duration	Effectiveness / Comments	Reference
0.2 mg l^{-1}	Immersion		Successful (n=3)	Janse, pers. com.
0.2 mg l^{-1}	Immersion		Successful (n=3). Once added, it will react with light.	Janse, pers. com.
0.2 mg l^{-1}	Immersion		Successful (animals did not like treatment) (n=3)	Janse, pers. com.
0.2 mg l^{-1}	Immersion		†Death (n=1)	Janse, pers. com.
1-5.0 mg kg^{-1}	PO (in food)	q24h q1-7d	Systemic mycosis.	Miller, pers. com.
2.5-10.0 mg kg^{-1}	PO, IM, IP		Systemic mycosis.	Miller, pers. com.
10-20.0 mg kg^{-1}	PO, IM, IP		Systemic mycosis.	Miller, pers. com.
1-2.0 mg kg^{-1}	IM, IP, or IV	One dose or as needed		Stoskopf, 1993.
2.0 mg kg^{-1}		Once		Mylniczenko, pers. com.
2.0 mg kg^{-1}	IM	q12h x2		Mylniczenko, pers. com.
2.0 mg kg^{-1}	IM, under Ax	Once		Mylniczenko, pers. com.
5.0 mg kg^{-1}	IP, IV	One dose or as needed		Stoskopf, 1993.
1.0 mg kg^{-1}	IV, Ax	Once	Through Ax	Mylniczenko, pers. com.
1.0 mg kg^{-1}	IV, Ax	Once	Through Ax	Mylniczenko, pers. com.
1.0 mg kg^{-1}	IV	Once		Mylniczenko, pers. com.
1.0 mg kg^{-1}	IP, IV, IM	One dose or as needed		Stoskopf, 1993.
0.735 mg kg^{-1}	PO (in food)	Once		Mylniczenko, pers. com.
0.735 mg kg^{-1}	PO (in food)	Once		Mylniczenko, pers. com.
0.735 mg kg^{-1}	PO (in food)	Once		Mylniczenko, pers. com.
20-30.0 mg kg^{-1}	IP, IV	One dose or as needed		Stoskopf, 1993.
7.5 ml kg^{-1}	IP	SID, 4d	Animal survived.	McEwan, pers. com.
	Topical		Topical wound cleansing agent. Do not use solutions with detergents.	Miller, pers. com.
0.02 mg kg^{-1}	PO, IM	OP SID, IM EOD		Stoskopf, 1993.
0.3 mg kg^{-1}	IM	One dose or as needed		Stoskopf, 1993.
2-3.0 mg kg^{-1}	IP, IM	One dose or as needed		Stoskopf, 1993.
2.0 mg kg^{-1}	IP, Ax	Once		Mylniczenko, pers. com.
1.0 mg kg^{-1}	IP	q48h x5		Mylniczenko, pers. com.
0.1 mg kg^{-1}	PO (in food)	q24h x7		Mylniczenko, pers. com.
2.0 mg kg^{-1}	PO (in food)	q24h x10		Mylniczenko, pers. com.
2.0 mg kg^{-1}	PO (in food)	q48h x21		Mylniczenko, pers. com.
5.0 mg kg^{-1}	IM, under Ax	q7d x2		Mylniczenko, pers. com.
1.7 mg kg^{-1}	PO (in food)	q96h (for 1month)		Mylniczenko, pers. com.
1.0 mg kg^{-1}	PO (in food)	q3d x3		Mylniczenko, pers. com.
1.0 mg kg^{-1}	PO (in food)	q3d x3		Mylniczenko, pers. com.
500.0 mg	PO (in food)	q24h x10		Mylniczenko, pers. com.
500.0 mg	PO (in food)	q24h x7		Mylniczenko, pers. com.
1000.0 mg	PO (in food)	q48h x21		Mylniczenko, pers. com.
14.0 mg kg^{-1}	PO (in food)	Once		Mylniczenko, pers. com.
14.0 mg kg^{-1}	PO (in food)	Once		Mylniczenko, pers. com.
Standard stock	Immersion	20sec (once)		Mylniczenko, pers. com.

is truly the pathogen (disease-causing agent). Beneficial bacterial populations are normally found in the gastrointestinal tracts of all animals and populations have been identified to exist in the musculature and organs of elasmobranchs, which may be critical for the metabolism of urea and other physiological processes (Knight et al., 1987). Careless treatment of an animal with antimicrobials could have an effect on the symbiotic relationship of these bacteria, resulting in consequences that are not yet fully understood. Under-dosing or inadequate treatment times can create additional problems, such as bacterial imbalance and resistance. It is therefore critical to carefully evaluate medication application to avoid these pitfalls.

(1) Aminoglycosides

Aminoglycosides provide bactericidal activity against many gram negative bacteria, with some effectiveness against Staphylococcus spp. Commonly used amino-glycosides include gentamycin, amikacin, and neomycin. Amino-glycosides can be nephrotoxic (i.e., toxic to the kidneys) and may cause temporary neurological dysfunction. It is important to use fluid therapy alongside this group of antibiotics to reduce nephrotoxic effects.

(2) Cephalosporins

Cephalosporins provide broad-spectrum activity and are effective against β lactamase-producing bacteria that can be resistant to other forms of antibiotics. Cephalosporins are poorly absorbed through the gastrointestinal tract, which may limit their usage in aquatic animals where parental administration is not an option. Commonly used cephalosporins include ceftazadime, ceftiofur sodium, and cefoxitin. Once absorbed, cephalosporins are distributed to tissues and fluids, with the exception of the central nervous system. This group of drugs is rarely toxic to the kidneys.

(3) Quinolones

Quinolones are relatively new to veterinary medicine and include enrofloxacin, levofloxacin, ciprofloxacin, as well as the immersion drugs naladixic acid, oxolinic acid, and sarafloxacin, most commonly used for aquatic animals. Quinolones have bactericidal activity against most gram-negative and many gram-positive bacteria. Willens et al. (1999) successfully applied trovafloxacin to female little skates (*Leucoraja erinacea*) at a dosage rate of 10-100 mg kg^{-1} OP. The drug did not adversely affect cartilage, as has been demonstrated in other quinolones in fast-growing young mammals. Plasma levels were present 144 hours post-injection and were still increasing, possibly due to the slow metabolism of the species or an inability to metabolize or excrete the drug. It should be noted that this drug has the advantage of providing a broader spectrum against gram-positive organisms; however, it has been associated with serious liver injury in humans and it is only recommended when safer, alternative antimicrobials are not effective (Burnham, 2002).

(4) Tetracyclines

Tetracyclines are available for both oral and parental administration. The most common tetracyclines used in aquatic animal medicine are oxytetracycline, doxycycline, and chlortetra-cyclines. This group offers a broad spectrum of activity against both gram-positive and gram-negative bacteria. Once administered, tetracyclines are quickly distributed through tissue and will penetrate the blood-brain barrier into the central nervous system. Tetracyclines are bacteriostatic, although at high concentrations they may be bactericidal. If given in bath form, the calcium and magnesium in saltwater will inactivate the drug. Therefore, when tetracyclines are used in seawater, a recommended dose of approximately four times the freshwater dosage is needed to compensate.

(5) Chloramphenicol

Chloramphenicol is available in both oral and parental formulations. It is a broad-spectrum antibiotic with action against both gram-positive and gram-negative bacteria. Chloramphenicol should not be administered simultaneously with penicillin, streptomycin, or cephalosporins, and it should not be administered to animals that may end up in the food chain. In extremely rare cases this drug has been associated with bone marrow disease in humans. Therefore, gloves should be worn when using this drug. Florfenicol, a relative of chloramphenicol, has been demonstrated to have a longer half-life and is a safer drug (Lobell et al., 1994). Florfenicol has been used in aquatic animals but the half-life has ranged from two hours in loggerhead sea turtles, *Caretta caretta*

(Stamper et al., 2003), to 14.7 hours in salmon, *Salmo salar* (Lobell et al., 1994).

(6) Sulfonamides

Sulfonamides are antibacterials that offer a broad spectrum of activity against gram-positive and gram-negative bacteria. Common sulfonamides include sulfadiazine, sulfamethoxyzole, and sulfadimethoxine, and are usually available in both a parental and oral form. These drugs are often combined with the antibiotics trimethoprim and pyrimethamine, to enhance their therapeutic index. Sulfonamides have been known to cause folic acid deficiencies when used for a long period of time.

(7) Metronidazole

Metronidazole is used for anaerobic bacteria as well as protozoal infections. Gloves should be worn when handling this drug as sensitivity reactions are quite common.

(8) Antimicrobial wound flushing agents

Iodine compounds are bactericidal, have high activity against viruses, are fungicidal and tuberculocidal, and are effective against bacterial spores. Iodine compounds can stain and corrode metal, and tinctures of iodine, containing alcohol, will dry the skin. These compounds should be used with caution and proper attention must be paid to drug concentration. Dilutions should be appropriate for the site or specific location for which the iodine is being used. Antibiotic dilutions are effective for disinfection of surgical sites. Alcohol is irritating to shark skin and its use should be avoided wherever possible.

(9) Antimycobacterial agents

Mycobacteriosis in fishes is caused by atypical mycobacteria, usually under sub-optimal environmental conditions. Once established, mycobacterium is difficult to control. Disinfection and quarantine are still considered the best methods of control. There is little published information available on the treatment of mycobacteriosis; however, combinations of rifampin, erythromycin, enrofloxacin, and streptomycin have proved to be effective treatments during limited clinical trials. Kanamycin may be effective, although there has been evidence of resistance. Mycobacterium can be insidious and difficult to eradicate, and many clinicians dispute that it should be treated at all. The need for improved management is mandatory.

Antifungals

The group of drugs known as the “azoles”, including itraconazole, fluconazole, and ketoconazole, have been documented as effective against fungi in other taxa. However, blood titers of itraconazole in treated bonnethead sharks (*Sphyrna tiburo*) were undetectable, following several weeks of continued dosing, casting some doubt on its effectiveness as an antifungal in sharks (Stamper, personal experience). Amphotericin B is effective against many fungi, but is relatively caustic and should be used with caution. No information has been documented about the use of amphotericin B in sharks.

Antiviral agents

The use of antiviral drugs in aquatic medicine is limited, as is the documentation of viruses in elasmobranchs. Antivirals are used routinely in many ophthalmic preparations, which are of limited use in fishes. Commonly used antiviral drugs are idouridine, vidaribine, and trifluridine. There are several drugs which are currently being studied for possible use against retroviral infections; however, their use in elasmobranchs is limited at this time.

Antiparasitics

Antiparasitic medications include drugs that affect protozoans, such as *Trypanosoma* spp., *Amyloodinium* spp., *Trichodina* spp., *Haemogregarina* spp.; coccidians, such as *Eimeria* spp.; microsporidia and myxosporidia; metazoans, including trematodes (flukes), nematodes (round worms), cestodes (tapeworms), and leeches; and various crustacean parasites, including branchiurans, isopods, and copepods. Antiprotozoan drugs include copper, metronidazole, chloroquine, and primaquine. Anticoccidials, such as diclazuril and toltrazuril, have been effective against many species of coccidia. There are many treatments for metazoans. For external flukes, salinity changes, organophosphates, levamasole, and praziquantel have all been used. For tapeworms, praziquantel and levamasole may be used. For nematodes, fenbendazole is usually effective but hematological problems related to this drug have

been reported in other species (Weber et al., 2002). Other organisms (e.g., isopods, copepods, and branchiurans) can be controlled by chitin synthesis inhibitors such as lufeneron or dimilin. For a more detailed discussion of antiparasitic agents, please refer to Chapters 24 and 25 of this manual.

Steroids and anti-inflammatory drugs

The use of steroids, by the authors, for elasmobranch medicine has been limited to treatments for shock, transport trauma, and anorexia. The use of non-steroidal anti-inflammatory drugs (NSAIDS), such as flunixin meglumine, has been limited to inflammatory mediation and promoting analgesia. There is little information available on the specific application of NSAIDS to aquatic animals. NSAIDS should be used with caution as they have many serious side effects in higher vertebrates, including gastrointestinal bleeding, nephrotoxicity, and specific effects on platelet aggregation. The use of NSAIDS for pain relief in aquatic animals has not been established at this time.

Shock and lactic acidosis

Shock and lactic acidosis are common medical challenges facing elasmobranchs. The following discussion outlines the pathophysiology of these two conditions.

Intracellular metabolism requires a narrow range of free hydrogen ion concentration (pH) within which enzymatic and biochemical processes may function efficiently and appropriately. Critical functions such a myocardial electrophysiology and cellular response to endogenous and exogenous chemical compounds (i.e., hormones and drugs) require a specific pH milieu. Significant deviations from these narrow ranges are poorly tolerated and may be life threatening. It is essential that the clinician have a thorough understanding of the homeostatic mechanisms working to maintain normal pH.

When an animal exceeds its normal exercise limits, body metabolism takes place under increasingly anaerobic conditions. A byproduct of these conditions is the formation of lactic acid, resulting in a blood-pH decrease. This process occurs quickly in pelagic species of elasmobranchs during periods of stress, capture trauma, anesthesia, immobilization, and a variety of disease processes (Ross & Ross, 1999). Benthic species are subject to the same metabolic effects, but usually to a lesser degree (Ross & Ross, 1999). In either case, if the problem is left unresolved, it should be considered life-threatening. Correction of an elasmobranch's acid-base status may occur in one of two ways, either through modification of respiratory status and/or metabolic modification with drug therapy.

(1) Sodium bicarbonate

It is not always practical to accurately assess depth of acidosis in field situations, in particular when causative agents have been acute and treatment is required urgently. Acid-base status is therefore usually modified using an empirical estimate (i.e., a best approximation) of the amount of treatment (e.g., sodium bicarbonate) required. A suggested treatment for all species is 0.088 mg kg^{-1} of sodium bicarbonate. This treatment may be administered slowly by IV or IP, diluted in an isotonic IV saline or 5% dextrose solution. It is important to bear in mind that rapid administration of sodium bicarbonate may be extremely dangerous, causing death through the severe derangement of intra- or extracellular ionic concentrations. The risk of provoking alkalosis, by over-correction of the bicarbonate deficit, suggests repeated fractional doses should be used during treatment of acidosis. Once severe symptoms have been controlled, the size of each fractional dose, and the frequency of administration, should be decreased in order to restore normal bicarbonate levels.

(2) Sodium acetate

Sodium acetate (Abbott Laboratories, Abbott Park, Illinois, USA) is a safe, effective alternative for treating acidosis (Miller, personal observation). Many of the inherent problems associated with over-correction using sodium bicarbonate may be avoided by using sodium acetate (Plumb, 1999). In practice, 40 mEq of sodium acetate is mixed with each liter of IV fluid and is administered at the rate of 20-30 ml kg^{-1} $hour^{-1}$. Sodium acetate has been used with good results in sterile water and 0.45% saline, with no observed adverse effects. It is most effective administered IV, but may be administered IP with satisfactory results.

(3) Steroids

Corticosteroid administration is theoretically beneficial for many reasons. These drugs stabilize

lysosomal membranes and inhibit lipid peroxidation. Glucocorticoids reduce cerebral edema formation and modulate the inflammatory response. If corticosteroids are employed, the timing of administration appears to be important. High-dose methylprednisolone has improved survivability of recently captured mammals (Nielson, 1999; Johnson and Murtaugh, 2000) and this technique has been successfully extrapolated for use in sharks (Stamper, personal observation). Methylprednisolone should be administered within six hours of capture. The recommended dosage regimen is 30 mg kg^{-1}, followed by 15 mg kg^{-1} two hours later. This dose may be repeated as required every six hours for the next 24-hour period. Alternatively, sodium prednisolone succinate (5-10 mg kg^{-1} IV) or dexamethasone (2 mg kg^{-1} IV) may be administered as a single dose, at time of capture or transfer.

Anabolic steroids have been used successfully to improve appetite, promote weight gain, and increase strength and vitality. For this reason their empirical use in elasmobranchs has been an adjunct to nutritional therapy for anorexia, unthriftiness, weight loss, cachexia, and general debility. Stanozolol is the most widely used steroid, typically administered at a dose of 0.55 mg kg^{-1}. Megasterol acetate (Mead Johnson Oncology Products, Bristol-Myers Squibb Co., New Jersey, USA) has been used with some success. However, in our experience, the use of anabolic steroids is rarely of long-term benefit and should only be an adjunct to other corrective measures. In addition, when using steroids, it is important to assess if desired effects will be outweighed by the potential undesirable effects (e.g., potassium depletion, reduced tissue repair, fluid retention, renal failure, weight gain, aggression, and infertility) previously observed in other taxa.

(4) Respiratory stimulants

Doxapram may be used to stimulate respiration following capture, transport, or anesthesia, to speed up recovery and reflexes in depressed animals. Doxapram is a general central nervous system stimulant, with all levels of the central nervous system affected. Doxapram will increase respiration rate, but systemic oxygenation may decrease as metabolism increases to support respiratory effort (Stoskopf, 1993a). Doxapram may be administered IV, IM, or applied topically to gill surfaces with satisfactory results. An initial dosage of 5-10 mg kg^{-1} is an acceptable range to begin resuscitative efforts. Doxapram should be used with caution as there is little supportive pharmacological data available on its use in elasmobranchs, and the potential risks and benefits should be weighed before application. It has been the experience of the authors that animals can become hyperactive and move with sudden and explosive behavior once this drug has been applied.

(5) Oxygenation

Pure oxygen administered at pressures greater than one atmosphere has application as a therapeutic agent, but can also have toxic effects. When holding or transporting a shark in a small volume of water it is important to monitor oxygen levels either directly, through measurement of dissolved oxygen, or by monitoring clinical conditions (e.g., oxygen saturation of the blood, stress responses, heart rate, respiration rate, etc.). When required, supplemental oxygen must be titrated into the system to maintain dissolved oxygen levels at >6.0 mg l^{-1} (depending on altitude and temperature). As an adjunct to oxygen therapy, supplemental aeration of the system must be maintained, accomplished by improving the flow dynamics of the treatment system, mechanical aeration, or bubbling compressed air into the system.

At elevated oxygen tensions, highly reactive forms of oxygen are present in abnormal concentrations and toxicity may result. Oxygen toxicity occurs at the cellular level via the destruction of membrane lipids and nucleic acids. Clinical signs of oxygen toxicity include depressed respiratory effort, behavioral changes, loss of equilibrium, and eventually death.

(6) Other emergency drugs

Atropine may be used as a treatment for organophosphate poisoning and slow heart rate, while epinephrine may be used for cardiovascular emergencies. Furosemide may be used to treat fluid buildup within the coelomic cavity and diazepam may be used to reduce seizures. The reader should be aware that these treatments are extrapolations of similar treatments applied to mammals and it is uncertain if they will be totally effective in elasmobranchs (Stoskopf, 1993a; Carpenter et al., 2001).

(7) Fluid Therapy

Fluid therapy is a critical but inexact component of medical care. Fluids are administered to restore normal hydration status, replace electrolytes and nutrients, and administer medications that must be diluted in large volumes. Application revolves around estimating the amount of fluid loss and consequently the amount of fluid that needs to be replaced. This determination can only be made through a thorough understanding of the recent clinical history of the patient, a physical examination, and a laboratory analysis. There are three values that should be calculated to determine the volume of fluid to be administered: the hydration deficit, the maintenance requirement, and current fluid losses.

The amount, type, and method of fluid administration must be determined on a case-by-case basis. There are a variety of commercially prepared fluids available, falling into one of several basic categories: crystalloid solutions, colloid solutions, hypertonic solutions, fluid additives, and parenteral vitamin/mineral products. Special training in fluid administration is mandated for the application of this type of therapy.

Fluid therapy needs to be addressed within the context of elasmobranch physiology, in particular the elevated plasma concentrations of urea, NaCl, and trimethylamine oxide, responsible for osmotic regulation. A solution of iso-osmotic saline has been formulated to successfully restore and stabilize the metabolic status of recently acquired animals (Andrews and Jones, 1990). An elasmobranch-balanced salt solution can be made by adding 8.0 g l^{-1} NaCl and 21.02 g l^{-1} urea to phenol red-free Hank's balanced salt solution (Andrews and Jones, 1990). The solution is most effective when administered IV, but satisfactory results may be obtained from IP administration. The formulation may be compounded in the laboratory, if resources are available, or with the assistance of a local pharmacist. The treatment solution is titrated to effect, and should only be administered by persons trained in its clinical application.

Hormones

Thyroxine-Na levothyroxine has been used in other taxa to compensate for hypothyroidism. Although not previously documented, this treatment may be of use in cases of goiter.

Reproductive drugs include vasotocin, oxytocin, gonadotropin, and GnRH, among others. These drugs may be applied to elasmobranchs, but little work has been documented on their use within this taxon.

Nutritional supplements

Vitamins have been supplemented in captive elasmobranch diets for years. Vitamins are divided into two basic groups, fat-soluble and water-soluble. Fat-soluble vitamins include vitamins A, D, K, and E, and need to be used with caution, since they have been demonstrated to build up to toxic levels. Water-soluble vitamins include vitamin C (ascorbic acid) and the B vitamins (e.g., thiamine, riboflavin, and folic acid).

Some of the better understood and documented mineral requirements include potassium iodide and iodate. Inadequate levels of these minerals will produce goiter in elasmobranchs. Goiter in an adult male spotted eagle ray (*Aetobatus narinari*) has been successfully treated using 9.0 mg kg^{-1} of potassium iodide PO EOD for a period of three weeks (Stamper, personal observation). Lugol's iodine, used in a continuous bath, is another possible treatment for goiter.

Other important minerals include calcium and magnesium; however, the therapeutic application of these minerals has not been well documented.

LEGAL AND LOGISTICAL CONSIDERATIONS

In the U.S., use of any of the aforementioned drugs is considered "off-label", meaning that the Food and Drug Administration (FDA) has not specifically cleared these drugs for use in elasmobranchs. However, the FDA has allowed provision for these drugs to be used when performed under the direction of properly licensed personnel. Each country may have different regulations governing the use of specific chemotherapeutics.

Antibiotics should be used in conjunction with culture and sensitivity tests to allow effective treatment. Drugs that are transferred to another vial must have the following information transferred: name of drug, drug concentration, expiration date, and patient name. Drugs must be discarded once the expiration date has been reached.

Many anesthetics and anabolic steroids are regulated by the Drug Enforcement Agency (DEA) and must only be used by personnel with DEA licensure. Meticulous records must be kept, including the lot number of the drug, the amount used, the species name and identification number of treated individuals, and personnel administering the drug. Access to, use of, and disposal of, these drugs must be carefully controlled and documented, as specified by the DEA.

RESEARCH AND THE FUTURE

Drug pharmacokinetic (i.e., distribution and concentration) and pharmacodynamic (i.e., how drugs work and where they work within the body) studies are critically needed to further the field of aquatic medicine. Although the costs of such trials may be expensive, it is often possible for aquariums to conduct and co-publish a study by partnering with a pharmacology laboratory within a university's veterinary school or medical school. The aquarium can be responsible for animal husbandry and sample collection, the university laboratory can analyze the samples, and both organizations can evaluate and publish the data.

Materials and methods

An example of a drug study is outlined below. It is imperative that the reader recognizes the following to be an example only. A pharmacologist should be contacted prior to any pharmaceutical study to carefully critique methodologies.

A minimum of seven animals should be used for each experimental group in a pilot study, and the number may need to be increased if variability is significant. Several days prior to the drug, each animal should be weighed, examined visually, and blood collected for an assessment of serum chemistries and a complete blood count using Natt-Herrick's solution (Campbell, 1988).

The elasmobranchs should be held individually in identical recirculating systems. Water parameters such as salinity, temperature, ammonia, nitrite, nitrate, calcium, etc. need to be monitored and recorded. The specifications of the recirculating system should be monitored and recorded, including pump types, tank sizes, tank configurations, flow rates, heating or cooling elements, etc. The addresses of manufacturers should be noted.

Assign each animal a blindly-drawn number and randomly divide the animals into IV, IM, IP, or immersion treatment groups of equal number. Each animal should receive a single dose of a known amount (mg kg^{-1}) of the test drug. Drug name, percentage of active ingredient, manufacturer name and address, etc., should all be recorded. Route of administration (IM, IV, IP or immersion) needs to be noted. Size of needles, syringes, and rate of delivery must be documented, and it must be demonstrated that the drug was successfully administered where claimed (e.g., for IV: "…after delivery the plunger of the syringe was retracted to note whether blood was present, prior to drug injection, to determine that the drug was placed in the desired compartment…").

Once the drug has been given, note the times of repeated blood sampling (e.g., blood collections of X cc were taken at 0 (pre-dose sample), 0.5, 1.5, 3, 6, 12, 24, 48, 96, and 120 hours post-injection). Note how much blood was drawn and in what way (e.g., "…for each blood collection, approximately 0.5 ml of blood was collected using a 1.0 ml tuberculin syringe with a 26 gauge needle. The syringe and needle interiors were rinsed with 0.1 ml of 1000 IU ml^{-1} sodium heparin solution as an anticoagulant…"). Explain how the sample was processed (e.g., "…blood was placed into polyethylene micro-centrifuge tubes which were capped and immediately submerged in ice water. The blood was then centrifuged to harvest approximately 0.3 ml of plasma which was placed in polyethylene micro-centrifuge tubes via micropipette…"). Finally, explain how the samples were collected (e.g., "…the tubes were capped and stored at -70 °C until HPLC analysis…"). Sample shipment should be coordinated with the lab to ensure expeditious delivery and appropriate analysis. Records detailing each animal's behavior and their physiological parameters should be maintained at all times.

REFERENCES

Andrews, J. C. and R. T. Jones. 1990. A method for the transport of sharks for captivity. Journal of Aquariculture and Aquatic Sciences 5: 70-72.

Ashley, L. M. and R. B. Ciasson. 1988. Circulatory System. In: Laboratory Anatomy of the Shark. 5th ed pp. 46-52. Ashley, L. M. and R. B. Ciasson (eds). McGraw-Hill Science/ Engineering/Math, New York, NY, USA. 98 p

Burnham, T. H. (Ed.) 2002. Drug Facts and Comparisons 2000, 5th edition. Facts and Comparisons, St. Louis, MO, USA. 3212 p.

Campbell, T. 1988. Avian Hematology and Cytology. Iowa State University Press, Ames, IA, USA. 104 p.

Carpenter, J. W., T. Y. Mashima, D. J. Rupiper, and J. K. Morrisey. 2001. Exotic Animal Formulary. W. B. Saunders, Inc., Philadelphia, Pennsylvania, USA. 423 p.
Evans, D. H. 1997. The Physiology of Fishes. 2nd Edition. D. H. Evans (ed.). CRC Press, New York, NY, USA. 519 p
Johnson, J. A. and R. J. Murtaugh. 2000. Craniocerebral Trauma. In: Kirk's Veterinary Therapy XIII. Bonagura, J. D. (Ed.). pp.178-186. W. B. Saunders, Philadelphia, PA, USA. 1308 p.
Knight, I. T., D. J. Grimes, and R. R. Colwell. 1987. Bacterial hydrolysis of urea in the tissues of carcharhinid sharks. Canadian Journal of Fisheries and Aquatic Science 45: 357-360.
Lobell, R. D., K. J. Varma, J. C. Jounson, R. A. Sams, D. F. Gerken, and S. M. Ashcraft. 1994. Pharmacokinetics of florfenicol following intravenous and intramuscular doses to cattle. Journal of Veterinary Pharmacological Therapy 17: 253-258.
Nielson, L. 1999. Chemical Immobilization of Wild and Exotic Animals. Iowa State Publishing, Ames, IA, USA. 342 p.
Noga, E. J. 1996. Fish Disease: Diagnosis and Treatment. Mosby, St. Louis, USA. 367 p.
Plumb, D. C. 1999. Veterinary Drug Handbook. Iowa State Publishing, Ames, IA, USA. 853 p.
Ross, L. G., and B. Ross. 1999. Anaesthetic & Sedative Techniques for Aquatic Animals. 2nd Edition. Blackwell Science Ltd, Oxford, United Kingdom. 259 p.
Stamper, M. A., M. G. Papich, G. A. Lewbart, S. B. May, and D. D. Plummer. 2003. Pharmacokinetics of florfenicol in loggerhead sea turtles (*Caretta caretta*) after a single intravenous and intramuscular injection. Journal of Zoo and Wild Animal Medicine 34(1): 3-8.
Stoskopf, M. K. 1993a. Shark Pharmacology and Toxicology. *In:* Fish Medicine, pp. 809-816. M. K. Stoskopf (ed.). W. B. Saunders, Inc., Philadelphia, Pennsylvania, USA. 882 p.
Stoskopf, M. K. 1993b. Anatomy and Physiology of Sharks. *In*: Fish Medicine. pp. 749-753. M. K. Stoskopf (ed.). W. B. Saunders, Inc., Philadelphia, Pennsylvania, USA. 882 p.
Stoskopf, M. K., S. Kennedy-Stoskopf, J. Arnold, J. Andrews, and M. T. Perlstein. 1986. Therapeutic aminoglycoside antibiotic levels in brown sharks (*Carcharhinus plumbeus*). Journal of Fish Disease 9(3): 379-395.
Weber, M. A., S. P. Terrell, D. L. Neiffer, M. A. Miller, B. J. Mangold. 2002. Bone marrow hypoplasia and intestinal crypt cell necrosis associated with fenbendazole administration in five painted storks. Journal of the American Veterinary Medicine Association 221(3): 417-419.
Willens, S., J. L. Dunn, D. J. St. Aubin, S. Frasca, W. Carter, and J. E. Burkhardt. 1999. Pharmacokinetics of oral trovafloxacin administration in the little skate. Proceedings of 30th Annual International Association for Aquatic Animal Medicine, May 2-5, 1999, Boston, MA, USA. 87 p. Veterinary Software Publishing Inc., O'Fallon, IL, USA.

PERSONAL COMMUNICATIONS

Janse, M. 2001. Burger's Ocean, Arnhem, 6816 SH, Netherlands.
McEwan, T. 2001. The Scientific Centre, Salmiya, 22036, Kuwait.
Mylniczenko, N. D. 2001. The John G. Shedd Aquarium, Chicago, IL 60605, USA.

The Elasmobranch Husbandry Manual: Captive Care of Sharks, Rays and their Relatives, pages 467-472.
© 2004 Ohio Biological Survey

Chapter 30
Necropsy Methods and Procedures for Elasmobranchs

GERALD L. CROW

Waikiki Aquarium,
University of Hawaii, 2777 Kalakaua Ave.,
Honolulu, HI 96815, USA.
E-mail: crow@waquarium.org

JAMES A. BROCK

95-430 Kamahana Place,
Mililani, HI 96789, USA.
E-mail: brockj001@hawaii.rr.com

Abstract: With declining populations of free-living elasmobranchs, collecting sharks and rays for public exhibition has become increasingly difficult. As a result, husbandry practices for captive elasmobranchs must be refined and improved continually to ensure the longevity of these animals. One critical aspect of husbandry is the understanding of life-threatening diseases through the use of detailed necropsy procedures. A skilled technician conducting a thorough necropsy gross examination can often provide a good diagnosis of the disease process or at least determine which organs were affected by the disease. When warranted, a complete necropsy with cultures, histology, tissue imprints, tissue and fluid stains, and SEM diagnostics can be used. The clinician should be familiar with experts in the various pathology fields and be prepared to ship tissue, parasites, and fluids to laboratories around the world for a full diagnostic workup.

Necropsy examination is an essential part of elasmobranch husbandry and should be integrated thoroughly into the husbandry program (including collecting and handling procedures, exhibit design, daily observation, water chemistry, nutrition, and all aspects of veterinary care). Elasmobranchs are susceptible to a number of diseases (Stoskopf, 1993; Crow, 1996) and careful, detailed necropsy procedures will bring about better understanding of these processes. This chapter greatly benefited from the published work of Reimschuessel et al. (1993) and Noga (1996). For more detail on the examination of elasmobranchs and disease methodologies please refer to Chapters 20-29 of this manual.

GENERAL METHODS

It is recommended that the clinician and key aquarium staff inspect all exhibits, in the morning and evening, to note behavioral signs and physical responses to the environment that may provide clues to potential health problems within the elasmobranch collection. Detailed computerized records should be maintained on treated animals, and previous pathology case history information should be available, for ready access, in the event of a necropsy exam.

Euthanasia

If an elasmobranch is showing agonal signs and the decision is made to euthanize it, attempts should be made to obtain key diagnostic samples while the animal is still alive (i.e., blood samples and tissue scrapings). Euthanasia techniques should result in a rapid loss of consciousness, followed by cardiac and respiratory failure, and ultimate loss of brain function (Anon., 2001).

Various methods can be employed to euthanize an elasmobranch (i.e., overdosing with anesthetics, severing of the spinal cord, etc.) and should take into consideration humane treatment and personal safety, as well as optimal sample collection. When using anesthetics as bath solutions, elasmobranchs should be left in solution for at least 10 minutes following cessation of gill movement (Anon., 2001). The decision to terminate life should only be taken when no veterinary procedures would improve the fish's condition.

Preparation

Animals should not be frozen as it renders tissue unsuitable for diagnostics. It is critical that the necropsy procedure is conducted as soon as possible after the fish's death. Elasmobranchs found dead for more than six hours, depending on the water temperature within the exhibit, are often autolyzed and will not provide useful cultures or usable tissues for histology. However, whenever possible, these animals should be subject to a gross exam which may still provide useful information.

An accurate diagnosis relies heavily on the experience of the clinician, supplies and media available, and the capability of the designated diagnostic laboratory. The examiner should be familiar with elasmobranch anatomy and, if needed, have a dissection manual available. A basic necropsy kit, support equipment, tissue sampling, and preserving fluids should be accessible at all times. A sample necropsy report form is provided in Chapter 36 of this manual.

A complete necropsy can entail considerable time and expense; therefore, each case should be carefully evaluated to determine the extent of the necropsy procedure. It is important to note that results from cultures and histopathology may take several days to weeks before they are completed. A careful gross exam can provide immediate information.

The prosector (person conducting the necropsy) should be familiar with the general anatomy of elasmobranchs (Figures 30.1 and 30.2) and have an understanding of normal versus abnormal appearance (i.e., color, size, consistency, etc.) of tissues and organs. Wet tissue mounts, scrapes, smears, and tissue imprints often provide useful

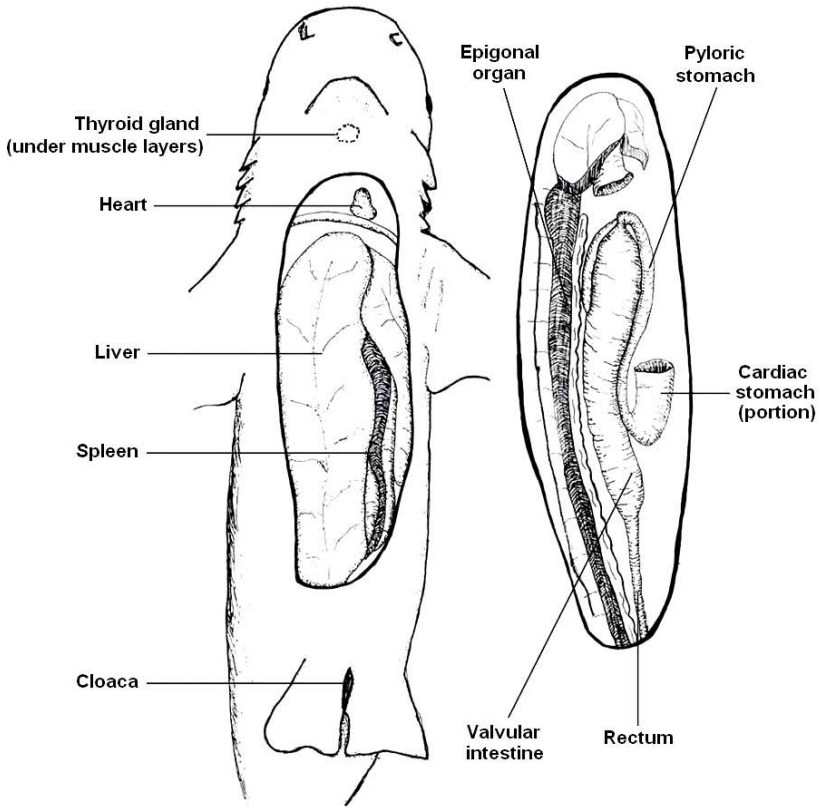


Figure 30.1. Basic internal anatomy of the blacktip reef shark (*Carcharhinus melanopterus*) showing the location of principal organs.

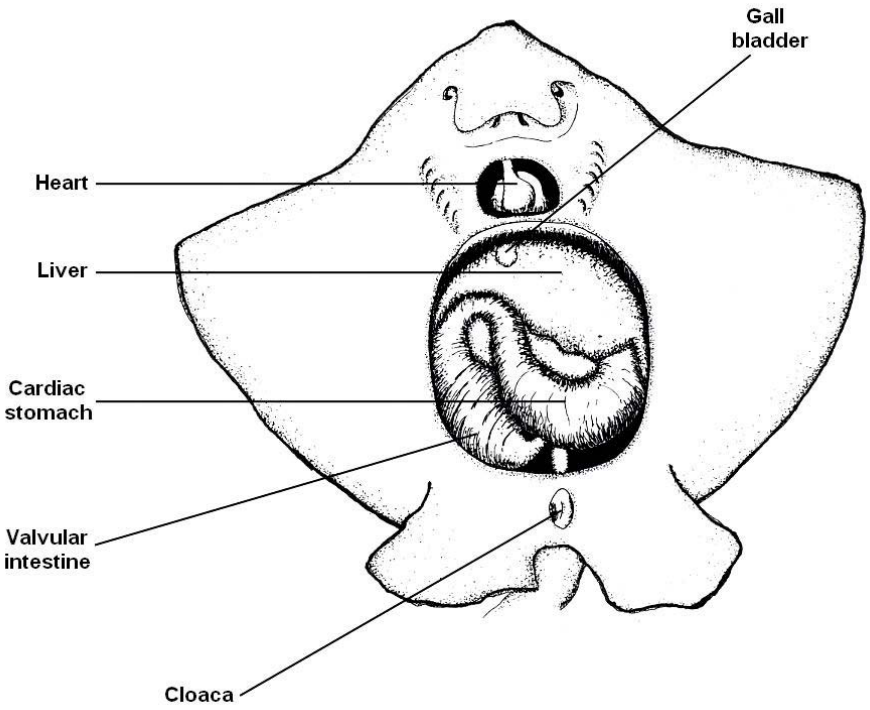


Figure 30.2. Basic internal anatomy of the brown stingray (*Dasyatis lata*) showing the location of principal organs.

information, and the evaluation of most organs in this way is encouraged. Thin sections of tissue need to be taken for wet mounts, in order for adequate light penetration. Small tissue samples (i.e., 1.0 cm^3 or less) need to be taken for histology, in order to allow penetration of fixative. Fournie et al. (2000) review the fixation of tissues. The recommended tissue to fixative volume ratio is 1:10.

Some common mistakes made during necropsy include: not wearing gloves (even though there are no published reports of mycobacterium infections in elasmobranchs, many potential diseases are present); cutting tissues too thick, preventing penetration of fixative; not including all tissues; and not taking multiple samples of each tissue, which, if desired, can be placed in different fixatives. Tissue fixatives are typically considered hazardous material. Therefore, a certified shipper of hazardous material must always be used to ensure proper handling and labeling of shipped samples and fixatives.

THE NECROPSY

The supplies needed for a thorough necropsy are listed in Table 30.1. Additional supplies and preservatives may be required if other tests are requested. Ideally, the necropsy supply kit should be ready for the evaluation of all potential disease etiologies: infectious (viral, bacterial, fungal, parasitic), trauma, tumors, toxins, metabolic, and nutritional. The contact details of some laboratories that routinely examine fish tissue have been provided in Table 30.2.

The following is a generalized procedure for organs to be examined during a necropsy. A regular sequence is recommended to ensure that each organ is examined. Attempts should be made to aseptically open any site that may require culture.

External examination

The external body should be closely inspected for any changes to normal body integrity. The specimen should be measured and weighed. The mouth should be inspected for any discoloration and blockage. The gills should be examined closely for signs of excessive bleeding and color changes. Gill clips should be taken and tissue samples examined under the microscope for evidence of parasites or gas super-saturation. Any skin lesions should be noted and samples of abnormal and normal tissue taken for histopathological examination. The cloaca should

Table 30.1. List of items suggested for use during an elasmobranch necropsy procedure. BHI = Brain Heart Infusion agar; TCB = Thiosulfate Citrate Bile Salts agar.

Vinyl or latex gloves
Calipers
Dissection scope
Binocular microscope with 800x
Two sizes of scissors
Forceps
Bone cutters
Scalpel blades
Slides/cover slips
Syringes 1cc and 5cc with needles
Scale and measuring tape
Labeled tissue containers for preserved tissues
Sterile loop or culturette with transport media
10% buffered formalin, Bouin's, or Davidson's solutions
Absolute methanol
Bacterial growth media (BHI and TCBS with 2% salts)
Fungal growth media (Sabouraud dextrose agar 2% salts)
Stains for slides (Geimsa, Diff-Quick, and Acid Fast)
SEM fixative (Millonig's buffer and glutaraldehyde)
Digital, slide, and video cameras

be examined for the presence of parasites, and any exudate or discharge should be collected for evaluation.

Internal examination

The brain should be the first internal organ sampled, as brain tissue deteriorates rapidly. The brain of elasmobranchs is surrounded by a cartilaginous case called the chondrocranium. The elasmobranch brain consists of five parts, the telencephalon (olfactory), diencephalon (pineal organ), mesencephalon (vision), metencephalon (cerebellum), and myelencephalon (hearing). Expose the brain by removing the skin just posterior to the eyes. The chondrocranium will be exposed as whitish cartilage. As the skin is peeled away at the posterior end of the chondrocranium there is an endolymphatic foramen. Just posterior to the foramen gently slice down to cut this cartilage without hitting the brain. Then either cut along the side of the chondrocranium with bone cutters or slice over the top of the chondrocranium to expose the brain. Cerebral spinal fluid should be checked for discoloration and a culture taken if excessive, or discolored, fluid is present. Fluid should be removed with a syringe and needle, and placed on a slide for examination. The fluid can be placed on a mini-tip culturette and inoculated onto media. The brain should be removed intact and, depending on size, placed directly in, or sectioned before placing in, fixative.

Eyes are often overlooked in the necropsy procedure. The eyes should be removed intact and placed in fixative for histological examination. Eyes should be slit for fixative penetration.

The thyroid gland is commonly ignored during necropsy. It is, however, critical to body function, affected by environmental stressors, and should

Table 30.2. A sample of diagnostic laboratories from around the world that specialize in the examination of fish tissue.

United States

University of California at Davis
Department of Medicine
School of Veterinary Medicine
Davis, CA 95616
(916) 752-3411

Department of Fisheries and Aquaculture
College of Veterinary Medicine
7922 NW 71 St.
Gainesville, FL 32606
(904) 392-9617

North Georgia Diagnostic Ass. Lab.
College of Veterinary Medicine
University of Georgia
Athens, GA 30602
(404) 542-5260

Fish Diagnostic Medicine
College of Veterinary Medicine
Drawer V
Mississippi State, MS 39762
(601) 325-3432

Aquatic Toxicology and Pathology Laboratory
Department of Pathology, 711 MSTF
University of Maryland School of Medicine
10 S. Pine St.
Baltimore, MD 21201
(410) 328-7230

Pathology Laboratory
Osborn Laboratories of Marine Science
New York Aquarium, NY Zoological Soc.
Boardwalk & West 8th Street
Brooklyn, NY 11224
(718) 265-3417

Fish Diagnostic Laboratory
Department of Avian and Aquatic Animal Medicine
College of Veterinary Medicine
Cornell University
Ithaca, NY 14853
(607) 253-3365

Joseph M. Groff, VMD, PhD
Department of Pathology, Microbiology and Immunology
Room 1149, Haring Hall
One Shields Avenue
School of Veterinary Medicine
University of California
Davis, California 95616
(530) 753-8739
e-mail: josephvmd@aol.com
(e-mail contact for correspondence preferred)

Zoo/Exotic Pathology Services
Dr. Drury Reavill
2825 KOVR Drive
West Sacramento, CA 95605
(916) 725-5100

Marine Pathology Laboratory
University of Rhode Island
Kingston, RI 02882
(401) 792-2334

Northwest ZooPath
18210 Waverly Drive
Snohomish, WA 98296
(360) 668-6003

Australia

CSIRO Livestock Industries
Australian Animal Health Laboratory
Private Bag 24
Geelong VIC 3220
Australia
(61) 352-275426

Yeerongpilly Veterinary Laboratory
Animal research Institute
665 Fairfield Road
Yeerongpilly QLD 4105
Australia
(61) 733-629440

Fisheries Western Australia
Locked Bag 39
Cloisters Square WA 6850
Australia
(61) 363-365216

Animal Health Laboratory
Food, Agriculture, and Fisheries Division
DPIWE
P O Box 46
Kings Meadows TAS 7249
Australia

Netherlands

Central Institute for Animal Disease Control
Fish Pathology
P O Box 65
8200 AB Lelystad
The Netherlands
(313) 202-38238

Utrecht University
Department of Veterinary Pathology
Section Pet Avian, Exotic An. and Wildlife
Yalelaan 1
3584 CL Utrecht
The Netherlands
(313) 025-34357

Canada

Fish Pathology Laboratory
Department of Pathology
Ontario Veterinary College
Guelph, Ontario
N1G 2W1 Canada
(519) 824-4120

be included in the exam. The thyroid gland in healthy elasmobranchs is a flattened organ located in loose connective tissue between the ventral side of the coracohydral and the medial side of the coracomandibular muscles. A general description of the location of the thyroid gland has been given in Figure 30.1.

To enter the body cavity, a midline incision is recommended. Place the elasmobranch on its back. Gently pull up a piece of skin, posterior to the pectoral girdle, with a pair of forceps and make a small incision with a scalpel blade. Keeping the skin elevated, cut toward the tail stopping just short of the cloacal area. It is important not to touch any internal organs with the scalpel or gloves when doing the cutting. A quick inspection of the organs prior to any manipulation of body contents should be done to observe any abnormalities. Cultures and fluid samples should be taken.

The liver is typically the most prominent organ in the body cavity of elasmobranchs. The color should be reddish/beige and the edges should be sharp and well-demarcated. Vitamin E deficiency typically creates rounded edges and a mushy texture that easily comes apart in your hand. If a liver infection is suspected, the external surface can be sterilized and the tissue sliced open for culture samples. Care must be taken during interpretation because *Vibrio* spp. are a normal part of the liver flora in elasmobranchs (Grimes et al., 1985). Liver tissue imprints can be made on slides and fixed in 100% methanol.

The gallbladder is a thin-walled greenish sac located at the junction of the left and right lobes of the liver. Parasites have been discovered in this organ, and fluid stains may be useful for disease diagnosis.

The spleen and pancreas are located alongside the pyloric stomach. The spleen should be bright red or maroon and the pancreas beige. Both organs should be inspected for any abnormalities. If disease is suspected, cultures and tissue imprints should be taken.

The reproductive tract should be examined for egg or sperm development and traced from the testis (male) or ovary (female) to the sperm sac (male) or uterus (female).

The epigonal glands and kidneys are located on both sides of the vertebral column. The kidney has been reported to contain bacteria as part of its natural flora (Grimes et al., 1985). Bacterial cultures should be taken if these organs are suspected in the disease process.

The stomach and valvular intestine should be opened and examined for abrasions, obstructions, lesions, and parasites. Stomach contents should be collected, rinsed, and placed in a petri dish or bowl for metazoan parasite identification using a dissection scope.

REFERENCES

Anon. 2001. 2000 report of the AVMA panel on euthanasia. Journal of the American Veterinary Medical Association 218: 669-696.

Crow, G. L. 1996. A review of the diseases and pathology of captive elasmobranchs. *In:* AZA Annual Conference Proceedings, September 17-21, Waikiki, Hawaii, p. 76-81. American Zoo and Aquarium Association, Silver Spring, Maryland, USA.

Fournie, J. W., R. M. Krol, and W. E. Hawkins. 2000. Fixation of fish tissues. *In:* The Laboratory Fish, p. 569-578. G. Ostrander (ed.). Academic Press, San Diego, California, USA.

Grimes, D. J., P. Brayton, R. R. Colwell, and S. H. Gruber. 1985. Vibrios as autochthonous flora of neritic sharks. Systematic Applied Microbiology 6: 221-226.

Noga, E. J. 1996. Fish Disease Diagnosis and Treatment. Mosby, St. Louis, Missouri, USA. 367p.

Reimschuessel, R. 1993. Postmortem examination. *In:* Fish Medicine, p. 160-165. M. K. Stoskopf (ed.). W. B. Saunders Co., Philadelphia, Pennsylvania. USA.

Stoskopf, M. K. (ed.). 1993. Fish Medicine. W. B. Saunders Co., Philadelphia, Pennsylvania, USA. 882 p.

The Elasmobranch Husbandry Manual: Captive Care of Sharks, Rays and their Relatives, pages 473-482.
© 2004 Ohio Biological Survey

Chapter 31
Husbandry of Freshwater Stingrays of the Family Potamotrygonidae

RICHARD ROSS

Institute for Herpetological Research
PO Box 50038,
Montecito, CA 93150, USA.
E-mail: stingrays1@worldnet.att.net

Abstract: Freshwater stingrays are often subjected to stressful conditions prior to importation and new specimens should be evaluated for signs of disease or stress. Recently transported specimens should be quarantined and appropriate medical treatments administered during this period. Once acclimatized, specimens can be maintained in communal aquariums with other fishes. In general, freshwater stingrays do not bother other fishes, if they cannot eat them, and they do not interact aggressively. Freshwater stingrays eat any kind of live food, and can be trained to eat a variety of fresh or freshly thawed dead foods. Frequent feedings of varied foods are necessary for optimal health. While many species of freshwater stingray can be maintained readily with basic husbandry techniques, some species have more specific needs and are less suitable for communal aquariums.

Tropical freshwater stingrays of the family Potamotrygonidae contain three genera (*Paratrygon*, *Plesiotrygon*, and *Potamotrygon*) and 18 species. These stingrays are endemic to South American rivers that drain into the Atlantic Ocean or Caribbean Sea. The range of many Potamotrygonid stingrays is restricted to a single basin or river system. Some species are even restricted to a single river. This high level of endemism means that Potamotrygonid stingrays are at high risk of endangerment through habitat destruction and overharvesting.

This chapter examines the husbandry of freshwater stingrays of the family Potamotrygonidae; specifically, assessment of newly imported stingrays; their requirements for water quality, habitat, feeding, and general husbandry; and diseases of freshwater stingrays, showing possible medication regimes.

ACQUISITION AND ACCLIMATIZATION

Importation

Commercially available freshwater stingrays are often larger than other ornamental fishes. While medium-sized stingrays generally withstand shipping well, large specimens, of >35 cm disk width (DW), are more sensitive to the stresses of capture, handling, and shipping. Long flights, delays in making connections, and poor water quality are responsible for significant mortalities. Stingrays are initially stressed while being captured and held by villagers, by being roughly handled during transport to exporters' facilities, and finally, by being kept in inadequate conditions before exportation. Once they arrive at importer's facilities, they may be kept for as little as one or two days before being shipped again. By the time they reach their ultimate destination, stingrays may have been subjected to two to three weeks of substandard conditions. It is therefore important to examine new stingrays for signs of stress, disease, and poor condition.

Assessing new specimens

The most important sign to look for in a newly imported stingray is curling of the disc, or margin of the fin. A healthy stingray always keeps its disc-margin flat to the substrate, except when actively moving around. A stingray that consistently holds

the edges of its fin elevated (i.e., the fin edges "curl" upwards during rest) will almost inevitably die. There are two possible exceptions to this: a stingray may be resting in a current of water that causes the fin margin to be elevated; or a stingray in good condition, at rest, may slowly undulate the fin, or disc, on either side of its tail. If in doubt, the stingray can be gently encouraged to move and observed as it settles into a resting position again. If the fin margin remains elevated after the fish has settled to the substrate, this is indicative of a stressed fish that is likely to die. In the early stages of this sign the trailing edge of the disc-margin will be affected first, on either side of the tail. When this sign spreads around the disc towards the front, death will soon occur. Aquarists should always be aware of this sign and be prepared to identify it at its earliest stage. When it occurs in an acclimatized specimen or long-term captive it is an ominous sign, indicating an overlooked problem.

Other signs of poor health or disease include a cloudy or milky film covering the body, rapid breathing while at rest, open sores on the dorsal surface, red or bloody sores on the underside of the fish, and areas of fungal infection on the skin (see below). While these signs may indicate disease or stress, they are not necessarily indicative of imminent death; curling of the disc margin is a far more serious sign. Stingrays in good health should have clear skin, and an almost velvety appearance. Light-colored patches on the skin, or an overall cloudiness or milky discoloration, are a sign of disease, especially fungal infections.

Unexplained mortalities

Occasionally, specimens that appear in good health may refuse food and eventually die. Unfortunately, these unexplained deaths are puzzling for the aquarist as causes may not be obvious. The most likely explanation for these deaths is exposure to extreme stress during capture and transport. Failure to provide fresh transport water can result in the accumulation of excess nitrogenous wastes (i.e., ammonia) and cause permanent damage to the kidney. Although there are no obvious visible signs, renal failure will inevitably lead to death which may take place several weeks after transport. Another possible cause of unexplained death may be permanent neurological damage from elevated ammonia concentrations. Additionally, where water quality is poor, dissolved oxygen levels may be too low, causing irreversible damage to the brain or other organs, and eventually resulting in death weeks later.

Acclimatization and quarantine

Rays that are severely stressed for brief periods may not show abnormal physical signs for 7-10 days. Therefore, care during the period following shipping can be critical. Stingrays that appear healthy, with no abnormal signs, may initially do well, only to deteriorate days later. Whenever possible, newly received specimens should be kept in a quarantine or isolation tank during this period, at least until they have been feeding for several days. Early signs of poor health are listlessness, cessation of feeding, failure to begin feeding, and of course, fin curl. Stingrays with any of these signs should be kept in tanks with high water quality, good filtration and aeration, and should immediately be started on an antibiotic treatment program (see below). Since stingrays may stop feeding during treatment, specimens should be kept in isolation after treatment, and not placed in communal tanks, until feeding is well established.

Newly acquired stingrays should be examined for weight loss. The tail and pelvic bones are areas where weight loss will be most apparent. The pelvic bones are located on the stingray's dorsal surface, on either side of the tail, where the tail joins the body. When visible, they appear as small tent-like elevations of the skin. The pelvic bones should not be visible on a stingray in good nutritional condition. Similarly, the tail should be full and thick, with no bony structure visible through the skin. Stingrays kept without food for long periods, either by exporters or retail shops, may show signs of weight loss. Recently imported stingrays may have lost weight during the weeks in transit without food. Once in captivity, these stingrays should begin feeding and regain lost weight quickly. When in doubt about a new specimen's status (e.g., a specimen within a retail shop) it can be offered food—an acclimatized stingray, in good health and kept in suitable conditions, will almost never refuse food. A stingray in good health, but showing signs of weight loss, is likely to thrive once in a supportive environment, and should readily regain lost weight. Such specimens should be maintained in an isolation tank, if possible, to eliminate competition for food by other animals. Specimens with visible pelvic bones must be fed a lot of food to re-establish normal weight.

When specimens first arrive, small amounts of food, either blackworms (*Lumbriculus variegatus*) or tubifex worms (*Tubifex tubifex*), can be left in the isolation tank, while watching the stingrays to see if they begin feeding. Quantities can then be gradually increased in order to establish the appropriate amount for each feeding session. Stingrays are active fish, having high energy demands and food should be given two to three times each day. Many weeks may be necessary to re-establish the normal body weight of previously starved animals.

Although healthy specimens may be ready to begin feeding a day or two after arrival, the constant activity in an exhibit tank may delay or prevent a new specimen from becoming acclimatized, even one in good health. Newly acquired specimens may take time to accept unfamiliar foods offered in captivity, while acclimatized specimens search constantly and aggressively for food, leaving little for a new specimen to eat. There is always a risk of introducing infectious agents to acclimatized specimens if newly acquired animals are placed directly into a communal exhibit. Therefore, as previously mentioned, quarantining of new specimens is always recommended.

HANDLING SPECIMENS

The spine and venom

The spine, or barb, of a stingray is a defensive weapon. Stingrays rarely attempt to sting, even when netted or manipulated during treatments. However, some taxa are more aggressive than others. For example, ocellate river stingrays (*Potamotrygon motoro*) are more likely to sting when being netted. Stingray envenomation is uncommon. Individuals who have been stung report that the venom is extremely painful, but none suffered serious long-term consequences. The primary risk from a stingray barb appears to be secondary infection through wound contamination. The fleshy sheath surrounding a spine may contain toxins or other proteins that promote secondary infection. Stingrays shed their spine about two to three times per year. Shed spines will be found at the bottom of an exhibit. These spines may have residual toxins and should be handled with care. Envenomation has occurred from dead stingrays that have been kept frozen.

It is common practice for exporters to place a piece of air hose or other plastic tubing over the barb to prevent injuries to workers and to prevent the spine from becoming caught in nets or perforating plastic shipping bags. If left over the spine this tubing can catch on objects in the tank, twisting the barb and putting stress on the tail. The spine may even be torn free, leaving an open wound at the barb insertion site. In some extreme cases, exporters have even been known to cut off the barb tip, which may cause damage to the spine sheath or tail. When plastic tubing is placed over the spine, it is often forced over the spine's protective sheath, damaging the tissue. Purulent necrotic material can accumulate in the tubing promoting infection and in some cases result in sepsis and premature shedding of the barb. It is recommended that plastic tubes be removed from barbs as soon as specimens are received. Removal of barbs can be done prior to removing the stingray from the shipping bag, or while it is in the quarantine tank. Brief anesthesia with MS-222 (e.g., Finquel®, Argent Laboratories, USA) can be helpful in removing tubing.

Plastic tubing is difficult to remove from the barb due to the backward-pointing teeth of the spine. The easiest way to remove tubing is to hold it with a forceps at one end, while cutting off the top of the tube with a razor or scalpel, starting from the end closest to the body. When the top has been cut away, the rest of the tubing can be spread apart and removed. After removing tubing, the area around the spine should be examined for signs of infection or bleeding. If such signs are present, the stingray should be given an antibiotic treatment (see below) and kept in an isolation tank until signs of infection have disappeared.

Catching and moving specimens

Catching and moving a stingray is complicated by two factors: stingrays are venomous, and the spine readily catches in the mesh of most nets. The easiest method to catch stingrays is to guide the specimen into a plastic bag. The bag is then raised, allowing most of the water to drain out. Another technique, used in open-top holding tanks, is to guide the stingray into a submerged tub, which is then gently raised out of the water. This method causes little stress to the stingray and is certainly less traumatic than netting. Once in a bag or tub the stingray can be readily transferred to another tank.

When a stingray must be captured and restrained for treatment, it is usually necessary to use a net. In this case, a fine mesh net must be used as this

reduces (but does not eliminate) the risk of catching the spine in the mesh. Chasing a stingray around the tank should be avoided. Stingrays are not agile swimmers and can be gently guided into a net. Once caught, stingrays can be raised to the surface for treatment. It is best not to remove the stingray from the water entirely, as this causes them to panic and thrash around in the net, risking entanglement of the spine.

If a stingray's spine becomes caught in the mesh of a net, it is sometimes possible to free the barb by gently pushing the mesh backwards toward the stingray's body. If this procedure fails, it is best to cut the entangled tip of the spine free using high-quality heavy duty scissors (or wire cutters). When the spine becomes caught, the fleshy sheath does not penetrate the net. This means that the tip of the spine is exposed and easily visible. Usually only the tip of the spine becomes caught in the net, so cutting off 6-12 mm of the barb will release the stingray. The net should be raised to just below the surface of the water and the spine should be cut as close as possible to the inside of the net. Cutting the tip of the spine does not injure the stingray. If it is not possible to cut the spine free, a piece of the net can be cut free to release the stingray. Never try to pull the net free, as this will only entangle it further. When a stingray feels itself restrained by the net, it will swim away vigorously, which will embed the net more tightly. Therefore, the net should be cut away as quickly as possible, leaving a small piece of mesh attached to the spine. This will not bother the stingray, and will fall off when the spine is shed. Forcefully pulling on the net may cause the entire spine to be torn from the tail. This action will leave an open wound in the tail, which will usually heal spontaneously, but must be monitored for signs of infection. The fleshy sheath and spine will re-grow in about six months.

WATER QUALITY

Water quality guidelines for freshwater stingrays do not differ greatly from the requirements of other South American fishes.

pH

The majority of freshwater ray species prefer water that is soft, and slightly acid. Stingrays thrive in a pH of 6.25-6.75, but most will tolerate a pH range of 5.0-8.0, if changes occur gradually. Newly imported specimens are more sensitive to inappropriate pH than acclimatized specimens. For example, new specimens that were listless and refused to feed suddenly became active and began feeding when pH was lowered from a range of 7.3-7.4 to 6.25 (personal observation).

Hardness

Although sensitivity to water hardness does vary between species, most stingrays tolerate moderate hardness levels. Conductivity, as measured by TDS (total dissolved solids), can be used as a guideline. TDS measurements in the range of 350-450 mg l^{-1} (=ppm) are usually acceptable; however, stingrays generally prefer softer water, in the range of 150-200 mg l^{-1}. Discus (=ceja and =manzana) (*Paratrygon aiereba*) and China stingrays (undescribed) will not generally tolerate hard water, requiring water that has a TDS <200 mg l^{-1}. However, once acclimatized, even these species will tolerate harder water.

Temperature

Water temperature for stingrays should normally be maintained in the range of 24.0-26.5 °C. Stingrays seem comfortable up to temperatures of 29.5 °C and appear to tolerate brief exposure to colder temperatures of 15.5-18.5 °C (as may occur during shipping) without permanent harm. However, chronic exposure to temperatures below 22.0 °C may cause anorexia and illness. Largespot river stingrays (=tiger) (*Potamotrygon falkneri*) require a higher temperature than other species (i.e., 25.5-26.5 °C). If the temperature falls below this range, anorexia will soon develop in this group. Since all other husbandry aspects will appear normal, in such cases, it is easy to overlook a drop in temperature as the cause of anorexia in largespot river stingrays, especially in a communal or mixed-species aquarium.

SUBSTRATE

In the wild, freshwater stingrays are found in areas where river bottoms are composed primarily of mud, silt, or extremely fine sand. Substrates composed of particles that are sharp or abrasive (e.g., angular sand particles) may damage the skin of stingrays and result in fungal or bacterial infections. Substrate should therefore be chosen carefully. Smooth gravel, such as medium or coarse aquarium gravel, is suitable. Stingrays will do well in bare-bottom tanks, although they

appear to be uncomfortable for a short period of time before becoming acclimatized to the lack of substrate. Stingrays use their pectoral fins to move slowly over the bottom. Without substrate they are unable to gain traction and slip when trying to use their pectoral fins. Eventually, however, stingrays in bare-bottomed tanks seem to adjust to these unusual circumstances.

Stingrays will bury themselves in substrate, especially when startled. Occasionally stingrays will bury themselves while inactive and lie with their eyes protruding. As stingrays become fully acclimatized they spend less and less time buried in the substrate, and eventually spend most of their time looking for food or exploring their surroundings. In general, delicate species, such as the discus, China, and largespot river stingrays do not do well on coarser substrate that is suitable for other taxa. This is probably due to their tendency to bury themselves more frequently, resulting in abrasions of the skin.

Substrate should not be too deep when used in aquarium systems without under-gravel filters, as anaerobic areas may develop where substrate becomes compacted. Additionally, uneaten food can accumulate in thick layers of gravel; although active stingrays will turn over shallow substrate while searching for food items and keep it relatively free of debris (i.e., if the substrate is not more than 35 mm deep).

FOOD AND FEEDING

Stingrays eat a wide variety of foods. Maintaining a varied diet is extremely important in captive animals, both for environmental enrichment and minimizing the risk of nutritional deficiencies. Stingrays are active and should be fed at least once a day, and preferably two to three times daily. The following are considered to be suitable food items:

1. Goldfish (*Carassius auratus*) or other small fishes.
2. Ghost shrimp (*Callianassa californiensis*), small crayfish (e.g., *Orconectes* spp., *Procambarus* spp., etc.), or grass shrimp (*Palaemonetes* spp.).
3. Californian blackworms (*Lumbriculus variegatus*) or other tubificid worms.
4. Nightcrawlers (*Lumbricus terrestris*), redworms (*Lumbricus rubellus*), or other commercially-raised earthworms.
5. Chopped pieces of raw fish and shrimp.

The first food for newly acquired stingrays should be tubificid worms as these seem to be most readily accepted and are small enough to be inadvertently ingested through the mouth or spiracle, thereby giving the stingray an opportunity to taste this unfamiliar food by chance. Once a stingray is observed to be readily feeding on tubificid worms, finely chopped night crawlers can be introduced in small quantities. Once recognized as food, these will be readily eaten by nearly all stingrays. Other types of food can then be tried.

Live foods, including blackworms or tubifex worms, may be fed in quantities sufficient to leave a small amount in the tank after a feeding session, allowing the stingrays to browse at leisure. However, when cleaning the substrate, note whether a significant amount of living worms are present as they may colonize the substrate, if left uneaten, and add to the tank's biomass. Chopped redworms or nightcrawlers, and any non-live, non-aquatic foods should be fed in smaller quantities, in order to avoid overlooked detritus decomposing in the tank.

Stingrays have relatively small mouths (e.g., a stingray of 25 cm DW would typically have a mouth of only 13-19 mm diameter) and food items must be chopped into small pieces so they can be readily ingested. If a stingray ingests a piece of food and repeatedly spits it out and ingests it again, this usually indicates that the food item is too large. Some stingray species, such as the antenna stingrays (*Plesiotrygon* spp.), have even smaller mouths, relative to their body size. Once acclimatized, stingrays can develop techniques for eating larger food items. For example, newly imported stingrays may have difficulty consuming small pieces of chopped nightcrawler, but over time they will learn to eat an entire worm by sucking it into their oral cavity. Additionally, newly acquired stingrays may ignore "feeder" goldfish. However, they quickly learn to chase down and consume "feeder" goldfish, even learning where they hide within the exhibit.

When feeding large quantities of "feeder" goldfish to obligate fish-eating species, such as the discus and China stingrays, it is wise to supplement with vitamin B1 every one to two weeks, at a dosage rate of 0.125 mg l^{-1} $week^{-1}$ (personal observation).

Stingrays will grow in proportion to the quantity of food given. Most imported species can grow to a large size (i.e., 120-150 cm DW). However, like most aquarium fishes, if fed small amounts of food regularly, their growth rate will be slower.

SPECIES-SPECIFIC HUSBANDRY REQUIREMENTS

Although stingrays are not aggressive fishes, keeping them in a communal aquarium can present problems. Any fish small enough to fit into a stingray's mouth may eventually become prey. Stingrays are otherwise tranquil animals. Keeping more than one specimen in the same tank generally does not present problems, even when size or species differences are a factor. Except for species requiring special conditions (see below), numbers of stingrays of different species and sizes may be kept together without difficulty as long as there is adequate food. Under these conditions, the aquarist should provide food at least twice a day and observe each specimen to be certain that all are obtaining sufficient food.

Large-eyed stingrays

The large-eyed stingrays include more commonly available species such as the ocellate river, Magdalena river (*Potamotrygon magdalenae*), porcupine river (*Potamotrygon histrix*), and the smooth back river (=teacup) (*Potamotrygon orbignyi*) stingrays, as well as the less common white-blotched river (*Potamotrygon leopoldi*) and bigtooth river (*Potamotrygon henlei*) stingrays.

The large-eyed stingrays are active, aggressive fishes that hunt for food and constantly move around an exhibit. For this group, the water temperature range should be 22-24 °C, nitrate levels should be <100 mg l^{-1}, and pH should be 6.25-6.75. The large-eyed stingrays should be fed two to three times daily with a variety of foods. In a community tank, all specimens should be observed carefully while feeding. Specimens not getting sufficient food can be fed directly with tongs.

The large-eyed stingrays make good display species as they are constantly active and are aggressive feeders. Adult specimens generally do well in display aquariums with catfish of the family Pimelodidae, or other predatory fish. On occasion, conflict can arise between stingrays and arawanas (*Osteoglossum bicirrhosum*), or large species such as redtail (*Phractocephalus hemioliopterus*) or gilded (*Zungaro zungaro*) catfish. These fishes have been known to chew the tails of stingrays.

Small-eyed stingrays include obligate fish eaters such as the discus, China, and coly (undescribed) stingrays. This group includes the long-tailed (=common) river stingray (*Plesiotrygon iwamae*) and black-tailed (=dwarf) river stingray (undescribed), collectively called the antenna stingrays. For husbandry purposes the small-eyed stingrays can be divided into two sub-groups: the antenna stingrays, and discus and China stingrays.

Antenna stingrays

Antenna stingrays have smaller mouths than most stingrays and therefore feed primarily on blackworms or tubifex worms. Small specimens (i.e., <25 cm) are often incapable of eating small pieces of chopped nightcrawler. Finely chopped redworms or blackworms may be given as an alternative food. Larger specimens will eat finely chopped nightcrawlers, and even small "feeder" goldfish.

Gravel substrates are satisfactory for antenna stingrays. Due to the extreme length of the tail, which can be several times the length of the stingray's disk, antenna stingrays require a larger surface area than other species.

The tail of the antenna stingray is delicate and specimens with damaged tails often die. If the tail is kinked or damaged near the tip, it may break off (or even remain intact) and heal, leaving a small bump where the damage occurred. However, if the tail is damaged, bent, or kinked, close to the body (especially where it changes from all white to variegated), it rarely heals, eventually falls off, and the stingray will probably die. The cause of death is unknown but may be related to infectious agents entering the tail stub. Wild-caught specimens with healed tails (i.e., broken and healed in the wild) are not at risk. The tail can be easily damaged by objects in the tank. Rocks, driftwood, or even aquarium equipment can catch the tail, causing it to kink or break. Powerheads, air-lift tubes, or other equipment is equally dangerous. A specimen may have its tail drawn into a powerhead intake, resulting in the tail becoming entangled and damaged by the impeller. Therefore, antenna stingrays are best kept in a tank without ornamentation or equipment.

The long-tailed river stingray is a difficult species to display for several reasons: the long, delicate tail is at constant risk of being broken or damaged; new arrivals are delicate and may refuse food unless they are held in a quiet aquarium; and acclimatized specimens can become aggressive.

The black-tailed river stingray seems to be less sensitive to tail injuries than the long-tailed river stingray. Kinks in the tail are not as likely to cause the tail to fall off and specimens with broken tails are not at risk of dying. The black-tailed river stingray is the only species that may rest with its disc margin elevated above the substrate when in good health. The black-tailed river stingray is unable to eat foods larger than blackworms or tubifex worms, although small grass or ghost shrimp may be eaten. The black-tailed river stingray is not large enough to turn over gravel to search for worms and therefore uneaten worms will soon colonize exhibit substrate. Prior to feeding, the substrate should be disturbed to see if worms are present. If so, the substrate should be agitated to free worms so they can be eaten.

Discus and China stingrays

The discus and China stingrays are generally more delicate than other stingrays. The China stingray was only recently described and the name seems to originate from the stingray's similarity to a Chinese coolie's hat. The discus, or ceja (eyebrow in Spanish), stingray gets its name from the dark markings over each eye. The manzana (apple in Spanish) stingray, which may be a morph of the discus stingray, has a shape reminiscent of an apple-half cut from top to bottom, the tail being the "stem" of the apple.

Discus and China stingrays require soft water. Their fin, or disc, is thinner and more delicate than those of large-eyed stingrays and seems to be sensitive to irritation from substrates. When maintained on substrate, China stingrays may thrive for short periods and then develop fungal infections and die. Discus stingrays survive on substrates, but may develop nicks and tears in their fin. Bare-bottom tanks work well for these species.

Discus and China stingrays have a habit of adhering to the side of an aquarium, making them interesting display species. However, they are ambush feeders and are poor competitors for food in displays with other fishes, making them difficult to display. Discus and China stingrays feed exclusively on "feeder" fishes and supplementation with B1 vitamins is therefore essential. These stingrays feed most effectively during the initial confusion when "feeders" are first dropped into an aquarium. If "feeders" escape the initial feeding frenzy, they may stay out of the range of the stingrays for days. These stingrays rarely survive for more than one and a half years and may die suddenly, even when feeding well and appearing to be robust of health (personal observation).

Largespot river stingrays

Largespot river stingrays are among the most challenging and delicate of the freshwater stingrays. They prefer acid pH in the range of 6.25-6.50. When pH rises to ≧7.0, their behavior changes, becoming quieter, feeding less aggressively, and spending more time beneath the substrate. Largespot river stingrays require a warmer temperature than other species, preferring a range of 25.5-27.5 °C. If too cool, these stingrays will become inactive, stop feeding, and will eventually sicken and die.

When newly acquired, largespot river stingrays prefer to feed nocturnally and remain quiet during the day. They are rarely tempted by "feeder" goldfish and often reject chopped nightcrawlers for many months following acclimatization. Even when well-acclimatized, they do not do well with aggressive species of stingrays. This species is best kept alone or with other largespot river stingrays. Newly imported specimens are often large and severely stressed from transport.

CLINICAL CARE

Healthy stingrays demonstrate typical patterns of activity, exploring the exhibit for food items, occasionally burying themselves in the substrate, and soon surfacing again to look for food. Discus stingrays often adhere to the side of the tank waiting for "feeder" fish, which they will trap against the tank wall. Although stingrays will periodically swim along the sides of an exhibit and the water surface, healthy stingrays rarely spend much time free-swimming. A stingray that spends most of its time swimming freely in the water, rather than along the substrate, may be demonstrating stress. Rapid breathing, decreases in activity, remaining under the substrate for long periods, and most importantly, inappetence, can all indicate stress and/or compromised health in a stingray.

Inappetence

Most species, if received in good condition, will begin feeding in about two to three days, although some specimens may take as long as one to two

weeks (especially China and largespot river stingrays). Stingrays are normally active and inquisitive and should begin exploring a tank and searching for food almost immediately. Stingrays that remain quiet for more than a few days, or refuse food after this time, should be closely monitored for signs of injury, stress, or illness. Stingrays that otherwise appear to be in good health, but do not begin feeding immediately, should be kept in an isolation tank until feeding has become regular. Shy or stressed specimens can starve, in the midst of abundant food, if kept in a tank with too many active fishes.

Healthy stingrays eagerly accept food, and between feedings are almost always searching for more food. Stingrays will rarely refuse food when offered, even if they have been recently fed. Therefore, refusal of food is often the first sign of a health problem in stingrays. If a stingray refuses food, even once, it should be examined carefully for signs of disease and water quality parameters should be thoroughly checked.

Dominance interactions

Intra- and interspecific dominance interactions may be responsible for loss of appetite. These interactions may be subtle or overt, and can be related to differences in species, size, and/or gender, etc.

Although uncommon, aggressive behavior can occur between stingrays. Adult males may bite each other, and males will bite females during courtship. Occasional bites of this nature are harmless. However, severe injuries to females have been known to occur, especially during courtship. Additionally, male-male interactions can sometime produce serious injuries. Such injuries usually occur around the disc margin and are characterized by abrasions or frayed fins. In the wild a subordinate stingray can escape a dominant specimen, but in an aquarium this is not possible. As a result, a subordinate specimen may be repeatedly injured and will eventually stop feeding and die. When aggression is present in an exhibit, the subordinate specimen must be watched closely to ensure it does not stop feeding or becomes listless and inactive. In addition to injuries around the disc margin, abrasions or bite marks may appear on the dorsal surface of a stingray. When such injuries appear with increasing frequency, specimens should be segregated. If intra-specific interactions are intense, they may result in loss of appetite and the eventual death of subordinate fish.

Catfishes (*Hypostomus spp*.) may chew on the dorsal surface of stingrays, causing serious abrasions and death. It is unknown whether catfish acquire some nutritional value from the skin or slime coat, or whether this is simply aggressive behavior. Although suckermouth catfish (*Hypostomus plecostomus*) can generally co-exist peacefully with stingrays, this group must be watched carefully for signs of aggression.

Fish lice

Stingrays in captivity are usually not susceptible to the common diseases of other tropical fishes. Occasionally, newly imported specimens may carry fish lice (*Argulus spp*.). These ectoparasites appear as small brown circular spots, about 2-3 mm in diameter, and are more readily seen on light-color stingrays. Fish lice are usually only observed in small numbers (i.e., 2-3) and if gently touched the parasite will skitter across the skin of the stingray. When on the dorsal surface, fish lice can be removed readily with forceps. When on the ventral surface, removal is more difficult as it is difficult to invert stingrays. In these circumstances, it is easier to pinch the parasite through a net, from underneath, in order to remove it. Sudden heavy outbreaks of fish lice may occur within an exhibit, without the addition of new stingray specimens. These outbreaks are probably caused by a few unobserved parasites successfully reproducing. Dimilin or diflubenzuron (e.g., Anchors Away®, Jungle Laboratories Corp., USA), a chitin inhibitor non-toxic to fishes, is effective at treating fish lice outbreaks at a dosage 2,650 mg l^{-1}. This treatment should be repeated once after a two week period.

Fungus

Fungal infection is the commonest disease of freshwater stingrays. The first obvious sign of an underlying health abnormality is often a fungal infection of the skin. Fungal infections appear to occur secondarily to external injuries, bacterial infections, or chronic stress. Fungal infections caused by the freshwater cotton wool fungus (*Saprolegnia* spp.) are often seen on the tail, especially where a piece of air tubing has been placed over the spine, or at the tip of the tail where minor injuries may have occurred during handling. These infections appear as small cotton-like tufts and generally are not difficult to cure. Treatment with antibiotics appears to be an effective means of eliminating fungal infections (see below). Since

fungi are generally not susceptible to antibiotics, it is likely that the successful treatment of fungal infections is a secondary effect of treating an underlying bacterial infection.

Antibiotics

Furanace (e.g., Nitrofurazone, Novalek Inc., USA) may be added directly to aquarium water to treat minor infections; however, injectable antibiotics are generally more effective and preferable. Enrofloxacin (Baytril®, Bayer Corp., USA) at 0.25 mg kg^{-1} (daily or every other day), or ceftazadime pentahydrate (Fortaz®, GlaxoSmithKline Inc., USA) at 10.0 mg kg^{-1} (every other day), administered intramuscularly for 7-10 days, may be used to treat bacterial infections secondary to external injury.

Rays requiring injection should be netted and held just below the surface of the water, to prevent excessive struggling. The process is facilitated if the spiracles remain submerged. If two people are available, the body of the stingray can be supported from below with one hand, while the injection is being given with the other hand. The second person can hold the net. Injections should be given intramuscularly. The preferred injection site is slightly to the right or left of the spine, about halfway between the line of the eye and the base of the tail. The needle should be inserted to a depth of ~5.0 mm, at a low angle, rather than perpendicular to the body. Care should be taken to avoid being within range of the spine when giving injections. Before handling or administering any form of medication it is advisable to seek veterinary advice.

SEX DETERMINATION AND REPRODUCTION

Stingrays, like other elasmobranchs, reproduce by internal fertilization. Male stingrays have claspers (modified pectoral fins located on either side of the tail) used to transfer sperm into the cloaca of the female during copulation. Gender can be readily determined in small or newborn specimens as claspers are present at birth. However, claspers remain small until stingrays become sexually mature, so it may be necessary to examine the ventral surface of a young specimen to verify clasper presence or absence.

While reproduction in freshwater stingrays has been accomplished in captive conditions, little is known about their reproductive strategies. Captive breeding has occurred primarily in the fall and winter in the northern hemisphere, with courtship behavior from September through December, and births occurring from November through January. Little is known about environmental cues that may stimulate reproductive behavior in stingrays, although possibilities include changes in the water temperature, pH, and photoperiod.

All freshwater stingrays are believed to bear live young. When captive bred, gestation periods have been about three months and litter sizes ranged from 1-12 offspring. Newborn stingrays may begin feeding within 24 hours of birth. Only the smallest foods should be offered (e.g., blackworms, tubifex worms, or pieces of finely chopped redworm). No negative interactions have been reported between adult and newborn stingrays. Other than concern for food competition, there appears to be no reason to separate newborn stingrays from parent females or other adults. Allowing newborns to remain in the water in which they were born is preferable whenever possible. Care must be taken to ensure that newborns are getting sufficient food in large community exhibits.

SUGGESTED READING

Alfred, E. R. 1962. Sharks, rays and sawfishes in Malayan freshwaters. Malayan Nature Journal 16 (4): 235.

Charvet-Almeida, P., M. L. G. de Araujo, R. S. Rosa, and G. Rincon. 2002. Neotropical freshwater stingrays: diversity and conservation. Shark News, 14: 1-2.

Chaudhuri, B. L. 1912. Freshwater sting-rays of the Ganges. Journal of the Proceedings of the Asiatic Society of Bengal (new series), 7: 625-629.

Compagno, L. J. V. and S. F. Cook. 1995a. The exploitation and conservation of freshwater elasmobranchs: Status of taxa and prospects for the future. *In:* The biology of freshwater elasmobranchs. M. I. Oetinger and G. D. Zorzi (eds.). Journal of Aquariculture and Aquatic Sciences, 7: 62-90.

Compagno, L. J. V. and S. F. Cook, 1995b. Freshwater elasmobranchs: A questionable future. IUCN Shark News 3: 4-6.

Compagno, L. J. V. and S. F. Cook. 1995c. Through the glass darkly: A troubled future for freshwater elasmobranchs. Chondros 6(1): 7-9.

Compagno, L. J. V. and T. R. Roberts. 1982. Freshwater stingrays (Dasyatidae) of Southeast Asia and New Guinea, with description of a new species of *Himantura* and reports of unidentified species. Environmental Biology of Fishes 7(4): 321-339.

Compagno, L. J. V. and T. R. Roberts. 1984a. Marine and freshwater stingrays (Dasyatidae) of West Africa, with description of a new species. Proceedings of the California Academy of Sciences, ser. 4, 43: 283-300.

Compagno, L. J. V. and T. R. Roberts. 1984b. Dasyatidae. *In:* Check-list of the freshwater fishes of Africa, p. 4-5. J. Daget, J. P. Gosse, and D. F. E. Thys van den Audenaerde (eds.). Museum Royal de L'Afrique Centrale, Tervuren, Belgium/Office de la Recherche Scientifique et Technique Outre-Mer, Bonde, France, 1.

Day, F. 1873. Report on the freshwater fish and fisheries of India and Burma. Office of the Superintendent of Government Printing, Calcutta. 307 p.

Jubb, R. A. 1967. Freshwater Fishes of Southern Africa. A. A. Balkema, Cape Town, South Africa. 248 p.

Monkolprasit, S. and T. R. Roberts. 1990. *Himantura chaophyraya*, a new giant freshwater stingray from Thailand. Japanese Journal of Ichthyology 37 (3): 203-208.

Roberts, T. R. 1978. An Ichthyological survey of the Fly River in Papua-New Guinea with descriptions of new species. Smithsonian Contributions to Zoology 281: 1-72.

Rosa, R. S. 1985. A systematic revision of the South American freshwater stingrays (Chondrichthyes: Potamotrygonidae). Unpublished doctoral dissertation. The College of William and Mary in Virginia, USA. 523 p.

Rosa, R. S. 1985b. Further comment on the nomenclature of the freshwater stingray *Elipesurus spinicauda* Schomburgk, 1843 (Chondrichthyes; Potamotrygonidae). Revista Brasileira da Zoologia, São Paulo 3(1): 27-31.

Rosa, R. 1996. Relaçao preliminar das especies de peixes (Pisces, Elasmobranchii, Actinopterygii) ameacadas no Brasil. Revista Brasileira da Zoologia. 13 (3): 647-667.

Smith, H. M. 1945. The freshwater fishes of Siam, or Thailand. Bulletin of the United States National Museum (188), 622 pp.

Smith, J. A. 1863. Notice of the *ukpam*, a large species (probably new) of stingray (*Trygon*, Cuv.), found in the old Calabar River, Africa. Proceedings of the Royal Physical Society of Edinburgh, 3: 64-69.

Tanaka, S. 1994. Research of freshwater elasmobranchs in Lake Nicaragua. Report of the Japanese Society for Elasmobranch Studies 31: 26-34. [In Japanese].

Taniuchi, T. 1979. Freshwater elasmobranchs from Lake Naujan, Perak River, and Indragiri River, southeast Asia. Japanese Journal of Ichthyology 25(4): 273-277.

Taniuchi, T. 1987. Investigational report on freshwater stingrays in West Africa. Studies on adaptability and evolution of freshwater elasmobranchs III. Investigation Committee of Freshwater Elasmobranchs, Nagasaki University, Japan. Report of the Overseas Scientific Investigation in Africa, March 1987: 23-45.

Taniuchi, T. 1992. Report on preliminary investigation of freshwater elasmobranchs in Mexico and Central America. Report of Japanese Society for Elasmobranch Studies 29: 33-49. [Japanese text with English abstract].

Taniuchi, T. and M. Shimizu. 1991. Elasmobranchs collected from seven river systems in northern Australia and Papua-New Guinea. *In:* Studies on Elasmobranchs collected from seven river systems in northern Australia and Papua-New Guinea, p. 3-10. M. Shimizu and T. Taniuchi (eds.). The University Museum, Nature and Culture No. 3(1991).

Taniuchi, T., T. T. Kan, S. Tanaka, and T. Otake. 1991. Collection and measurement data and diagnostic characters of elasmobranchs collected from three river systems in Papua New Guinea. *In:* Studies on Elasmobranchs collected in seven river systems in northern Australia and Papua-New Guinea, p. 27-42. M. Shimizu and T. Taniuchi (eds.). The University Museum, Nature and Culture No. 3(1991).

Taniuchi, T. T., M. Shimizu, M. Sano, O. Baba, and P. R. Last. 1991. Descriptions of freshwater elasmobranchs collected from three rivers in northern Australia. *In:* Studies on Elasmobranchs collected from seven river systems in northern Australia and Papua-New Guinea, p. 11-26. M. Shimizu and T. Taniuchi (eds.). The University Museum, Nature and Culture No. 3(1991).

The Elasmobranch Husbandry Manual: Captive Care of Sharks, Rays and their Relatives, pages 483-486.
© 2004 Ohio Biological Survey

Chapter 32
Husbandry of Tiger Sharks, *Galeocerdo cuvier*

ANDY DEHART

National Aquarium In Baltimore
501 E. Pratt St.
Baltimore, MD 21202, USA.
E-mail: adehart@aqua.org

Abstract: Tiger sharks (*Galeocerdo cuvier*) have rarely been kept successfully in captivity. Some of the important factors to consider when keeping this species, as for most elasmobranchs, include exhibit size and design, diet, and medical care. This article presents a brief overview of the husbandry requirements for tiger sharks, based on information drawn from the experiences of several institutions that have attempted to maintain this species.

Tiger sharks (*Galeocerdo cuvier*), have rarely been kept in captivity, and very few have been maintained with any long-term success. Recent advances in aquarium science are now making it possible to maintain this delicate species for longer periods of time. The key factors to successfully exhibiting tiger sharks include an understanding of their unique captive and natural behavior, and recognition of their special needs related to exhibit and habitat design, specimen acquisition, daily husbandry, and medical care.

This article should serve as a general guideline for maintaining tiger sharks in captivity. Since only a few tiger sharks have been kept for an extended period of time, it is important to note that this guideline reflects a good deal of extrapolation based on scant information obtained during past attempts to keep this species. The record for maintaining a tiger shark in captivity is 4.5 years, and counting, at the Acuario de Veracruz, Veracruz, Mexico (Marin-Osorno, pers. com.). Four other institutions have been able to keep tiger sharks in an exhibit for over two years: Sea World, Orlando, USA; Epcot Center's Living Seas Pavilion, Orlando, USA; Atlantis Resort, New Providence, Bahamas; and the Henry Doorly Zoo, Omaha, USA (Crow and Hewitt, 1988; Davis, pers. com.; Kaiser, pers. com.).

CAPTIVE BEHAVIOR

Tiger sharks are notable for their unique behavior in captivity. Once in a captive environment, most specimens quickly develop a pattern of swimming along the perimeter of the display (Crow and Hewitt, 1988; Seligson and Weber, 1990; Dehart and Stoops, 1998). Some specimens orient their body parallel and adjacent to smooth sections of the exhibit, such as the acrylic windows or concrete walls. These individuals may rub their pectoral fins, the lower portion of their rostrum, and the bottom of their caudal fins, on the smooth surfaces. A specimen kept at the Henry Doorly Zoo was observed to spend substantially more time rubbing the walls of the display while the aquarium was open and crowded with people (Dehart & Stoops, 1998). In addition to the tiger shark, the 1,710 m^3 Atlantic coral reef display, complete with a tunnel bisecting the tank, contained a lemon shark (*Negaprion brevirostris*), six sandbar sharks (*Carcharhinus plumbeus*), and a number of Caribbean teleosts. It is believed that increased rubbing of the walls during visiting hours was the result of excess noise generated by the public as they moved through the tunnel. The tiger shark maintained at Epcot Center's Living Seas Pavilion spent nearly 60% of its time adjacent to the perimeter of the exhibit (Seligson and Weber, 1990).

Tiger sharks typically avoid other species of sharks and appear to require larger areas of individual swimming space within an exhibit (Crow and Hewitt, 1988). Atlantis Resort maintained three tiger sharks in a 5,206 m^3 display with multiple elasmobranch and teleost species. More than any other species within the exhibit, the tiger sharks tended to stay away from their own species (Kaiser, pers. com.). Acuario de Veracruz, however, has successfully maintained two tiger sharks together for over a year and both continue to do well (Marin-Osorno, pers. com.). Tiger sharks appear to be indifferent or oblivious to divers in the water. At both the Henry Doorly Zoo and Epcot Center's Living Seas Pavilion tiger sharks have been known to bump divers, seemingly unaware of their presence (Davis, pers. com.).

SYSTEM DESIGN

Exhibit size, shape, and structure are all critical for tiger sharks. Tiger sharks tend to swim at the surface so depth is not an important factor for these sharks, but adequate surface area is critical. One of the specimens kept at Atlantis Resort would spend a large portion of the day in a shallow lagoon area of the exhibit, having dimensions: 30 m x 15 m x 1.5 m deep (Kaiser, pers. com.). The exhibit should be of an irregular shape, with no corners more acute than 135°.

All exhibit surfaces should be covered with display elements, such as rockwork or artificial coral, to prevent the shark from swimming too close to smooth surfaces. At the Henry Doorly Zoo, half of the exhibit was covered with rockwork, while the other half was a smooth, black, concrete wall. When the shark swam near artificial rockwork she did not rub her fins or body. However, rubbing was observed adjacent to smooth concrete walls. The décor at Atlantis Resort was one continuous wall of angular rockwork, with no smooth areas. In this display, tiger sharks stayed away from the perimeter and no problems of continuous rubbing were observed (Kaiser, pers. com.). A brief description of the five facilities that have successfully maintained tiger sharks for over two years has been summarized in Table 32.1.

Catwalks or other structural items should be located >60 cm above the surface of the exhibit, to prevent tiger sharks from rubbing their dorsal fin on these structures. Reducing stocking density will lower stress imposed on tiger sharks and promote long-term survival of this species.

Table 32.1. A summary of the basic exhibit parameters for five institutions that have successfully maintained tiger sharks, *Galeocerdo cuvier* , showing: exhibit shape, exhibit dimensions, exhibit volume, water systems, and specimen longevity.

Aquarium	Exhibit shape	Dimensions (meters)	Volume (m3)	Water system	Longevity
Acuario de Veracruz (Veracruz, Mexico)	Kidney shaped	27 x 16 x 4.5 deep	919	Natural supply closed system	4.5 + years
Disney's Living Seas (Orlando, Florida, USA)	Circular	61 diameter x 8 deep	21,660	Artificial supply closed system	3.3 years
Sea World of Florida (Orlando, Florida, USA)	Dumb-bell	38 x 12 x 5.5 deep	2,300	Artificial supply closed system	3.25 years
Atlantis (New Providence, Bahamas)	Circular tank + shallow lagoon	Exhibit: 45 x 27 x 4 deep Lagoon: 30 x 15 x 1.5 deep	5,206	Natural supply open system	2.5 years
Omaha's Henry Doorly Zoo (Omaha, Nebraska, USA)	Square	21 x 17 x 5 deep	1,710	Artificial supply closed system	2.25 years

SPECIMEN ACQUISITION

Acquisition of appropriately sized specimens, followed by a carefully planned transport, are key factors in successfully keeping tiger sharks. Smaller specimens seem to acclimate better to captivity. Four of the institutions listed in Table 32.1 acquired new specimens measuring between one and two meters total length (Crow and Hewitt, 1988; Davis pers. com.; Kaiser pers. com.)

Tiger sharks appear to be less resistant to transport conditions than other carcharhinids and every attempt should be made to limit transport time to five hours or less, and certainly not more than 12 hours. It is important to fast specimens for five days prior to shipping. A tiger shark acquired by the Henry Doorly Zoo regurgitated food into its transport container during shipping and polluted the water. It is advisable to handle this species as little as possible, so as not to compromise their protective mucous layer.

Two specimens that are now successfully being held at the Acuario de Veracruz were brought back to a holding, or staging, pen immediately after capture by long-line. These animals were allowed to acclimatize to captivity for two to eight months in the staging pen, before they were successfully moved into the exhibit (Marin-Osorno, pers. com.).

DAILY HUSBANDRY

Dietary planning, feeding, and water chemistry are all important factors to consider for the daily husbandry of tiger sharks.

Long-term success maintaining tiger sharks depends on a successful feeding strategy. For tiger sharks, in mixed species exhibits, a separate feeding station is recommended and specimens should be fed at the end of feeding sessions. Many of the specimens held in captivity began eating shortly after acquisition, but some refused food for extended periods. While fresh or frozen fish is the typical diet for this species in captivity, evidence from a specimen held at the Durban Aquarium suggests that mammalian or avian flesh might elicit a feeding response when a diet of fishes fails (Van De Elst et al., 1983).

Because tiger sharks are timid, they will often refuse to eat at the main feeding station with other sharks. A dedicated feeding station makes it possible to condition tiger sharks to accept food when other sharks are not eating. Although the quantity of food and method of feeding is different for every institution, it is believed that this species should be fed more frequently, and fed a higher percentage of food per body weight, than other species (Crow and Hewitt, 1988). It would not be unusual to feed these sharks 10-20% of body weight week^{-1}. As with other species of elasmobranchs, vitamin supplements (e.g., Mazuri® Vita-Zu Sharks/Rays vitamin supplement tablets, PMI Nutrition International, Missouri, USA) are important. In addition to vitamin supplementation, the tiger shark held at the Henry Doorly Zoo was given 400 IU of vitamin E and 10-15 fish oil caplets (i.e., menhaden or cod liver oil) every feeding session. Many of the tiger sharks in captivity have demonstrated feast or famine behavior, gorging themselves for a long period of time only to cease eating entirely for an extended period (Davis, pers. com.; Kaiser, pers. com.).

Adequate life support systems and optimal water chemistry are essential to successfully maintain tiger sharks. According to Crow and Hewitt (1988) the optimal temperature range for this species is 23-29 ^{o}C, with a mean of 26 ^{o}C. Dissolved oxygen (DO) seems to play a critical role in the longevity of this species. The swimming behavior of a specimen maintained at the Henry Doorly Zoo markedly improved when DO was elevated to 105-110% saturation (Dehart and Stoops, 1998). Elevated dissolved oxygen levels were achieved by using pure oxygen, as opposed to atmospheric air, to feed the exhibit's ozone generator; decreasing the risk of gas bubble disease that may have resulted from hyper-aeration.

MEDICAL ISSUES

Abrasion from rubbing against the walls of an exhibit is a constant concern for tiger sharks. Abrasions can become an entry point for bacterial or fungal infections. Bacterial infections have been combated with enrofloxacin (Baytril®, Bayer Corp., USA) at a dosage of 5 mg kg^{-1} daily (SID) administered in food (PO). If the specimen will not eat every day, 10 mg kg^{-1} every other day (EOD) will suffice.

Fungal infections are typically harder to treat. A *Fusarium* spp. infection of a specimen maintained at the Henry Doorly Zoo was treated with ketoconazole (e.g., Nizoral™, Janssen Pharmaceutica Products, Titusville, USA) at a dose of 5 mg kg^{-1} SID PO, with moderate success (Dehart & Stoops, 1998). Early detection and

treatment of these problems will help prevent systemic complications.

CONCLUSIONS

The information given in this paper represents observed trends for the few attempts at maintaining tiger sharks in captivity. Few of these attempts have had long-term success and there is still much to learn. This species represents a challenge to the aquarium community but its future is promising, provided the needs for swimming space, diet, and medical care can be adequately met. It is important to understand that a facility interested in maintaining this species must dedicate a great deal of resources to this animal. An aquarium should not try to keep this species on the basis that one is available. Tiger sharks are best suited to large systems with a large surface area for the animal to swim freely. A low stocking density and low numbers of other large shark species will increase the chances of successfully keeping this species.

ACKNOWLEDGEMENTS

I would like to thank the National Aquarium in Baltimore and Omaha's Henry Doorly Zoo. Special thanks go to Steve Kaiser, Jane Davis, Gary Stoops, Kathy Vires, Raul Marin-Osorno, and Valerie Lounsbury for their valuable information and assistance along the way.

REFERENCES

Crow, G. L. and J. D. Hewitt. 1988. Longevity records for captive tiger sharks (*Galeocerdo cuvier*) with notes on behavior and management. International Zoo Yearbook 27: 237-240.

Dehart, A. H. and G. L. Stoops. 1998. Husbandry observations, and treatment of fungal infection in a tiger shark, *Galeocerdo cuvier*. Drum and Croaker 29: 9-13.

Seligson, S. A. and D. J. Weber. 1990. Alterations in established swimming habits of carcharhinid sharks at the Living Seas Pavilion. Journal of Aquaculture and Aquatic Sciences 5(4): 105-111.

Van De Elst, R. P., J. A. Ballard, A. P. Bowmaker, G. Cliff, P. A. Garratt, and G. D. Thurman. 1983. Force feeding of a captive tiger shark (*Galeocerdo cuvieri* (Lesuer)) and its response to different food types presented. Journal of Aquaculture & Aquatic Sciences 3(4): 71-72.

PERSONAL COMMUNICATIONS

Davis, J. 2001. SeaWorld Florida, Orlando, FL 32821, USA.

Kaiser, S. 2001. Atlantis Resort, New Providence, Bahamas.

Marin-Osorno, R. 2001. Acuario de Veracruz, Veracruz, CP 91170, Mexico.

The Elasmobranch Husbandry Manual: Captive Care of Sharks, Rays and their Relatives, pages 487-491.
© 2004 Ohio Biological Survey

Chapter 33
Husbandry of Spotted Ratfish, *Hydrolagus colliei*

HELEN TOZER

Living Elements Ltd.
Unit 13-7228 Progress Way,
Delta, B. C. V4G 1H2,Canada.
E-mail: livingelements@telus.net

DOMINIQUE DIDIER DAGIT

The Academy of Natural Sciences
1900 Benjamin Franklin Parkway,
Philadelphia, PA 19380, USA.
E-mail: dagit@acnatsci.org

Abstract: Most holocephalans occur in the deep waters of the continental shelf and slope, and as a result are unlikely candidates for captivity. The spotted ratfish (*Hydrolagus colliei*) is one notable exception occurring in near-shore waters, in the northern part of its range, off the western coast of Canada and the USA. In recent years new knowledge on the biology of this species, as well as increased experience in techniques for capture and captive care, has resulted in an increasing number of spotted ratfish in public aquariums. As with any animal in captivity, optimal success with this species occurs when habitats, diets, and tank-mates are matched to conditions in their natural environment. Exhibit temperature for ratfishes should have the range 8-12 °C. Ratfish are quite tolerant of low salinity, but prefer 28-33 g l^{-1}. Ratfish are quite sensitive to ammonia and nitrite. Low lighting, low-profile substrates of rock, mud, or sand, and rounded walls, are all required for ratfish. Ratfish eat a wide variety of foods including live clams and shrimp, fresh and frozen prawns, crabs, fish, and squid, and even gel food. Protocols for keeping the spotted ratfish may be used when keeping related species (e.g., the ghost shark, *Callorhinchus milii*, and the cape elephantfish, *Callorhinchus capensis*).

Living holocephalans (Subclass: Holocephali) are the closest relatives of sharks, skates, and rays, and are comprised of three families (Callorhinchidae, Rhinochimaeridae, and Chimaeridae), each distinguished by a unique snout morphology. Holocephalans, commonly referred to as chimaeroid fishes or ratfishes, are an ancient lineage that evolved over 300 million years ago and the morphology of living representatives differs little from their fossil ancestors. Biologically and evolutionarily the chimaeroid fishes are an exciting lineage, almost 'living fossils'. While aquariums display ever increasing numbers of shark and ray species, members of the Chimera family have been placed largely on the side-lines as display animals. Much of the bias against keeping captive holocephalans is their typically deep-water habitat, and the relative lack of information about their collection and transport. The spotted ratfish (*Hydrolagus colliei*) is one notable exception, occurring in relatively shallow water in the most northerly parts of its range. In addition, three species of elephantfishes of the Family Callorhinchidae, a monogenetic family

restricted to the Southern Hemisphere, occur in shallow water during part of their life cycle and have been successfully kept in captivity.

GENERAL BIOLOGY

Most holocephalans are found in coldwater habitats on continental shelves and slopes, but the spotted ratfish, ranging from Southeast Alaska to Baja California, including the northern Gulf of California, can be found in quite shallow water (i.e., 6.0-18.5 m) in the northerly areas of its range. In British Columbia the depth of spotted ratfish is seasonal, remaining mostly below the thermocline (7.2-8.9 °C), except during the spring when animals move up into the shallows to feed at night. In Puget Sound, smaller ratfish move from deep water by day to much shallower water at night (Quinn et al., 1980). Several hypotheses have been suggested to explain this diel migration, including predator avoidance and exploitation of food resources in shallow water. Alternatively, it may be a means to regulate ambient light conditions, as ratfish have an all-rod retina and no means to regulate the amount of light entering their eyes (Quinn et al., 1980). Spotted ratfish prefer low relief habitat and are often found over rocky, mud, and gravel bottoms. Spotted ratfish occur singly or in aggregations, with sexes often aggregating separately as has been observed in the Gulf of California (Mathews, 1975). During mating events aggregations can be large.

The spotted ratfish is distinguished by a body that is reddish-brown to silver ventrally, and marked with distinct white spots on the head, trunk, and tail. The lateral line shines an iridescent gold and the eye is a striking green color. The head is blunt with a slightly protuberant snout and the body tapers to a whip-like tail. As with all holocephalans, the spotted ratfish possesses three pairs of tooth plates, two pairs in the upper jaw and a single pair in the lower jaw. The first dorsal fin is preceded by a stout venomous spine that can inflict a serious wound (Halstead and Bunker, 1952). A single gill opening, on each side, is located just anterior to the base of the pectoral fin. Spotted ratfish are a relatively small, slender species compared to other holocephalans. Females grow larger than males reaching a total length of ~97 cm. There is no reliable method to age spotted ratfish and there is little maturation data available. Females and males become sexually mature at ~25.5 cm and ~20.5 cm, snout-to-vent length, respectively (Love, 1996). Snout-to-vent is used to measure length, as wild ratfish often have broken tails.

In addition to the prerequisite claspers, males have a small club-like structure on their forehead, called the tenaculum (Figure 33.1), used during mating to grasp the female's pectoral fin, with its opposable tip and cluster of sharp denticles, while the pelvic clasper is inserted into her cloaca (Powell, pers. com.). In addition, males have a pre-pelvic tenacula, a blade-like structure armed with a row of sharp denticles, located in a pouch

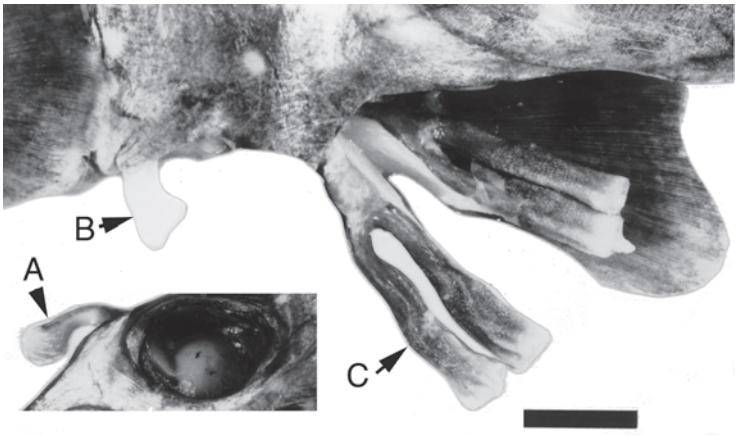


Figure 33.1. Images of a male spotted ratfish (*Hydrolagus colliei*) showing the following clasping organs: (**A**) the frontal tenaculum (inset); (**B**) the pre-pelvic tenaculum, shown everted from its pouch; and (**C**) the bifurcate pelvic clasper.

anterior to the pelvic girdle. This structure is probably used for grasping and positioning the female during copulation (Figure 33.1). Female ratfish can spawn year round, probably peaking in the summer and fall (Sathyanesan, 1966). Regardless of size or age, females will only produce two egg cases at a time. The egg cases are violin or spindle shaped with ridges and stiff hair-like structures jutting out along the edges of the case (Figure 33.2). Based on observations in aquariums, egg extrusion seems to be a long process (i.e., 18-36 hours) and the eggs can remain attached to the female via long filaments for two to six days (Sathyanesan, 1966). Once deposited on the ocean floor, embryos may remain within the egg case for up to a year. On hatching, young ratfish are a miniature version of their parents.

Figure 33.2. Violin or spindle-shaped egg cases being extruded from the cloaca of a female spotted ratfish (*Hydrolagus colliei*).

At all life stages ratfish seem to be opportunistic feeders, with no single food making up more than 25% of their diet. Common food items include shrimp, clams, worms, fishes, brittlestars, cephalopods, nudibranchs, amphipods, and seastars (Johnson and Horton, 1972; Quinn et al., 1980; Macpherson and Roel, 1987). Food is detected using both olfaction and weak electroreception, via sensory pits on the head (Hart, 1973; Fields and Lange, 1980).

CAPTURE AND TRANSPORTATION

Ratfish are more active during the night and twilight, so these represent the best times for collection. Ratfish are sensitive to net trauma, so wherever possible they should only be corralled with nets and then collected on SCUBA using plastic bags. If a net is used to move ratfish, it should be soft and knotless. Careless use of nets can damage the dorsal spine and males can entangle and damage their frontal tenaculum on fiber nets.

Transportation of ratfish is best achieved by using a large cooler, 90% filled with chilled seawater. Reduced surge and a dark environment appear to be important considerations. A hole drilled into the lid of the cooler permits the addition of an air-stone for aeration. Avoid using unlined Styrofoam containers as startled ratfish may impale their dorsal spine in the lid and remain out of the water for extended periods (Bruecker, pers. com.). During longer transports water quality may deteriorate and should be replaced with chilled seawater to dilute metabolic toxins and maintain optimal water temperatures. To prevent a build-up of nitrogenous wastes, ammonia sponges (e.g., AmQuel®, Novalek Inc., USA) may be added to the transport container when fresh seawater is not available. To buffer subsequent drops in pH, sodium bicarbonate, at a dose of 100 mg l^{-1}, may be used (Correia, 2001).

Handle ratfish with care as the dorsal spine can inject a weak venom known to cause pain and swelling for up to a week (Boyle, pers. com.). Poorly handled ratfish may exhibit a serious condition called “bloody eye”, the result of a trauma to the efferent pseudo-branchial artery, a major blood vessel to the brain that runs directly below the orbit of the eye (Didier, 1994; Didier, 1995). If the eyes are badly blood-shot, it is likely that the ratfish will die within a few days. If the damage is mild, keeping the animal in a calm and quiet environment may allow it to recover and thereafter survive for a long time.

EXHIBITION AND CAPTIVE CARE

Ratfish are cruisers, using their large pectoral fins, like wings, to propel themselves through the water. Thus, ratfish need room to glide and turn. An exhibit of 1.5 m x 1.5 m x 1.0 m (depth) should provide three to four adult ratfish plenty of room. Ratfish do not have scales, so exhibit walls should be smooth to prevent abrasion. In addition, ratfish prefer rounded or curved walls, as they are less likely to collide with gently-curving surfaces (Travers, pers. com.; Godsell, pers. com.). However, cylindrical tanks should be avoided as the resultant spiral flow causes fish to turn into

the side and suffer abrasions of the pectoral fins and snout. In the wild, spotted ratfish are found in relatively low current areas, so exhibit water currents should be slow, otherwise specimens will start to spiral. Ratfish have been known to leap out of tanks, so jump-screen should always be provided. Substrate should be low-profile sand, gravel, or rock.

Exhibit temperature should have the range 8-12 °C. Ratfish can tolerate temperatures a couple of degrees higher than 12 °C, once acclimatized, but longevity may be compromised. Ratfish are quite tolerant of low salinity, but prefer 28-33 g l^{-1} (parts per thousand). Ratfish are sensitive to ammonia and nitrite, so biological filtration must be adequate to keep these nutrients in check.

Light is probably the single most important factor in controlling ratfish behavior. As ratfish have all-rod retinas, the eye is susceptible to high illumination, the optical signals from dazzled animals overwhelming their brain and leading to aberrant behavior. If light levels are too high, ratfish will exhibit unusual behavior such as spy-hopping or spiraling at the surface. Artificial light should be muted using filters or screens over the exhibit. If ambient light is used, the tank should never be exposed to direct sunlight.

Intraspecific aggression is a problem in overcrowded exhibits. Examples of fin-nipping, and larger ratfish charging and crowding smaller animals during feeding have been observed. In addition, tail-biting and pectoral nipping are exhibited by males during courtship and mating. Females are usually larger than males and will often exhibit dominance in a captive group, out-competing tank-mates for food. A highly dominant female may need to be removed if she compromises the health of the other individuals in an exhibit.

If exhibit conditions are appropriate, ratfish should settle down quickly and start eating within two to five days. Food items can include live clams and shrimp, fresh and frozen prawns, crabs, fish, and squid, and even gel diet. A vitamin supplement (e.g., Mazuri® Vita-Zu Sharks/Rays vitamin supplement tablets, PMI Nutrition International, Missouri, USA) is recommended if dead or frozen food is to be a staple diet. Ratfish can be fed at any time of the day, but are generally nocturnal and crepuscular feeders. Ratfish prefer to eat from the bottom but may take a while to find food items, so competitive, gluttonous tank-mates should be avoided.

Ratfish do well with tank-mates that they don't normally eat or that won't eat them. Less potent anemones (e.g., *Metridium* spp.) chitons, unpalatable starfishes (e.g., leather stars, *Dermasterias imbricata*), large crabs, orange seapens (*Ptilosarcus gurneyi*), and smaller fishes can all make good tank-mates for ratfish. Fishes such as the kelp greenling (*Hexagrammos decagrammus*) and large bottom-dwelling sculpins (e.g., the buffalo sculpin, *Enophrys bison*) should be avoided, as they tend to out-compete ratfish for food. Elasmobranchs, even small bottom-dwelling sharks, should be avoided as they will prey on ratfish (Didier, 1994).

Ratfish are quite hardy and not prone to disease. Regardless, prophylactic antibiotics are recommended, to prevent secondary infections of the skin and fins, following difficult transports. Ratfish should not be treated with drugs or chemicals unsuitable for scale-less fishes or elasmobranchs. Ratfish carry a species-specific gut-parasite (Simmons and Laurie, 1972; Billin, pers. com.). If a new animal fails to thrive following acclimatization and introduction, a worming treatment may be advised.

Although it is not possible to age ratfish with any certainty, they are a relatively long-lived species. Several facilities have successfully maintained wild-caught ratfish for up to eight years. In some cases captive ratfish have mated, produced eggs, and, at least in one case, produced viable offspring (Amemiya, pers. com.; Sasanuma pers. com.).

OTHER CHIMAEROIDS

In general, the methods described above can be applied to other species of shallow water holocephalan (e.g., members of the Family: Callorhinchidae). Both the ghost shark (*Callorhinchus milii*) and cape elephantfish (*Callorhinchus capensis*) have been successfully kept in captivity using similar protocols. If done with extreme care it is possible to capture these ratfishes in nets—e.g., trawling, etc. (Didier, 1994). Alternatively, egg capsules may be obtained in trawl nets or from gravid females and hatchlings reared in captivity (Duffy, pers. com.). Netting may be the only method to capture ghost sharks in New Zealand, as they are rarely observed by divers, while cape elephantfish have been caught on SCUBA in South Africa. *Callorhinchus* spp. tend to be more selective

feeders than the spotted ratfish. For example, the ghost shark was observed to feed almost exclusively on the surf clam (*Maorimactra ordinaria*), or related bivalves, despite the presence of other food items. Examining the gut contents of commercially caught fishes will help better understand the normal diet of a given species.

CONCLUSION

With increasing experience and research, ratfish are now no harder to obtain and maintain than their elasmobranch cousins. Ratfishes have many odd anatomical features and weirdly endearing behaviors to enthrall even the most jaded visitor. In addition, ratfishes are surrounded with sufficient mystique and folk-lore to appeal to even the most demanding of marketing departments. Aquarists responsible for ratfish grow attached to them and their weird ways, and thoroughly enjoy taking care of them. Ratfish are well worth investing some in-house research as a potential for an interesting and unusual display.

ACKNOWLEDGEMENTS

The following people are thanked for sharing their ratfish experiences and expertise: Danny Kent and John Fisher of the Vancouver Aquarium Marine Science Centre; Dr. Kiyonori Nishida of the Osaka Ring of Fire Aquarium; Kerry Gladish and Neil Allen of Landry's Downtown Aquarium; Kelly Travers and Nina Godsell of the Weymouth Sealife Park; Shinichi Sasanuma and Kentaro Amemiya of Tokyo Sealife Park; and Philip Bruecker and Mike Boyle of Living Elements, Ltd.

REFERENCES

Correia, J. P. S. 2001. Long-Term Transportation of Ratfish, *Hydrolagus colliei*, and Tiger Rockfish, *Sebastes nigrocinctus*. Zoo Biology 20: 435-441.

Didier, D. A. 1994. Capture, care and behavioral observations of two species of chimaeroid fishes: *Hydrolagus colliei* and *Callorhinchus milii*. Chondros 5(1): 1-4.

Didier, D. A. 1995. Phylogenetic Systematics of Extant Chimaeroid Fishes (Holocephali, Chimaeroidei). American Museum Novitates, No. 3119, 86 p.

Fields, R. D. and G. D. Lange. 1980. Electroreception in the ratfish (*Hydrolagus colliei*), Science 207: 57-548.

Halstead, B. W. and N. C. Bunker. 1952. The venom apparatus of the ratfish, *Hydrolagus colliei*. Copeia 1952(3): 128-138.

Hart, J. L. 1973. Pacific Fishes of Canada. Fisheries Research Board of Canada, Bulletin 180, 740 p.

Johnson, A. G. and H. F. Horton. 1972. Length-weight relationship, food habits, parasites, and sex and age determination of the ratfish, *Hydrolagus colliei* (Lay and Bennett). Fisheries Bulletin 70: 421-429.

Love, M. 1996. Probably More Than You Want to Know About the Fishes of the Pacific Coast. 2nd Edition. Really Big Press, Santa Barbara, California, USA. 386 p.

Macpherson, E. and B. A. Roel. 1987. Trophic relationships in the dermersal fish community off Namibia. South African Journal of Marine Science 5: 585-596.

Mathews, C. P. 1975. Note on the ecology of the ratfish, *Hydrolagus colliei*, in the Gulf of California. California Fish and Game 61: 47-53.

Quinn, T. P., B. S. Miller, and R. C. Wingert. 1980. Depth distribution and seasonal and diel movements of ratfish, *Hydrolagus colliei*, in Puget Sound, Washington. Fisheries Bulletin 78: 816-821.

Sathyanesan, A. G. 1966. Egg-laying of the chimaeroid fish, *Hydrolagus colliei*. Copeia 1966: 132-134.

Simmons, J. E. and J. S. Laurie. 1972. A study of Gyrocotyle in the San Juan Archipelago, Puget Sound, USA, with observations on the host, *Hydrolagus colliei* (Lay and Bennett). International Journal of Parasitology 22: 59-77.

PERSONAL COMMUNICATIONS

Amemiya K. 2003. Tokyo Sealife Park, Tokyo 134-8587, Japan.

Billin, K. 2003. Landry's Downtown Aquarium, Houston, TX 77002, USA.

Boyle M. 2003. Living Elements Ltd., Delta, B. C. V4G 1H2, Canada.

Bruecker P. 2003. Living Elements Ltd., Delta, B. C. V4G 1H2, Canada.

Duffy, C. 2003. New Zealand Department of Conservation, Hamilton, 2001, NZ

Godsell, N. 2003. Biological Services and Weymouth Sealife Park, Weymouth DT4 7SX, UK

Powell D. 2003. Monterey Bay Aquarium, Monterey, CA 93940, USA.

Sasanuma, S. 2003. Tokyo Sealife Park, Tokyo 134-8587, Japan.

Travers, K. 2003. Biological Services and Weymouth Sealife Park, Weymouth DT4 7SX, U. K.

The Elasmobranch Husbandry Manual: Captive Care of Sharks, Rays and their Relatives, pages 493-497.
© 2004 Ohio Biological Survey

Chapter 34
Notes on Reproduction of the Zebra Shark, *Stegostoma fasciatum*, in a Captive Environment

KAY KUNZE

Omaha's Henry Doorly Zoo,
3701 S. 10th St.,
Omaha, NE 68107, USA.
E-mail: Kkunzeomahazoo@cs.com

LEE SIMMONS

Omaha's Henry Doorly Zoo,
3701 S. 10th St.,
Omaha, NE 68107, USA.
E-mail: LSimmons@omahazoo.com

Abstract: A pair of zebra sharks (*Stegostoma fasciatum*) successfully reproduced in the Shark Reef exhibit, Henry Doorly Zoo. Mating behavior was similar to that described for the nurse shark (*Ginglymostoma cirratum*) (Klimley, 1980; Carrier et al., 1994) and chain dogfish (*Scyliorhinus retifer*) (Castro et al., 1988). Between September 1998 and September 2000, 80 fertile eggs were laid. Mean incubation time was 152.5±26.5 days, newborns had a mean TL of 30.2±0.8 cm, and mean weight of 92.2±14.0 grams. Increased water temperature resulted in decreased incubation time (R^2=0.887, n=6).

The zebra shark (*Stegostoma fasciatum*) is a tropical, inshore shark of the Indo-West Pacific (Compagno, 1984). It is a hardy shark and a popular species for public exhibition. Zebra sharks are oviparous, laying eggs in large, dark brown or purplish-black cases (17cm long x 8 cm wide x 5 cm thick). These cases have fine, lateral tufts of hair-like tendrils which serve to anchor the cases to the substrate (Compagno, 1984).

The Shark Reef exhibit at the Henry Doorly Zoo (Omaha, Nebraska, USA) is an irregular, oval shaped tank having a depth range of 4.6-5.8 m and a volume of 3,400 m^3 (1,700 m^3 of which is on exhibit). The tank incorporates a public walk-through tunnel across one third of the exhibit floor. It is decorated with many artificial corals, covering one wall, a number of simulated coral bomboras, and patch reefs. The exhibit is maintained at a temperature range of 24.5-25.5 °C. Artificial seawater is used. The lighting regime is computer controlled and a 12:12 light:dark photoperiod is maintained. Sunrise and sunset are simulated every day and ambient light is allowed to fall on the exhibit at night. The exhibit contains six sandbar sharks (*Carcharhinus plumbeus*), five southern stingrays (*Dasyatis americana*), and a variety of Caribbean reef fishes.

Two adult zebra sharks, caught off northeastern Australia, were added to the Shark Reef exhibit in November of 1996: a female of 210 cm total length (TL), weighing 46.0 kg; and a male of 199 cm TL, weighing 33.0 kg. The male had a hardened-clasper length of 203 mm (measured from the point of flexion to the tip). Shortly after the zebra sharks were added to the exhibit they showed signs of breeding and data related to reproduction were recorded. This empirical information has been presented below.

COPULATION

Pre-copulatory behavior was observed shortly after the zebra sharks were introduced into the exhibit and recurred throughout the year. The male shark was observed biting the pectoral fins and tail of the female for periods of several hours. The zebra sharks would generally behave in one of two ways: either swimming through the water column, with the male biting the tail of the female; or lying motionless on the bottom of the exhibit with the male holding the female by the pectoral fin or tail. These behaviors were often intense and on one occasion it was considered necessary to separate the sharks. Pre-copulatory behavior was occasionally followed by copulation. Mating behavior was similar to that described for the nurse shark (*Ginglymostoma cirratum*) (Klimley, 1980; Carrier et al., 1994) and chain dogfish (*Scyliorhinus retifer*) (Castro et al., 1988). During mating the female became listless. As the male bit her pectoral fin she would sink to the exhibit floor, sometimes falling on her back. The male would then wrap his body around the female and insert one clasper into her cloaca. Copulation would last two to five minutes and the male would then release the female. Following copulation the sharks would frequently swim to separate areas.

EGG-LAYING

Mating was observed on several occasions and on the 23rd of September 1998, the first egg was laid. The female continued to lay eggs until early February, 1999. Egg-laying was repeated during the 1999-2000 season and the 2000-2001 season. Egg-laying behavior was similar to that described for the chain dogfish (Castro et al., 1988). As egg-laying approached, tendrils began to appear from the cloaca of the female and she would start to slowly circle vertical structures. The

Table 34.1. Data recorded during the incubation and hatching of zebra sharks (*Stegostoma fasciatum*) at the Henry Doorly Zoo, showing basic water parameters, incubation parameters, hatch success rates, and neonate morphometrics. Data has been sorted by increasing water temperature.

System name	System volume	Mean water temperature (°C)	Mean pH	Mean [NH_3] ($mg.l^{-1}$)	Number of fertile eggs	Eggs hatched	Hatch success rate
WT (2000)	$5.7m^3$	23.0 ± 0.4	8.00 ± 0.10	0.00 ± 0.01	7	0	0%
SR (1999)	$3{,}400m^3$	24.4 ± 0.8	8.30 ± 0.07	0.01 ± 0.01	35	3	9%
SR (2000)	$3{,}400m^3$	24.5 ± 0.8	8.25 ± 0.07	0.01 ± 0.01	9	5	56%
QS (1999)	$1.9m^3$	25.6 ± 0.2	8.19 ± 0.12	0.01 ± 0.01	4	3	75%
FR2 (2001)	$1.9m^3$	26.0 ± 0.7	8.49 ± 0.09	0.01 ± 0.06	6	3	50%
FR1 (2001)	$1.9m^3$	26.2 ± 0.6	8.32 ± 0.15	0.00 ± 0.00	10	2	20%
QS (2000)	$1.9m^3$	26.6 ± 0.4	8.19 ± 0.07	0.01 ± 0.01	9	6	67%

System name	Number of eggs with embryo	First embryo visible (days)	Mean yolk diameter at 15 weeks (mm)	Mean incubation time (days)	Mean neonate TL (cm)	Mean neonate weight (g)	Neonate gender (m.f.u)
WT (2000)	6	43	35.0 ± 5.0	-	-	-	-
SR (1999)	11	40	38.0 ± 5.5	195 ± 2.6	31.1 ± 0.9	73.5 ± 7.8	3.0.0
SR (2000)	6	38	31.0 ± 0.8	167 ± 2.9	28.8 ± 1.9	91.9 ± 4.4	3.2.0
QS (1999)	4	35	33.3 ± 1.3	159 ± 3.8	29.6 ± 0.6	77.0 ± 2.8	1.2.0
FR2 (2001)	3	26	25.0 ± 2.0	137 ± 3.6	30.5 ± 1.3	101.4 ± 3.5	0.3.0
FR1 (2001)	4	23	26.0 ± 2.8	134 ± 2.8	30.2 ± 3.1	106.3 ± 7.8	0.2.0
QS (2000)	7	30	18.2 ± 4.5	123 ± 2.2	30.8 ± 1.9	103.0 ± 7.7	3.3.0

tendrils would become entangled within the vertical structure and the egg would be pulled from the oviduct. The female would then stop circling and resume normal swimming behavior. Eggs were frequently found attached to artificial corals. The time interval between the first and second egg ranged from a few minutes to a few days. Up to six eggs were laid per day. In 1998-1999, 46 eggs (39 fertile) were laid in a 112-day period. In 1999-2000, 26 eggs (25 fertile) were laid in 49 days, while 18 eggs (16 fertile) were laid in 24 days during 2000-2001.

INCUBATION

Shortly after deposition, eggs were moved from the Shark Reef exhibit into smaller incubation systems, having different temperature regimes (Table 34.1). Eggs were never exposed to air. Each egg was placed in a bucket while underwater, transferred to the incubation system, and added to the new system while underwater. Power heads (Maxi-Jet PH, Aquarium Systems, USA) were added to each incubation system to increase water circulation. On one occasion an egg was deliberately left in the Shark Reef exhibit, but it disappeared within two weeks. Before eggs were placed in the incubation systems, most of the tendrils were removed to prevent neonate entanglement on hatching. A few tendrils were retained, allowing eggs to be suspended below the surface of the water by tying them to a pipe lying across the top of the tank.

HATCHING

Approximately two to four weeks after the yolk sac was no longer visible, neonates would hatch. If an egg did not hatch within this period, it was generally the result of the neonate getting stuck within the egg-case and dying. On some occasions egg-cases were cut open to aid the emergence of the neonate. The neonates did not appear to be adversely affected by this procedure, even if a small portion of the yolk sac remained. No medical treatments were performed on the external yolk sac or neonates, although these specimens were closely monitored. All aided neonates survived.

DESCRIPTIVE STATISTICS

Throughout the incubation and hatching period, data were recorded for water quality, yolk and embryo development, incubation times, hatch success rates, neonate morphometrics, and neonate gender (Table 34.1).

Water chemistry was maintained at safe and constant levels in accordance with data published by Michael (2001). Water temperatures were measured daily using a dissolved oxygen meter (OxyGuard Handy Meter, Point Four Systems Inc., USA) rounded to the nearest 0.1 °C. Ammonia (NH_3), nitrite (NO_2), and nitrate (NO_3) were measured weekly using colorimetric titration (Permachem Reagents, Hach Company World Headquarters, USA). pH was measured weekly using a portable pH meter (Model 410A, Orion Research Inc., USA). During 2001 the incubation systems exhibited signs of pH instability. It was known that elasmobranchs are sensitive to pH shifts (Stoskopf, 1993) so phosphoric acid (H_3PO_4) (Phosphoric Acid 75%, Em Science, USA) was added to the incubation systems to maintain a constant pH of 8.0.

Eggs were checked weekly for embryo development and yolk diameter was measured. Measurements were taken using plastic calipers while illuminating the egg case with an underwater flashlight (UK 400, Underwater Kinetics, USA). To minimize error the egg was placed as level as possible with the flashlight underneath, pointing straight up. This process reduced shadows cast by the yolk and allowed more accurate measurement. On hatching, neonates were weighed using a digital platform scale (XE Series Model 3000, Denver Instrument Company, USA) and TL was measured (as per Compagno,1984).

The period of incubation before first embryos became visible ranged from 23-43 days, with a mean of 33.6±7.5 days. Total incubation time ranged from 123-195 days, with a mean of 152.5±26.5 days. 27.5% of the fertile eggs hatched. The sex ratio of hatched sharks was 10 males to 12 females. Neonates had a TL of 26.7-33.0 cm, with a mean of 30.2±0.8 cm, and their weight ranged from 68.0-117.6 grams with a mean of 92.2±14.0 grams (Table 34.1).

Data recorded during incubation suggested a relationship between temperature and embryo development. This possibility is consistent with observations in other species (Kormanik, 1993; Wourms, 1977). Increasing water temperature resulted in significantly decreased yolk diameters at week 15 (R^2=0.629, n=7) and decreased incubation times (R^2=0.887, n=6), and appeared to have a weaker influence on hatch success rates

and neonate size (Table 34.1). The highest hatch success rate (75%) occurred at a mean temperature of 25.6 °C, while the lowest hatch success rate (0%) occurred at a mean temperature of 23.0 °C. Eggs incubated at this lower temperature produced an embryo but it died after 112 days of incubation. The nature of recorded data introduced artifacts of uncontrolled variables, shifting time-lines, and, in some cases, a degree of measurement subjectivity. Great care should therefore be exercised when interpreting these results.

REARING

After hatching, the neonates would be disoriented and swim in spirals. In some cases this behavior lasted for three to six months and the neonates occasionally injured themselves on exhibit decoration. When maintained in restricted systems neonates were observed accidentally biting each other and rubbing against exhibit walls. When these circumstances arose, the exhibits were appropriately modified to provide sufficient and unimpeded swimming space.

Neonates were indifferent to food when it was initially introduced. A feeding stick was constructed and food presented directly underneath their mouths. This feeding technique induced the sharks to feed with greater enthusiasm, even within minutes of hatching. Neonates were fed twice daily for the first four to six months. A variety of food items were given to the young zebra sharks, including: shrimp (*Metapenaeus dobsoni*), clams (*Mercenaria mercenaria*), little tunny (*Euthynnus alletteratus*), Atlantic mackerel, (*Scomber scombrus*), and squid (*Loligo opalescens*). These food items were cut into bite-sized pieces and soaked in a vitamin supplement (Vita Fish™, Marine Enterprises International, USA). Once neonates were feeding well, they were broadcast fed once per day.

In 1999, the pups were placed in a display tank with fluctuating water temperatures (22.3-29.0 °C). Bluestreak cleaner wrasse (*Labroides dimidiatus*) were later added to the exhibit to control ectoparasites on other tank inhabitants. The wrasse, along with some millet butterflyfish (*Chaetodon miliaris*) and Pacific double-saddle butterflyfish (*Chaetodon ulietensis*), began picking at the eyes of the sharks. Other institutions have reported similar behavior for raccoon butterflyfish (*Chaetodon lunula*), foureye butterflyfish (*Chaetodon capistratus*), and bannerfish (*Heniochus* spp.). The eyes of the neonates clouded over and hemorrhaged. Infection spread to the brain causing meningitis and ultimately death. One of the sharks was moved to a tank with higher water temperatures (>24.0 °C) and survived. Since this incident zebra sharks have never been maintained in water temperatures below 24.0 °C or held with problematic fishes.

Table 34.2. Anti-helminthic treatments administered to the Shark Reef exhibit, Henry Doorly Zoo, between June 1999 and September 2000.

Date of treatment	Medication administered	Treatment duration	Dosage
Jun 1999	Trichlorfon	8 weeks	0.35 mg l^{-1} for week one; 0.45 mg l^{-1} for week two; and 0.55 mg l^{-1} for the final five weeks.
Oct 1999	Trichlorfon	3 weeks	0.55 mg l^{-1}
Feb 2000	Praziquantel	Once only	2.0 mg l^{-1}
May 2000	Trichlorfon	4 weeks	0.25 mg l^{-1} for weeks one and two, and 0.27 mg l^{-1} for weeks three and four.
Sep 2000	Praziquantel	Once only	2.0 mg l^{-1}

Many of the zebra shark pups have been transferred to other institutions and 17 of the original 22 remain alive to this day.

MEDICAL TREATMENTS

During pre-copulatory behavior the pectoral fins of the adult female were frequently injured to the point where medication was deemed necessary. At these times she was treated with oral enrofloxacin (Baytril®, Bayer Corp., USA) at a dosage rate of 408 mg day^{-1} (~8.9 mg kg^{-1} day^{-1}) for a period of two weeks.

Following an outbreak of *Neobenedinia melleni* during June of 1999, a series of dimethyl phosphonate (Trichlorfon or Dylox® 80, Bayer Corp., USA) and praziquantel (Praziquantel 100%, Professional Pharmacy Services Inc., USA) treatments were administered to the Shark Reef exhibit (Table 34.2). These treatments could have influenced reproductive success in the zebra sharks and also influenced the development of embryos incubated within the exhibit. Between September and October of 2000 the adult zebra sharks died. The cause of death is unknown but it is possible that sensitivity to the anti-helminthic treatments may have played a role.

ACKNOWLEDGEMENTS

Appreciation is extended to Gary Stoops and Kim Cook for assistance in the collection of data. We would like to thank Kendra Coufal, Sean Putney, Kathy Vires, and Mark Smith for assistance with the revision of this manuscript.

REFERENCES

Carrier, J. C., H. L. Pratt, Jr., and L. K. Martin. 1994. Group reproductive behaviors in free-living nurse sharks, *Ginglymostoma cirratum*. Copeia 1994(3): 646-656.

Compagno, L. J. V. 1984. Sharks of the World. FAO Species Catalogue, Vol. 4, Parts 1 and 2. Food and Agricultural Organization of the United Nations, United Nations Publications, New York, USA. 655 p.

Castro, J. I., P. M. Bubucis, and N. A. Overstrom. 1988. The reproductive biology of the chain dogfish. Copeia 1988(3): 740-746.

Klimley, A. P. 1980. Observations of courtship and copulation in the nurse shark, *Ginglymostoma cirratum*. Copeia 1980(3): 878-882.

Kormanik, G. A. 1993. Ionic and osmotic environment of developing elasmobranch embryos. Environmental Biology of Fishes 38: 233-240.

Michael, S. W. 2001. Sharks and Rays. T. F. H. Publications, Inc., Neptune City, New Jersey, USA. 256 p.

Stoskopf, M. K. (ed.). 1993. Fish Medicine. W. B. Saunders Company, Harcourt Brace Jovanovich, Inc. Philadelphia, Pennsylvania, USA. 882 p.

Wourms, J. P. 1977. Reproduction and development in chondrichthyan fishes. American Zoologist 17: 379-410.

The Elasmobranch Husbandry Manual: Captive Care of Sharks, Rays and their Relatives, pages 499-504.
© 2004 Ohio Biological Survey

Chapter 35
Assessing Reproductive Potential and Gestation in Nurse Sharks (*Ginglymostoma cirratum*) Using Ultrasonography and Endoscopy: An Example of Bridging the Gap Between Field Research and Captive Studies.

[Originally published: Zoo Biology, 22(2): 179-187. Reprinted with permission: Wiley-Liss, Inc.]

JEFFREY C. CARRIER

Department of Biology - Albion College,
4705 Kellogg Center,
Albion, MI 49224, USA.
E-mail: jcarrier@albion.edu

FRANK L. MURRU

Kerzner International,
1000 South Pine Island Road,
Suite 800,
Plantation, FL 33324 USA
E-Mail: Frank.Murru@kerzner.com

MICHAEL T. WALSH

SeaWorld Orlando,
7007 SeaWorld Drive,
Orlando, FL 32821, USA.
E-mail: mtwdsm@Earthlink.net

HAROLD L. PRATT JR.

Mote Marine Laboratory
24244 Overseas Highway
Summerland, FL 33042 USA
E-Mail: wpratt@mote.org

Abstract: Over a period of three years, five reproductively active female nurse sharks (*Ginglymostoma cirratum*) from a wild, actively mating population of nurse sharks were captured, confined, and periodically examined through the course of gestation to determine the gestation period and characterize paternity. In the final year of the study, candidate animals were first evaluated in the field using ultrasonography, and selected animals were then transported from the study site to holding facilities at SeaWorld Adventure Parks in Orlando, Florida, USA. Periodic monitoring of the animals was conducted using ultrasonography, endoscopy, and routine blood analysis. Gestation was determined to be

a minimum of 131 days, multiple paternity was shown for two individual litters, and ultrasonography and endoscopy were shown to be useful adjuncts to assessment of pregnancy and monitoring of gestation in this species, though poor survival of offspring and small litter size may be a consequence of handling, transport, and the use of invasive procedures such as endoscopy.

While several species of large sharks have successfully mated and given birth in captivity, few studies beyond that of Klimley (1980) have systematically detailed the behavioral interactions between mating animals in captivity and few have followed the reproductive physiology of captive animals. Similarly, comprehensive field studies of the reproductive behavior and biology of large sharks are rare (Pratt and Carrier, 2001), but the most complete study to date has suggested that behaviors noted in captive animals often differ significantly from those of wild populations (Carrier et al., 1994).

While it may be desirable to conduct studies of natural behaviors such as courtship and mating outside of the captive environment, such studies cannot be controlled and monitored, as is possible in captivity, and are usually limited in scope because of their inability to observe animals through time. Field observations of individual animals generally do not extend beyond the momentary events of courtship and copulation, and therefore cannot determine even the most basic information such as the time of gestation. Physiological changes that accompany pregnancy and that can be detected by routine blood chemistries are not possible in field studies. Finally, because nursery grounds are incompletely understood and poorly described for most species of sharks, assigning neonates to specific maternal or paternal parents for purposes of pedigree analysis is complicated and would require observers to be present at birth to make immediate captures of mother and littermates.

For the past eleven years, the reproductive behavior of a population of nurse sharks (*Ginglymostoma cirratum*) in the westernmost islands of the Florida Keys has been systematically studied (Carrier, et al., 1994; Pratt and Carrier, 2001). These sharks mate during June at a very specific location that has been known to scientists since the beginning of the last century (Gudger, 1912). Nurse sharks in the area have been tagged by the authors (JC and HLP) as a part of this ongoing study, and 189 individuals are readily recognizable by unique tags and/or scar patterns. Males have been observed to return to the mating grounds annually and the study has shown that the females are on a two-year reproductive cycle of mating and parturition. Castro (2000) has supported this finding by independent studies utilizing dissection of gravid females taken throughout the year. Studies of nurse shark migration indicate that this species does not show extensive movements (Carrier, 1985; Carrier and Luer, 1990; Kohler et al., 1998). The presence of neonates and juveniles in all seasons indicates that the area is a nursery ground for this species as well as a mating ground.

Though the study has revealed the mechanics of mating and copulation and the complex behaviors associated with mating (Pratt and Carrier, 2001), it has been unable to directly measure gestation period and has been unable to evaluate paternity from this population because of the inherent inability of a field study to follow individual animals continuously, from mating through parturition. Like most sharks, nurse sharks are long-lived and mobile, and consequently not restricted to areas where they can be constantly observed.

To attempt to determine answers to these questions, a collaborative project was initiated with SeaWorld Adventure Parks (Orlando, Florida, USA) to capture actively mating females at the study site and transport selected animals to holding facilities in Orlando where they would be held and monitored through gestation and later returned with surviving offspring to the study area at the completion of the captive portion of the project. Since ultrasonography has been used previously as a diagnostic and investigative tool in large elasmobranchs by one of the authors (Walsh et al., 1993), though not for studies of reproduction, ultrasounds were performed at the field site in the final year of the study to improve the probability that animals that would be utilized for the study were likely to be fertile.

METHODS

Female sharks, that had been observed in mating activities prior to capture, were selected for capture in mid-June. Five animals were transported and held in captivity over the course of three years. Two were taken in Year 1 of the

study; one was taken during Year 2; and two were taken in Year 3. Since mating in this species often occurs in waters less than 2 m deep, animals could be captured using a heavy mesh beach seine (30 m x 2 m deep) by surrounding a mating pair and restraining them within the net when the mating event terminated. Males were measured and tagged, blood and tissue samples were obtained, and the animals were immediately released. Females were transferred to a nylon/vinyl sling and either placed in a temporary enclosure at the study site, or transferred directly to a chartered research vessel where they were measured and tagged and placed into a live animal transport unit (3 m x 1 m x 1 m deep) with oxygen-supplemented, constant flow circulation.

Sonography was performed in the field in the final year of the study using a Pie Medical Scanner Model 200 with an ASP-18 3.5 MHz Linear Probe in an attempt to identify egg cases *in utero*. The criteria used for animal selection were to choose those animals whose sonography revealed the greatest number of egg cases present in the paired uteri and who had been observed to mate numerous times throughout the observation period.

Female nurse sharks that were selected for further study were transported by boat to Key West, Florida, where they were then transferred to specially equipped trucks and taken to SeaWorld facilities at Orlando. Upon arrival, they were immediately transferred to non-display, quarantine pools (either indoor, circular pools, measuring 12 m in diameter by 1.5 m in depth, or in-ground, outside pools, measuring 10 m in length by 4.5 m in width with a depth of 1.5 m). The salinity was maintained at 30-32 g l^{-1} (=ppt) and the temperature kept at 25 °C. Before evidence of parturition, females were separated and sequestered in separate pools. In the final stages of gestation, beginning in mid-October, a false bottom constructed of polyvinylchloride (PVC) frames with square openings measuring approximately 10 cm were added to the pools. This permitted spent egg cases to fall to the bottom through the openings and allowed neonates to seek refuge and reduce the possibility of becoming prey to females.

Female sharks were fed mackerel and other fish twice weekly to an amount equivalent to 3-5% body weight. To monitor the progress of gestation, animals were removed monthly by sling to a transport unit (3 m x 1 m x 1 m deep) and placed supine under light anesthesia (MS222 (e.g., Finquel®, Argent Laboratories, USA)) at a dosage of 50 mg l^{-1} (=ppm)). No further restraint was required.

Blood analyses and ultrasonography were conducted monthly. Examination by endoscopy was performed at two-month intervals during the same time and under the same anesthesia regime that sonography was performed. Endoscopy was accomplished using a Corometrics Model CMH-150 Illuminator and Storz Xenon 300 light source with a Hopkins Telescope (5 mm x 29 cm, 0°) coupled to a Storz Veterinary Video Camera (Hi8). The endoscope was inserted in the cloaca and into the common vagina and anteriorly past the left or right uterine sphincter muscle, and advanced slowly to visualize, illuminate and ultimately photograph the intrauterine environment. Ultrasonography was performed using the same instrument as previously described for the field assessment.

Nurse shark development is characterized as aplacental viviparity (ovoviviparous) and young are known to hatch from eggs held internally in the paired uteri (Castro, 2000). Empty or non-viable egg cases are released precociously and young are presumably born two to three weeks following the shedding of the case (Perry Gilbert, pers. com.). Hence, once egg cases were observed to be present in the holding pools, visual inspections of the tanks by divers underwater and surface observers were conducted regularly, several times each day. Egg cases, aborted young, or neonates were then removed from the tank and held in smaller, isolated aquaria where conditions could be more closely controlled and monitored. All observations of shed egg cases, aborted embryos, and births were recorded for calculations of estimates of gestation. Neonates were offered fresh clams and shrimp to satiation.

RESULTS

The first two females in Year 1 produced no egg cases or young. The third animal (Year 2) produced a few egg cases in October and November, but no young were observed. Figure 35.1 depicts a sonography image of eggs in utero. Both of the animals selected in Year 3 produced egg cases, aborted young with egg sacs still attached, and gave birth to young that lived for short durations as summarized in Table 35.1. However, only one neonate animal survived to release (9754-8). All five adult females were returned to the study site and released during the summer following their capture.

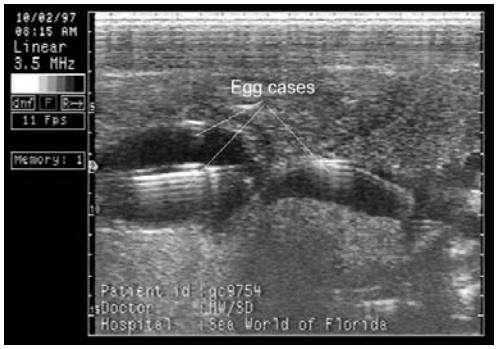


Figure 35.1. Ultrasound images of egg cases.

Figure 35.2. Intrauterine image from endoscopy showing embryo post-hatching, estimated to be 124 days post-mating. Uterine folds are evident in upper right.

The intrauterine endoscopy of both Year 3 animals revealed the presence of egg cases and debris from disintegration of unfertilized eggs. The final endoscopy was performed on the 2nd of October and it is at this time that an emergent embryo is visible (Figure 35.2) in animal 9750. Simultaneous ultrasounds were performed to verify position and orientation though the thick skin of female nurse sharks limits image quality (Figure 35.3). Movement of the embryo as recorded on the videotape of the procedure indicates it was alive at the time of the procedure.

The length of gestation ranged from 131 to 204 days. A closer examination of Table 35.1 shows that the average total length (TL) of this litter was 21.7 cm. The largest animal was the last one to be born (9750-5, 23.3 cm). Littermates from animal 9754 averaged 22.9 cm TL and showed better survival.

DISCUSSION

The recovery of egg cases in Year 2 provided a preliminary estimate of the time of gestation, allowing time to prepare the false bottom and establish observation protocols for animals in Year 3. The successful recovery of egg cases, aborted embryos, and neonates in Year 3 suggests that protocol was successful.

Table 35.1. Year 3 births and egg case recoveries for Animals 4 and 5. Gestation estimate presumes mating begins when maternal (or adult) females first appear in study site showing evidence of mating scars, and is set at June 1. N/A = data not recorded.

Female ID	Offspring ID	Date of Birth	Days lived (days)	Gender	Total length (cm)	Total weight (g)	Gestation estimate (days)
9750	Unhatched	09 Oct	0	M	N/A	N/A	131
9750	9750-1	11 Oct	0	F	21.4	131.0	133
9750	9750-2	11 Oct	0	N/A	N/A	N/A	133
9750	9750-3	21 Oct	0	N/A	20.0	96.2	143
9750	9750-4	16 Dec	0	F	22.0	N/A	199
9750	9750-5	21 Dec	3	N/A	23.3	81.9	204
9754	9754-1	09 Oct	2	F	21.5	70.0	131
9754	9754-2	09 Oct	2	F	22.7	118.5	131
9754	9754-3	10 Oct	3	F	21.7	113.4	132
9754	9754-4	11 Oct	1	F	23.6	120.6	133
9754	9754-5	11 Oct	2	F	21.9	89.8	133
9754	9754-6	11 Oct	2	F	22.1	108.0	133
9754	9754-7	24 Oct	1	N/A	23.0	87.4	146
9754	9754-8	30 Oct	Released in June	M	24.0	N/A	152
9754	9754-9	13 Nov	0	M	24.0	N/A	166
9754	9754-10	19 Nov	9	N/A	25.0	92.3	172

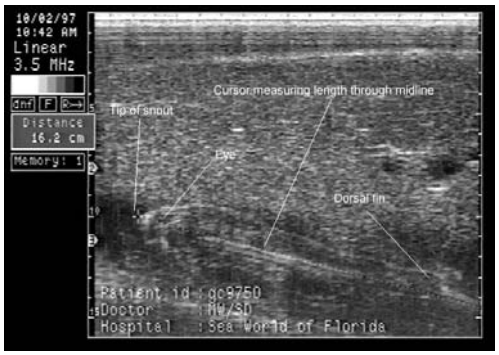


Figure 35.3. Sonograph of embryo, post-hatching, estimated to be 124 days post-mating.

Castro (2000) has reported litter sizes ranging between 21 and 50 embryos in this species and an average total length at birth of 28.0-30.5 cm. The small size at birth and poor survival in the present study do not compare well to Castro, though the natural mortality of neonates is unknown. The differences observed in this study could be attributable to stresses related to capture and handling, to the use of endoscopy at a particularly critical time in gestation, to excessive handling, or to infectious agents introduced during the endoscopic procedures. Despite the appearance of a hatchling during the endoscopic procedure, it could be the case that the large number of births and aborted embryos that followed within one to two weeks may have been because this invasive procedure may have interrupted the normal course of development. Furthermore, it is unlikely that the single survivor (9750-5) was the embryo observed in utero since it was born 80 days following the procedure and, as noted previously, birth is generally thought to occur within 10-14 days following intrauterine hatching. The imaging produced from an endoscope in nurse sharks is far superior to the use of ultrasound, for its level of resolution is comparatively superb. However it may increase embryo mortality and should be evaluated prior to use as an assessment procedure. Other species whose skin thickness is less disruptive to the propagation of ultrasound might benefit from using only ultrasound and avoiding endoscopy. This is especially important when the monitoring of the progress of gestation is important to isolate gravid females, or when other issues related to captive breeding or animal husbandry are critical and may serve to minimize the risk of inducing spontaneous abortions or premature births.

The small litter sizes of six and ten in this study, compared to the median range of 34 reported by Castro (2000), likely resulted from early removal of active females from the field. Only those ova in transit from the ovary to the oviducal gland would have been candidates for fertilization when the sharks were captured, and removing animals from the presence of males prevented further introduction of sperm, despite continued production of ova, and a normal litter size under these conditions would therefore be unlikely. Additionally, it is probable that nurse shark ova are produced serially during estrus, and ovulation might continue for several weeks. Individual large yolky ova may therefore be fertilized on different occasions, depending upon what sperm might be present at the time, as they descend through the oviducal gland. Since ovulation continues over a period of many weeks, this may explain the range of births from early October through late December and implies that the period of mating may last somewhat longer than the authors have reported previously. Later births would presumably result from matings that occurred later in the mating season. This conclusion is further consistent with the embryos shown in various developmental stages in utero as shown by Castro (2000). Early embryonic death may also explain small litter sizes though the current study could not confirm that possibility.

Tissue samples taken from all offspring as well as maternal samples and subsequent DNA analyses indicated multiple paternity in each litter (Saville et al., 2002). This is an outcome expected from field observations of females mating with multiple male partners and is consistent with ovulation that continues for several weeks when opportunities to mate with multiple males may be more likely. This might also be interpreted as indirect evidence of the absence of sperm storage (Pratt, 1993) or, alternatively, low sperm viability in this species. There could therefore be an actual need for multiple matings in order to fertilize successive groups of ova, and paternity may simply be determined by whatever sperm is present when ova are candidates for fertilization.

CONCLUSIONS

1. Gestation period was determined to be at least 131 days and may range to as long as 207 days.

2. Field sonography, when combined with visual observations of mating, improves the probability of obtaining animals that were likely to be in the early stages of pregnancy.

3. Intrauterine endoscopy was a superior technique for visualizing the progress of gestation, but may carry risks that include spontaneous abortion or accelerated gestation and could be exacerbated by excessive or rough handling or introduced infectious agents.

4. Utilizing research opportunities to juxtapose field studies of shark reproduction with captive studies of reproduction avoids the criticism associated with the introduced artificiality of drawing conclusions from observations of behaviors made under captive conditions, while maximizing the monitoring made possible by the captive facility. Nevertheless, issues of transport and maintenance as well as captive conditions that vary from natural conditions may produce results that are not fully consistent with natural observations.

ACKNOWLEDGEMENTS

The authors wish to express their appreciation to SeaWorld Adventure Parks of Orlando, Florida, USA, for providing logistical support for the field portion of this study as well as providing facilities and staff for captive maintenance of the animals. We particularly wish to thank Dr. S. Dover for his assistance with endoscopy and J. Kerivan and R. Davis and all of the aquarists who participated in capture and maintenance of nurse sharks. We also wish to thank C. Perry for her clinical expertise and for her management of the blood chemistries performed in the field and clinical settings throughout the study. We are additionally grateful to P. Taylor and the staff of the Dry Tortugas National Park for their suggestions and support, and the staff at the NOAA-NMFS-NEFSC Narragansett Laboratory for help with shark tags, equipment and support. Finally we wish to thank C. Carrier and T. Pratt and their very able assistance and continued support throughout the study. Funding was provided to JCC in part by Hewlett-Mellon Faculty Development Funds from Albion College and the W.W. Diehl Endowed Professorship of Albion College, and to HLP from NOAA's Highly Migratory Species Management Division.

REFERENCES

Carrier J. 1985. Nurse sharks of Big Pine Key, Florida (USA): An investigation of growth and movements, and a comparison of several types of external tags, *In:* Proceedings of the 5th International Coral Reef Congress, Tahiti, French Polynesia, Volume 6, p.655-660. C. Gabrie and V. Harmeli (eds.). Antenne-Museum-EPHE, Moorea, French Polynesia.

Carrier, J. C. and C. Luer. 1990. Growth rates in the nurse shark *Ginglymostoma cirratum*. Copeia 1990(3): 686-692.

Carrier, J. C., H. L. Pratt, Jr., and L. K. Martin. 1994. Group reproductive behavior in free-living nurse sharks, *Ginglymostoma cirratum*. Copeia 1994: 646-656.

Castro, J. I. 2000. The biology of the nurse shark, *Ginglymostoma cirratum*, off the Florida east coast and the Bahama Islands. Environmental Biology of Fishes 58: 1-22.

Gudger, E. W. 1912. Summary of work done on the fishes of Tortugas. Carnegie Institute Washington Yearbook. 11: 148-150.

Klimley, A. P. 1980. Observations of courtship and copulation in the nurse shark, *Ginglymostoma cirratum*. Copeia 1980: 878-882.

Kohler, N. E., J. G. Casey, and P. A. Turner. 1998. NMFS Cooperative Shark Tagging Program, 1962-93: An atlas of shark tag and recapture data. Marine Fisheries Review 60: 1-87.

Pratt, H. L., Jr. 1993. The storage of spermatozoa in the oviducal glands of Western North Atlantic sharks. Environmental Biology of Fishes 38:139-149.

Pratt, H. L., Jr., and J. C. Carrier. 2001. A review of elasmobranch reproductive behavior with a case study on the nurse shark, *Ginglymostoma cirratum*. Environmental Biology of Fishes 60: 157-188.

Saville, K. J., A. M. Lindley, E. G. Maries, J. C. Carrier, and H. L. Pratt, Jr. 2002. Multiple paternity in the nurse shark, *Ginglymostoma cirratum*. Environmental Biology of Fishes 63(3): 347-351.

Walsh, M. T., F. S. Pipers, C. A. Brendemuehl, and F. L. Murru. 1993. Ultrasonography as a diagnostic tool in shark species. Veterinary Radiology and Ultrasound 34(3): 213-218.

The Elasmobranch Husbandry Manual: Captive Care of Sharks, Rays and their Relatives, pages 505-514.
© 2004 Ohio Biological Survey

Chapter 36
Record-keeping for Elasmobranch Exhibits

MAX JANSE

Burgers' Zoo,
Antoon van Hooffplein 1,
6816 SH Arnhem, Netherlands.
E-mail: m.janse@burgerszoo.nl

JANE DAVIS

2020 North Avenue of the Stars,
The Living Seas, Walt Disney World,
Lake Buena Vista, Fl 32830, USA.
E-mail: Jane.Davis@disney.com

Abstract: Record keeping is critical to providing state of the art aquatic animal care. Aquarium management depends on accurate and up-to-date information about animal collections. Recorded data is used by aquarists for husbandry programs and research projects, while administrative staff use data to fulfill reporting requirements for legal documentation and permits. Management of elasmobranchs will improve more rapidly, if data can be shared efficiently between institutions; sample record-keeping templates have been provided accordingly.

Standardized, comprehensive, and accurate record-keeping is important for all animal care facilities. The maintenance of records is crucial for basic animal management and welfare; compliance with regulatory agencies; and to achievement of basic conservation, research, and education goals. Sharing data will improve husbandry practices, increase breeding success, and support conservation initiatives. The objective of this chapter is to outline the basic record-keeping requirements of an aquarium that maintains elasmobranchs.

REGISTRATION

Registration refers to the process of assigning a unique registration number to an animal upon its acquisition and incorporation into a collection.

Individual registration

It is recommended that each individual elasmobranch be registered. The most extensive record-keeping can be done with individually recognizable animals (refer to Chapter 9 of this manual). Data that should be recorded when an animal is initially acquired and registered has been outlined in Table 36.1.

Group registration

When individual identification is not possible, registration may be made for a group which may be identified by a number of different parameters (e.g., sex, species, exhibit or life support system (LSS), age class, etc.). The collection management procedures at the aquarium will

determine what defines an appropriate group. Group registration should include the information outlined in Table 36.1, for individually registered animals, plus group composition (i.e., sex ratio, age class ratio or total number in group).

RECORDS

Routine (daily and weekly)

Hard-copy daily records should be filed systematically to facilitate retrieval. An example daily record form is presented in Appendix 36.1. All staff should use standardized terminology when reporting observations to facilitate analysis and historical interpretation. The data for water quality and LSS may be recorded on a weekly or monthly record form (Appendix 36.2). The number and type of water quality and LSS parameters are dependent on the aquarium management system.

Non-routine

In addition to routine record-keeping practices, there are many events that may require further data collection, for example:

1. Medical treatments
2. Histological analyses
3. Parasitological analyses
4. Anesthesia
5. Postmortems or Necropsies
6. Nutritional assays
7. Animal training
8. Scientific research

Sample record forms for medical treatments and postmortems are given in Appendix 36.3 and Appendix 36.4, respectively.

COMPUTERIZATION

Computerized record-keeping is preferred as it facilitates data storage and analysis. Many zoos and aquariums use their own database or spreadsheet program in order to meet their unique requirements.

Individual animal record-keeping

One animal registration program, available for use with animals that are individually identifiable, is the Animal Records Keeping System (ARKS)

Table 36.1. Data to record when an individually identifiable animal is initially acquired

Data fields or item	Description of data to record
Registration number	Unique number assigned to individual or group
Taxonomic Classification	Class, family, genus, species, subspecies
Gender (m / f / u)	
Date of birth	
Location of birth	
Lineage	Sire, Dam, and their respective institutions.
Identifiers	Tags, transponders, physical markings, etc.
Acquisition date	
Acquisition source	Wild caught, captive born, etc.
Acquisition type	Purchase, donation, loan, birth, etc.
Terms of agreement	
Collection type	
Shipping information	
Permits	Federal, local, transport, CITES, etc.

(Flesness and Mace, 1988). ARKS is supported by the International Species Inventory System (ISIS). Founded in 1974, ISIS is an international non-profit organization serving 550 zoological institutions members from 54 countries. ISIS (www1) serves as a central repository for animal data which is annually pooled together and distributed to members (Flesness, 2001). The newest version of ARKS (v 4.0) is Windows-based and has some group registration possibilities, though extensive data analyses on a population level are not possible. A large portion of required fish taxonomy is included in the taxonomic list provided with ARKS.

ISIS provides an application, SPARKS, for maintaining single-species studbooks, that may be used for demographic and genetic analyses (Flesness and Mace, 1988). MedARKS, another ISIS product, is a medical record-keeping system that makes use of the ARKS inventory. MedARKS can be used to record clinical notes, lab results, anesthesia, parasitology, and fecal records (Flesness and Mace, 1988).

Group animal record-keeping

Although groups of animals can be registered in the ARKS database, this application is limited in tracking dynamics within and between groups. Computerized Registration for Captive Invertebrates (CERCI), developed by the London Zoological Society (London, UK), is a computerized record-keeping system designed specifically for grouped species such as invertebrates, fishes, and amphibians. CERCI provides analysis of demographic and genetic data (Burlingham-Johnson et al., 1994). The latest version is called Ectolink (Anon., 2000).

There is currently an effort underway to develop a new global animal database system that can handle both groups and individuals, as well as integrate data from medical, inventory, behavioral, nutritional, and other animal husbandry management information. The International Animal Data Information Systems Committee (IADISC) is spearheading this effort, known as the Zoological Information Management System (ZIMS). The goals of this initiative include the development of standards for animal data management, and the creation of an integrated and computerized animal management system (www2).

CONCLUSION

In conclusion, each facility must decide which method of record-keeping works best for the resources they have available. Standardized records would provide consistency among facilities during data collection and allow a more effective and meaningful exchange of information. Captive animal management for elasmobranchs would progress more rapidly if data can be shared and used in a timely and efficient manner.

ACKNOWLEDGEMENTS

We wish to thank Pete Mohan and Sue Dubois for their additions to this chapter. Thanks to Heather Hall and Paul-Pearce Kelly for their information on the CERCI database.

REFERENCES

Anon. 2000. Ectolink, a Web-enabled Conservation Database for Invertebrates, Fish and Amphibians. Zoological Society of London, London, England. 99 p.

Burlingham-Johnson, A., D. Clarke, and P. Pearce-Kelly. 1994. CERCI: a computer system for the demographic and genetic analysis of captive invertebrates, fish and other populations of colony animals. International Zoo Yearbook 33: 278-283.

Flesness, N. R. and G. M. Mace. 1988. Population databases and zoological conservation. International Zoo Yearbook 27: 42-49.

Flesness, N. R. 2001. ISIS Annual report 2000. International Species Information System (ISIS), Apple Valley, MN 55124-8151, USA. 8 p.

INTERNET RESOURCES

www1 www.isis.org

www2 http://www.zims.org

Appendix 36.1: Daily data record sheet for an elasmobranch exhibit

Daily animal record						
Aquarium number						Date
Aquarium name						
Temperature						
Collection						
Species	Reg. number	In	Out	Transfer	Mortality	Medical treatment
Food						
Species / specimen	Food (type 1)	Amount	Food (type 2)	Amount	Supplementation	
Behavior and Health						
Species / specimen	OK	Specification				
	☐					
	☐					
	☐					
	☐					
	☐					
	☐					
Remarks						
Refer record number(s):						

Appendix 36.2: Weekly data record sheet for water quality and LSS

Weekly water quality and LSS records								
Aquarium number							Week starting	
Aquarium name								
Water Quality								
	Units	Mon	Tue	Wed	Thu	Fri	Sat	Sun
Temperature	°C							
Salinity	‰							
pH								
Dissolved oxygen	%							
NH_4^+ (ammonia)	mg l^{-1}							
NO_2^- (nitrites)	mg l^{-1}							
NO_3^- (nitrates)	mg l^{-1}							
PO_4^- (phosphates)	mg l^{-1}							
ORP (oxidative redox potential)	mV							
TRO (total residual oxidants)	mg l^{-1}							
Heavy metals (optional)	µg l^{-1}							
LSS (life support system)								
	Units	Mon	Tue	Wed	Thu	Fri	Sat	Sun
Seawater out	m^3							
Seawater in	m^3							
Demi-water in	m^3							
Sand filter	#							
Backwash	y / n							
Pressure	kPa							
Ozone	mg l^{-1}							
UV								
Surface skimmer								
Protein skimmer								
Salt Addition	kg							
Remarks								

Appendix 36.3: Data record sheet for medical treatment

Medical treatment report			
Report number		Species	
Name of aquarist		Reg. number	
Aquarium number		Gender	
Aquarium name			
Problem description			
Disease name			

Medical treatment						
Treatment number	Date	Medication	Dose (mg kg^{-1})	Conc. (mg l^{-1})	Method	Duration

Results	
Date	Observations

Remarks (LSS changes, recent introductions, husbandry observations, etc.)	
Post-mortem report number	

Appendix 36.4: Data record sheet for post-mortem

Post mortem - I			
Specimen data			
Date		Common name	
Report number		Species name	
Necropsied by		Registration #	
Aquarium name		ISIS / ZIMS #	
Aquarium #		PIT #	
Origin aquarium #		Gender	
Time found		Total length (cm)	
Time since death		Weight (kg)	
Sent for histopathology?		☐ Yes	☐ No
Entered in Database?		☐ Yes	☐ No
Preliminary observations			
Water quality parameters		☐ Observed	☐ Not observed
☐ Cloudy	☐ Discolored	☐ Foul	☐ Surface scum
☐ Temp.	☐ pH	☐ Salinity	☐ Oxygen
☐ Ammonia	☐ Nitrites	☐ Copper	☐
☐	☐	☐	☐
LSS parameters		☐ Observed	☐ Not observed
☐ Normal	☐ Air supply off	☐ Water off	☐ Electrical failure
☐	☐	☐	☐
History		☐ Observed	☐ Not observed
☐ Scratching	☐ Rubbing	☐ At surface	☐ On bottom
☐ Erratic swimming	☐ Fighting	☐ Rapid ventilation	☐ Jumped out
☐ Injured	☐ Predation	☐ Not eating	☐ New arrival
☐	☐	☐	☐
Treatment history			
Samples taken			
Pictures		☐ Yes	☐ No
Blood		☐ Yes	☐ No
Biopsies		☐ Yes	☐ No
Swab cultures		☐ Yes	☐ No
		☐ Yes	☐ No
Digital photo:		File name:	

Post mortem - II			
Preliminary laboratory examination			
Body surface		☐ Normal	☐ Swelling
☐ Growths	☐ HLLE	☐ Open lesions	☐ Necrotic areas
☐ Cysts	☐ Nodules	☐ Spots	☐ Expothalmos
☐ Cloudy skin	☐ Cloudy eyes	☐ Fungus	☐ Parasites
☐ Discoloration	☐ Hemorrhage	☐ Decomposed	☐ Scarred
☐	☐	☐	☐
Gills - general		☐ Normal	☐ Hemorrhagic
☐ Cysts	☐ Parasites	☐ Mucus	☐ Flared
☐ Decomposed	☐ Scarred	☐	☐
Gills - filaments		☐ Normal	☐ Swollen
☐ Fused	☐ Clubbed	☐ Cottony tufts	☐
Gills - coloration		☐ Deep red	☐ Pale red
☐ Pale pink	☐ White	☐ Brown	☐
Fins		☐ Normal	☐ Opaque
☐ Frayed	☐ Parasites	☐ Hemorrhagic	☐ Decomposed
☐ White spots	☐ Black spots	☐ Eroded	☐
Body cavity		☐ Normal	☐ Ascites
☐ Spots	☐ Fatty infiltration	☐ Cysts	☐ Tumor
☐ Worms	☐ Hemorrhagic	☐ Edema	☐
Liver		☐ Normal	☐ Not observed
☐ Spots	☐ Inflammation	☐ Parasites	☐ Cysts
☐ Fatty	☐ Friable	☐ Hemorrhagic	☐ Decomposed
☐	☐	☐	☐
Color:		Consistency:	
Gall bladder		☐ Normal	☐ Not observed
☐ Green-yellow	☐ Black	☐ Clear	☐ Enlarged
☐ Ruptures	☐ Decomposed	☐	☐
Spleen		☐ Normal	☐ Not observed
☐ Spots	☐ Swollen	☐ Cysts	☐ Parasites
☐ Shriveled	☐ Decomposed	☐	☐

Post mortem - III			
Preliminary laboratory examination			
Stomach		☐ Normal	☐ Not observed
☐ Fused	☐ Swollen	☐ Cysts	☐ Parasites
☐ Mucus	☐ Decomposed	☐	☐
Intestine		☐ Normal	☐ Not observed
☐ Empty	☐ Filled with food	☐ Filled with mucus	☐ Hemorrhagic
☐ Nematodes	☐ Trematodes	☐ Edema	☐ Decomposed
☐	☐	☐	☐
Cloaca		☐ Normal	☐ Not observed
☐ Bloody	☐ Swollen	☐	☐
Pancreas		☐ Normal	☐ Not observed
☐ Spots	☐ Swollen	☐ Cysts	☐ Shriveled
☐ Decomposed	☐ Parasites	☐	☐
Color:		Consistency:	
Kidney		☐ Normal	☐ Not observed
☐ Pinpoint spots	☐ Swollen	☐ Large	☐ Small
☐ Pustules	☐ White	☐ Gray	☐ Decomposed
☐	☐	☐	☐
Thyroid		☐ Normal	☐ Not observed
☐ Spots	☐ Swollen	☐ Cysts	☐ Parasites
☐	☐	☐	☐
Gonads		☐ Normal	☐ Not observed
☐ Male	☐ Female	☐ Egg-bound	☐ Swollen
☐ Mucus	☐ Decomposed	☐	☐
Central Nervous System		☐ Normal	☐ Not observed
☐ Swollen	☐ Cysts	☐ Parasites	☐ Edema
☐ Hemorrhagic	☐ Decomposed	☐	☐
Other		☐ Normal	☐ Not observed
☐	☐	☐	☐
☐	☐	☐	☐

Post mortem - IV			
Histopathological tissues collected			
☐ **Skin**	☐ **Fin**	☐ **Gills**	☐ **Brain**
☐ **Heart**	☐ **Spleen**	☐ **Liver**	☐ **Stomach**
☐ **Intestine**	☐ **Kidneys**	☐ Spiral valve	☐ Rectal gland
☐ Eyes	☐ Esophagus	☐ Cartilage	☐ Nares
☐ Gall bladder	☐ Pancreas	☐ Muscle	☐ Lateral line
☐ Mouth	☐ Gonad	☐	☐
Laboratory		Date sent	
Contact		Date returned	

Bacterial and Fungal cultures collected - tests requested					
☐ Kidneys	☐ Aerobe	☐ Anaer.	☐ Fungi	☐	☐
☐ Liver	☐ Aerobe	☐ Anaer.	☐ Fungi	☐	☐
☐ Spleen	☐ Aerobe	☐ Anaer.	☐ Fungi	☐	☐
☐ Stomach	☐ Aerobe	☐ Anaer.	☐ Fungi	☐	☐
☐ Intestine	☐ Aerobe	☐ Anaer.	☐ Fungi	☐	☐
☐ Brain	☐ Aerobe	☐ Anaer.	☐ Fungi	☐	☐
☐ Blood	☐ Aerobe	☐ Anaer.	☐ Fungi	☐	☐
☐ Gonad	☐ Aerobe	☐ Anaer.	☐ Fungi	☐	☐
☐	☐ Aerobe	☐ Anaer.	☐ Fungi	☐	☐
☐	☐ Aerobe	☐ Anaer.	☐ Fungi	☐	☐
Laboratory			Date sent		
Contact			Date returned		

Parasitological examination			
Parasite location		Parasite ID	
Parasite location		Parasite ID	
Parasite location		Parasite ID	
Parasite location		Parasite ID	

Conclusions (inc. probable cause of mortality) and Remarks

The Elasmobranch Husbandry Manual: Captive Care of Sharks, Rays and their Relatives, pages 515-519.
© 2004 Ohio Biological Survey

Chapter 37
Census of Elasmobranchs in Public Aquariums

BETH FIRCHAU

Virginia Aquarium and Marine Science Center,
717 General Booth Boulevard,
Virginia Beach, VA 23451, USA.
E-mail: BFIRCHAU@vbgov.com

WARREN PRYOR

Biology Department,
University of Saint Francis,
2701 Spring Street,
Fort Wayne, Indiana, 46808, USA.
E-mail: Wpryor@sf.edu

JOÃO P. CORREIA

Oceanário de Lisboa,
Esplanada D. Carlos I - Doca dos Olivais,
1990-005 Lisboa, Portugal.
E-mail: jpcorreia@oceanario.pt

Abstract: Ever since animals have been kept in captivity there has been a need to record the composition of collections for management and development purposes. In an effort to record the composition of elasmobranch collections and enhance communication between facilities holding elasmobranchs, the American Elasmobranch Society (AES) Captive Elasmobranch Census (CEC) and the European Union of Aquarium Curators (EUAC) Fish and Invertebrate Taxon Advisory Group (FAITAG) Taxonomic Database (TD) were created in 1991 and 1999, respectively. The CEC boasts a strong participation, averaging 78 facilities worldwide and reporting on an average of 3,710 specimens and >140 species each year. The most numerous shark, ray, and chimera species maintained in CEC aquaria between 1992 and 2001 were the whitespotted bambooshark (*Chiloscyllium plagiosum*), southern stingray (*Dasyatis americana*), and spotted ratfish (*Hydrolagus colliei*), respectively, accounting for >16% of all elasmobranchs maintained. The 2000 CEC reported reproductive activity in >33% of the elasmobranchs maintained in the 91 participating facilities. The TD surveyed 31 facilities in 2002 and recorded 1,010 specimens and 82 species of elasmobranchs. The most numerous shark, ray, and chimera species maintained in TD aquariums were the smallspotted catshark (*Scyliorhinus canicula*), thornback ray (*Raja clavata*), and spotted ratfish (*Hydrolagus colliei*), respectively, accounting for >36% of all elasmobranchs maintained. The 2002 CEC reported reproductive activity, research, and specialized husbandry techniques for 15, 8, and 24 species of elasmobranchs, respectively.

Ever since animals have been kept in captivity, there has been a need to record the composition of collections for management and development purposes. Periodic inventories are vital to understanding the dynamic nature of collections and helping to determine the focus of an institution's propagation and acquisition strategies, husbandry regimes, educational programs, public relations campaigns, and conservation agendas.

In recent years, the public aquarium industry has undergone extensive growth, due to advances in aquarium science and increased public interest in the aquatic environment. In response to this interest and the industry's improved ability to propagate, collect, transport, and maintain a wider range of species, collections managers worldwide have found it necessary to communicate with each other to maintain an economic and environmental focus on their work. The compilation of inventories and databases, from a variety of different facilities, has proven to be an excellent way of aiding this communication.

The American Elasmobranch Society (AES) and European Union of Aquarium Curators (EUAC) conduct and compile elasmobranch censuses, national and international in scope, on an annual basis. These efforts are referred to respectively as the AES Captive Elasmobranch Census (CEC) and the EUAC Fish and Invertebrate Taxon Advisory Group (FAITAG) Taxonomic Database (TD). The development, purpose, use, trends, and future goals of these two efforts are the subject of this chapter.

CAPTIVE ELASMOBRANCH CENSUS (CEC)

In June of 1989, at the annual meeting of the AES, Demski and Scott (1989) suggested that increased communication was essential to both improved research efforts and the development of successful breeding programs for captive elasmobranch populations. Warren Pryor (Fort Wayne Children's Zoo, Fort Wayne, Indiana, USA) was inspired by this presentation and implemented a regional census, collecting data on elasmobranch species held at facilities within Midwestern USA. In July of the same year, after surveying zoos and public aquariums from seven Midwestern states, Pryor compiled and distributed the first AES Great Lakes Regional Inventory. Dependent on voluntary participation and compiled on a typewriter, the inventory recorded 137 elasmobranch specimens, representing 27 species, held at 14 institutions. In 1989, a second survey was completed by the same facilities and an additional 47 specimens were added to the inventory (Pryor, 1989).

Pryor conducted a third census during 1990. While attending the 1990 annual meeting of the AES, Pryor suggested that a national census be developed, giving a synopsis of his efforts and calling on members for their support (Pryor, 1990a). Later that year Pryor (1990b) published the first combined Central and Great Lakes Regional Elasmobranch Inventory, surveying 25 institutions and recording over 200 elasmobranch specimens representing more than 55 species.

In 1991 the AES president, Jack Musick, officially established the CEC as an ad hoc committee of the Society. With the assistance of volunteer regional coordinators (i.e., Beth Firchau, Columbus Zoo and Aquarium, Columbus, Ohio; Alan Henningsen, National Aquarium in Baltimore, Baltimore, Maryland; John Morrissey, Hofstra University, Hempstead, New York; John Rupp, Point Defiance Zoo and Aquarium, Tacoma, Washington; Tom Schmidt, Sea World, Orlando, Florida; and Kathy Vires, Henry Doorly Zoo, Omaha, Nebraska) the first national CEC was published in 1991 (Pryor, 1991). The 1991 CEC recorded 1,659 specimens, representing 65 species from 53 facilities. The 1991 CEC included elasmobranchs held in facilities located in the Caribbean. Contact information for each participating facility was included. Thus, the AES CEC was born.

Over the next two years, Pryor recruited additional facilities to participate in the annual CEC and expanded its reach to include regional coordinators and facilities from Canada, the Far East, and France. Survey return rates typically approached 100% and new facilities were added each year. In March of 1994, Pryor stepped down as chair of the CEC committee and Beth Firchau took his place.

Firchau's first goal as CEC committee chair was to expand participation within the USA, and to include more facilities from throughout the world. The international CEC of 1995 included 2,674 specimens, representing 103 species, from 64 facilities—drawn from 24 states of the USA and 12 additional countries (Firchau, 1995).

From 1995 until the present, Firchau, with the assistance of many regional coordinators, has built the CEC into an increasingly valued information resource, issuing annual national CECs and biennial international CECs.

Role and organization of the CEC

The AES CEC was developed as a tool to improve communication between public aquarium professionals, specifically with regard to elasmobranch husbandry and health-management. It has grown to be an internationally recognized resource for aquarists, curators, the media, researchers, the medical community, government agencies, and conservation organizations. The CEC boasts a strong participation, averaging 78 facilities worldwide each year of survey, and reports on an average of 3,710 specimens, representing more than 140 species.

The CEC is compiled each year, with the help of regional coordinators, and is guided and managed by the CEC committee chair. Each year facilities throughout the USA are invited to participate in the CEC, while international facilities are invited to participate on alternating years. Institutions are asked to provide information about species and numbers of individual elasmobranchs held in their collections. Sponsoring institutions absorb costs associated with contacting facilities, publishing, and distributing the CEC. Facilities that have participated in the CEC are not charged for the finished report. Information contained in the CEC is the property and responsibility of the CEC committee and the AES.

The CEC began as, and continues to be, a voluntary effort, detailing sensitive information. The information within the CEC must therefore be managed with care. In some cases, live animal collections at participating facilities are considered to be the assets of a private organization. To maintain the privacy of participants, the CEC chair and contributors must be discrete about how they use and distribute CEC information. A breach in trust between voluntary participants and the CEC committee would harm the latter's ability to effectively perform its role. Information drawn from the CEC is normally only distributed to participating facilities. Requests for CEC information by government agencies, conservation and environmental organizations, advocate coalitions, and other non-CEC groups may be granted. Each request is reviewed carefully and completely by the CEC committee chair and then forwarded to CEC participants for their ultimate consent. All CEC participants are encouraged to follow this guideline.

Observed trends in the CEC

Participation in the CEC continues to grow and diversify. With a repeat contribution rate reaching >95% nationally, and ~75% internationally, credible trends in collection size and composition may be inferred. The diversity of elasmobranch collections recorded in any one year, between 1992 and 2001, tended to be low, averaging ~147 species per CEC. Collection composition seemed to be somewhat connected to the geographical location of the participating facility. The most numerous shark, ray or skate, and chimera species maintained in aquariums were the whitespotted bambooshark (*Chiloscyllium plagiosum*), the southern stingray (*Dasyatis americana*), and the spotted ratfish (*Hydrolagus colliei*), respectively. These three species accounted for >16% of all elasmobranchs held in captivity. The nurse shark (*Ginglymostoma cirratum*) was another commonly maintained species.

Broadening the scope of the CEC

During the last 10 years, the CEC has included topical surveys to gain some insight into the state of captive elasmobranch husbandry. The 1999 national CEC included a survey of elasmobranch husbandry protocols from aquariums throughout the USA. Diet composition, feeding protocols, health-management protocols, acquisition techniques, quarantine regimes, exhibit dimensions, and collection compositions, from 38 institutions, were recorded. The survey illustrated a diverse approach to elasmobranch exhibition, husbandry, and health management. The results of the survey have since been used by exhibit designers, collection managers, public-relations specialists, veterinarians, and other public aquarium professionals to assist with the development of elasmobranch exhibits, to develop education and conservation programs, and to promote improvements in the husbandry of elasmobranchs.

The 2000 international CEC included a survey of reproductive activity within elasmobranch collections (i.e., had copulation, gestation, or birth been observed?). Of the 91 participating facilities, >33% reported reproductive activity, mostly occurring in *Chiloscyllium* spp. and *Raja* spp. The results of the survey have since been consulted to help facilities develop elasmobranch breeding programs.

Future of the CEC

As concern over the sustained use of global elasmobranch populations increases and public

interest in sharks and rays grows, there will be an increased desire for aquariums throughout the world to display elasmobranchs. The AES CEC will be used increasingly by managers to develop exciting and educational collections, and to prioritize and organize captive propagation programs. The CEC committee desires that the Census remain a respected and important resource for elasmobranch husbandry personnel and researchers around the world. To this end, the CEC committee is committed to expanding the Census and remaining responsive to the changing communication and information needs of public aquariums.

TAXONOMIC DATABASE (TD)

In January of 1999, members of the EUAC met at the Chester Zoo (North of England Zoological Society, Chester Zoo, UK) with the objective of structuring the taxon advisory group for European aquariums, or the FAITAG. The mission of the FAITAG was as follows (Hall, pers. com.):

"…to establish coordinated breeding programs as a means of increasing public awareness of fishes and aquatic invertebrates, with an emphasis on the threats to endangered species and their habitat and in conjunction with promoting positive initiatives within the natural environment…"

During the meeting it became apparent that a database detailing fishes exhibited at EUAC aquariums would be an invaluable tool. Mark Smith (Oceanário de Lisboa, Lisbon, Portugal) and João Correia (Oceanário de Lisboa, Lisbon, Portugal) volunteered to initiate and develop the program, which became known as the FAITAG Taxonomic Database (TD).

Role and organization of the TD

The role of the TD is encapsulated by the mission statement (Smith, pers. com.):

"…The mission of the TD (Taxonomic Database) is to compile, analyze and distribute information about the entire collection of fishes, aquatic invertebrates and aquatic plants maintained within European aquariums. Specifically, to produce a list of all species, using an agreed-upon taxonomic nomenclature, and to facilitate the exchange of information about the source, provenance, breeding, husbandry, research and conservation activities related to those species, within each respective institution…"

To structure the database, Smith and Correia initially established a simple spreadsheet where each record (row) corresponded to a single species within a single institution. The objective was to create an electronic database whereby data sets were easily transmissible (i.e., in ASCII or text format) to any part of the world, and easily incorporated into a software package for analysis and interpretation. Each record contained 18 standardized data fields detailing taxonomy, gender, provenance, reproduction, specialized husbandry, and research activities. Since the creation of the TD in 1999, EUAC member participation has been encouraged and the database has been updated continually. Under the guidance of the TD chair (Correia since 2001), the TD has grown into a valued husbandry and communication tool.

Observed trends in the TD

Participation in the FAITAG TD has grown rapidly since its inception in 1999. As of this writing, the database includes data from 31 aquariums throughout Europe and reports on 1,010 specimens and 82 species of elasmobranchs. The most numerous shark, ray, and chimera species maintained in EUAC aquariums were the smallspotted catshark (*Scyliorhinus canicula*), the thornback ray (*Raja clavata*), and the spotted ratfish (*Hydrolagus colliei*), respectively, accounting for >36% of all elasmobranchs. The 2002 TD reported reproduction, research, and specialized husbandry techniques for 15, 8, and 24 species, respectively.

Future of the TD

The future of the TD relies heavily on continued and broadening participation. Members of the EUAC are frequently urged to take part.

In recent years, non-EUAC aquariums have been invited to participate. Until recently, results drawn from the database have only been made available to participating EUAC institutions. However, steps are being taken to make the TD available to all EUAC members (via www.EUAC.org), and non-EUAC-affiliated institutions via Fishbase (www.fishbase.org) and the IUCN (International Union for the Conservation of Nature and Natural Resources).

PARTICIPATION

To take part in the AES CEC please contact the AES through their web site at www.flmnh.ufl.edu or e-mail the CEC chair on bfirchau@vbgov.com

To take part in the EUAC TD please contact the database coordinator on jpcorreia@oceanario.pt

REFERENCES

Demski, L. S. and S. W. Michael. 1989. Reproductive biology of captive elasmobranchs: Needs and strategies. *In:* Proceedings of the 5th annual meeting of the American Elasmobranch Society, June 17-23, 1989, San Francisco, California, USA. Abstract, p. 8.

Firchau, B. 1995. The American Elasmobranch Society International Captive Elasmobranch Census. Columbus Zoo and Aquarium, Ohio, USA. 23 pp.

Pryor, W. 1989. American Elasmobranch Society: Great Lakes Regional Inventory. Fort Wayne Children's Zoological Society, Indiana, USA. 3 pp.

Pryor, W. 1990a. Toward a national elasmobranch inventory. *In:* Proceedings of the 6th annual meeting of the American Elasmobranch Society, June 14-20, 1990, Charleston, South Carolina, USA. Abstract, p. 19.

Pryor, W. 1990b. American Elasmobranch Society: Great Lakes Regional Inventory. Fort Wayne Children's Zoological Society, Indiana, USA. 6 pp.

Pryor, W. 1991. American Elasmobranch Society: Captive Elasmobranch Inventory. Fort Wayne Children's Zoological Society, Indiana, USA. 27pp.

PERSONAL COMMUNICATIONS

Hall, H. 2001. Zoological Society of London, London NW1 4RY, UK.

Smith, M. 2002. Oceanário de Lisboa, 1990-005 Lisboa, Portugal.

The Elasmobranch Husbandry Manual: Captive Care of Sharks, Rays and their Relatives, pages 521-531.
© 2004 Ohio Biological Survey

Chapter 38
Education and Elasmobranchs in Public Aquariums

SUZANNE M. GENDRON

Ocean Park Hong Kong,
Aberdeen, Hong Kong, SAR CHINA.
E-mail: Suzanne.Gendron@oceanpark.com.hk

The primary reason elasmobranchs are kept in captivity is to act as advocates for their taxon and their native habitats. Through entertaining yet educational experiences at public aquariums, guests are inspired to support conservation efforts for the inhabitants of the oceans, and in particular elasmobranchs. Education has been an integral part of aquariums since their inception, but the central message, and methods of imparting knowledge, have gradually changed. Aquariums have moved away from teaching natural history toward teaching conservation advocacy, and likewise, from simplistic teaching techniques toward imaginative and interactive education programs. The key educational message of today is the promotion of a sustainable use of our natural resources. This message will be more effectively conveyed if aquariums can forge an emotional connection between the visitor and nature.

Aquariums exhibit more than elasmobranchs. When discussing the evolution of education in aquariums, and the general guidelines used to develop programs, exhibits, and graphics, it is often difficult to separate elasmobranch-specific elements from the fundamentals of marine education. Thus, examples given in this chapter are often generalized, though elasmobranch-specific examples are given wherever possible. The important role of education in aquariums can be summarized no better than through the words of the Senegalese ecologist and poet Baba Dioum (in Rodes and Odell, 1992):

"...public education is even more important than captive propagation in the conservation of a species, for in the end we will conserve only what we love. We will love only what we understand. We will understand only what we are taught...".

THE DEVELOPMENT OF EDUCATION IN AQUARIUMS

Since their inception in 1853, with the opening of an aquarium at the London Zoo (London Zoological Society, London, UK), public aquariums have been regarded as places of educational value (Taylor, 1993; Kisling, 2001; Nightingale, 2001; Van den Sande and Jouk, 2001).

The first aquariums typically contained single species in water-filled glass enclosures. Soon thereafter, community exhibits of bony fishes, sharks, invertebrates, and seaweeds were displayed. These exhibits were often accompanied by basic information (e.g., species identification, fish physiology, etc.), and visitors could easily observe the interaction of aquarium inhabitants (Kisling, 2001).

Over the next 150 years, public aquariums proliferated throughout Europe and the USA. The educational approach of these aquariums remained rooted in scientific teachings and classroom-like settings, even though their primary aim shifted more toward recreation (Ohara and Genjirou, 2001; Sonnenschein, 2001; Van den Sande and Jouk, 2001). However, in the last two decades, public aquariums have shifted their aim back toward education, research, and conservation, using entertainment as a means to facilitate these objectives (Nightingale, 2001; Würtz, 2001).

Today, zoo and aquarium associations throughout the world advocate the promotion of environmental education to their visitors and local communities. The philosophies of these regional associations have been summarized in Table 38.1. The American Zoo and Aquarium Association (AZA) requires at least one full-time educator to be employed by an aquarium before awarding AZA accreditation, emphasizing their dedication to the role of environmental education. Each regional association has an education committee, and the International Zoo Educators Association (IZEA) has members from zoos and aquariums in over 30 countries.

WHY IS EDUCATION ESSENTIAL?

It is clear from the aforementioned that education should be an important element of any modern aquarium. Despite this understanding, some individuals continue to regard aquariums as simply an entertaining diversion and a means to attract tourists (Simard, 2001). Aquariums are often used as a catalyst for redevelopment projects and the revitalization of city centers (e.g., Osaka City, Osaka, Japan; Chattanooga, Tennessee, USA; Long Beach, California, USA; Barcelona, Catalonia, Spain). This commercially driven inception is common in South-East Asia and China, where many oceanaria and aquariums start out as for-profit ventures (e.g., SeaWorld Indonesia, Jakarta, Indonesia; Underwater World, Nanjing, China). So, more specifically, why should education be included as part of a modern aquarium?

Before the public will take up environmental stewardship (i.e., the wise management of our natural resources), they need to understand the consequences of their actions and the options available to solve conservation challenges (i.e., empowerment). Armed with this information, the public can effectively become advocates for conservation and the sustainable use of the world's natural resources. Modern aquariums are well positioned to provide this educational role, through a variety of intimate encounters, especially where overburdened school systems are trying to teach science with increasingly limited resources (Simard, 2001). Animals in aquariums are thus environmental ambassadors, inspiring a respect for life and the environment and advocating conservation (Nightingale, 2001; Ohara and Genjirou, 2001; Simard, 2001; Sonnenschein, 2001; Würtz, 2001). As an adjunct to this more sublime motivation, in a world where public opinion about animals in captivity ranges between extremes, conservation education still provides aquariums the best justification for maintaining living collections (Norton et al., 1996; Nightingale, 2001).

WHAT SHOULD BE TAUGHT?

Before discussing key messages to be promoted in the early 21st Century, it is important to review what messages have been promoted over the past 40 years and how effective these campaigns have been.

Fascination through fear

Historically, when aquariums educated the public about elasmobranchs, they focused their efforts on taxonomy, biology, and natural history. Many elasmobranchs were simply ignored because they did not inspire the fear and fascination necessary to attract visitors. Furthermore, some aquariums actively influenced the public into believing that sharks were vicious, man-eating predators to be feared and eliminated. For example, in 1977, SeaWorld San Diego (California, USA) displayed a large, frozen, great white shark (*Carcharodon carcharias*), accompanied by graphics explaining that the shark was caught in waters frequented by bathers and a list detailing the contents of the shark's stomach, engendering fear in visiting patrons. When SeaWorld San Diego opened its Shark Aquarium exhibit in 1978, the graphics detailed differences between fishes and sharks, identified animals in the exhibit, and displayed little else except a prehistoric shark (*Carcharodon megalodon*) jaw. Background music was ominous and suggestive of dangers lurking beneath the waves. An already fearful public, whose attitude had been shaped by Peter Benchley's book *Jaws* and its cinematic adaptation, were easy prey for this type of exhibit. When *Jaws* was released in Hong Kong, one restaurant prominently displayed a sign that read: "…Get your revenge here! Shark-fin soup…" (Burrell, pers. com.) Any means to rid the world of sharks was considered justified. In the years since *Jaws* was first published, sharks have been killed out of fear in the guise of sport.

Increased understanding

Of course fear was not the only motivation to kill sharks. Sharks were harvested for protein, for traditional Chinese medicines, and, increasingly, for their fins. By the early 1980's, shark numbers had been dramatically impacted. If all shark

Table 38.1. Regional zoo and aquarium associations, showing excerpts of their philosophies and their website addresses.

Regional zoo and aquarium association	Association website address	Association philosophy
American Zoo & Aquarium Association (AZA)	www.aza.org	Excellence in animal care and welfare, conservation, education, and research that collectively inspire respect for animals and nature. Strengthen and promote conservation education programs for our public and professional development of our members.
Australasian Regional Association of Zoological Parks and Aquaria (ARAZPA)	www.arazpa.org.au	Harness the collective resources of zoos and aquariums to help conserve biodiversity in the natural environment. Provide exemplary learning opportunities that connect people with nature, enabling the community to better understand and contribute to a future where humans live in balance with the natural world.
European Association of Zoo and Aquarium (EAZA)	www.eaza.net	Promote education, in particular environmental education.
International Zoo Educators Association (IZE)	www.izea.net	Expand the educational impact of zoos and aquariums worldwide. Improve education programs in the facilities of its members, and provide access to the latest thinking, techniques, and information in conservation education.
South East Asian Zoo Association (SEAZA)	www.seaza.org	Increase public knowledge of, and participation in, the environmental conservation needs of South East Asia, and the world, and respect for the welfare of animals through awareness programs. Educate guests on the preservation of the natural environment and share the goals of conservation, education, recreation and research with our public.
World Association of Zoo and Aquariums (WAZA)	www.waza.org	Guide, encourage and support the zoos, aquariums, and like-minded organizations of the world in animal care and welfare, environmental education and global conservation.

species were to survive into the future, the message of fear had to change and the impact of fisheries had to be better understood.

Aquariums started to use hard data to demonstrate the low risk of shark attack. Statistics comparing the probability of death by shark attack (1 in 300 million) to death by bee stings (1 in 5.5 million) and lightning (1 in 19 million), were frequently used to put attacks into perspective. In addition, it was shown that only a few percent of the ~380 shark species were implicated in attacks. Popular actors were recruited to foster the public perception of sharks as victims and to encourage their protection. In this way, aquariums began to promote a different message, i.e., "…sharks, the misunderstood and maligned victims…".

Through continued research, the importance of sharks as an apex predator became better understood. It was demonstrated that prey animals could proliferate and overpopulate, putting pressure on resources and increasing the risk of epidemics, should sharks be removed from an ecosystem (Levington, 1982). In addition, removing apex predators could eliminate an important control on other predator species, resulting in unpredictable changes to prey composition and abundance (Campbell, 1987). Thus, prey fish populations could succumb more easily to epizootics, or other predators could overpopulate and devastate fish populations (Springer and Gold, 1989; Pauly et. al, 1998). The sum result of these changes would be an increased pressure on a marine fishery already in global crisis. The message promoted by aquariums became one of: "…sharks, an integral part of the marine eco-system that must be protected…".

The new message

Whereas the public perceives that ocean resources are infinite, it must be effectively conveyed that this is definitely not the case, and that ocean resources are limited (Vallette, 2001). At the time of printing, over 70% of the world's fisheries are unsustainable. The K-selected life history strategy of sharks, and their associated slow reproductive rates, make them particularly susceptible to fishing pressures (Rose, 1996). The need for a new message has thus emerged: "…ocean resources must be managed in a sustainable manner and everyone must take responsibility for preservation of the environment...". This message translates into the following concrete objectives: (1) limiting fishing, when necessary, to preserve species populations; (2) avoiding the unnecessary take of any species; (3) avoiding habitat destruction and fragmentation; and of course (4) recycling, reducing waste, and reusing products. Public awareness of these issues will help promote informed decisions.

Legislative bodies only act when pressured by their constituents. As part of their educational mandate, aquariums should advocate managed sustainable fisheries, habitat protection, and pollution controls, with local, regional, and national governments, and even international organizations. Furthermore, the public should be made aware that conserving the environment and protecting biodiversity is not only the responsibility of governments, aquariums, and like-minded institutions, but the obligation of every individual (Vallette, 2001).

When conveying the message of sustainability, it is always important to understand cultural context and exercise cultural sensitivity. The practice of shark-finning has drawn criticism for being both inhumane and unsustainable. Yet shark-fin soup has been a traditional Chinese delicacy, served to honor important guests, since the Ming Dynasty (Fowler et al., 2002). Thus, despite the existence of alternatives to shark fin, a western NGO (WildAid Foundation Singapore) was condemned as a cultural imperialist when it advocated sustainable fishing practices and requested people to stop eating shark-fin soup (Mackay, pers. com.). A more positive response was received in Hong Kong when a local dive club campaigned against an ad promotion that included shark-fin soup as a giveaway. The promotion was stopped when the company responsible became aware of the conservation implications and the attitude of at least some of their local public (Darvell, pers. com.). Another area requiring cultural sensitivity is the dialog between public aquariums and hobbyists. While an understanding of basic biology and husbandry is necessary to maintain elasmobranchs, the home aquarist must also be apprised of the responsibilities and ethics of keeping sharks and rays. Communication with the hobbyist community must be informative, but not patronizing, if it is to be effective.

There will always be a need to provide the public with basic information about sharks, skates, and rays. Species identification and life history information will provide a good basis upon which to build other important messages. Additional

information can include elasmobranch biology and physiology, marine and estuarine ecology, and the social, ethical, environmental, and economic implications of sustainable fisheries around the world.

One of the most frequently asked questions continues to be: "...what danger do sharks pose to humans?...". This question implies a pre-conceived negative impression of elasmobranchs. Aquariums must engender respect for elasmobranchs and build positive emotional connections by de-bunking the many myths that surround sharks and their relatives (e.g., that sharks seek out and attack people, that medicines made from sharks cure or prevent cancer, and that sharks can re-grow their fins once removed). In every case, information must be presented in an eye-catching and intuitive manner, must be simple to understand, must be relevant to the visitor, and must be culturally sensitive (Parsons, 1995). Importantly, all information must be presented in a positive manner so as to avoid turning away potential advocates, allowing them to reconnect with their environment.

INSPIRING STEWARDSHIP

Ecophobia vs. biophilia

An interesting phenomenon occurred during the 60's and 70's. Schools, zoos, aquariums, and museums felt it crucial to teach young children environmental issues by scare-mongering. Typical messages included the despair of disappearing rainforests, the horror of polluted waterways, and the irrevocable disappearance of wild places and animals. Children were exposed to many doom-and-gloom scenarios. It was the educators intent that such knowledge would help children grow to be environmentally responsible adults. Instead, children suffered from ecophobia (i.e., fear of ecological problems and the natural world), leaving them feeling helpless, unable to make a difference, and disconnected from nature. Ecophobia replaced biophilia (i.e., an innate attraction to live plants and animals) and few children grew up exploring nature and the environment, but rather sought solace through technology. The legacy of doom-and-gloom leads many visitors, not just children, to feel that positive change is unlikely and therefore that nothing can be done (Sonnenschein, 2001). With people increasingly experiencing ecophobia and disassociating from the environment, conservation messages did not appear to accomplish the goal of engendering respect and inspiring stewardship. In his book *Beyond Ecophobia*, Sobel (1996) eloquently advocates the value of instilling wonder in young visitors, long before they are taught about the terrible state of the natural world and appealed to save it. In recent years, educators have found that an intimate, emotional connection with nature is a more effective means of inspiring future stewardship. There are many obvious ways an aquarium can forge emotional bonds between their visitors and the animals. However, not only must the public develop emotional bonds with the animals, the public must be connected (or reconnected) with nature before they can be encouraged to take up the role of environmental steward. It is only when people care that they will take the time to learn and understand conservation issues, and assume stewardship.

What does the visitor know?

In order for aquarium exhibits and education programs to engender respect and inspire stewardship, aquarium staff must understand their audience. Only then can staff design a range of exhibits and publications to appeal to, and attract, different ages, cultures, and learning styles (e.g., To what is the aquarium visitor emotionally attached? What does the visitor value, believe, and perceive about the oceans and elasmobranchs?). Understanding the visitor is essential to designing effective aquarium exhibits and education programs.

With this question in mind, a cooperative of aquariums, zoos, museums, and conservation organizations formed The OCEAN Project, where OCEAN refers to Ocean Conservation through Education, Awareness, and Networking (www1). In November of 1999, The OCEAN Project commissioned a telephone survey to better understand prevailing attitudes, values, knowledge, and connections to the ocean. It was found that U.S. citizens knew little about how the oceans functioned, the health of the oceans, or how their own actions could jeopardize the oceans. Even though there was an awareness that the oceans could become threatened and were vulnerable, they did not yet believe that the oceans were in any immediate danger (www1).

In 2001 the AZA commissioned a multi-institutional study to analyze the overall impact of zoos and aquariums on the conservation knowledge, attitude, and behavior of their visitors. It was found that the conservation attitude of 5th

and 6th grade students was closely linked to their environment and experience (www2). Urban children tended to have naive views toward wildlife, as they lacked knowledge of, and experience with, animals in the wild. Finding ways for urban children to gain these experiences, through immersion, interpretation, and interaction, provides a valuable means to reconnect them with the natural world and promote conservation.

How does the visitor learn?

Of equal importance to understanding what the visitor already knows, and what the visitor feels, is understanding how the visitor learns. To be effective, program and exhibit designs must consider and allow for the learning characteristics of different age groups. Young children have different motor, cognitive, language, and social skills, when compared to older children (Table 38.2). Very young children will enjoy dressing and acting like sharks as their first exposure to elasmobranchs, while young adults would appreciate diving with rays and skates, or observing sharks underwater from within a protected cage. In general, most visitors respond well to material produced for the 8-10 year-old age bracket. Parents can interpret the graphics for younger children, foreign visitors will more easily understand the information, and children older than seven years will comprehend material at this level.

INFLUENCING THE PUBLIC

Aquariums have numerous opportunities to influence their visitors, local communities, local governments, and even foreign governments. In the USA alone 120 million people visit zoos and aquariums annually, exceeding the number attending all major sporting events combined. In addition, governments throughout the world are encouraging schools to use aquariums and zoos as learning forums. The opportunities to influence public opinion are numerous.

Practices for a positive impact

Dierking et al. (2001) outline important generic ideas to consider when creating new exhibits, programs, and other educational materials, suggesting that "…the most important thing zoos and aquariums can do to positively influence visitors [is to] be clear about [the] message, communicate it directly and succinctly, have staff and volunteers reinforce it personally, and build long-term relationships…". The means by which these objectives can be achieved include:

1. Concrete suggestions for ways people can facilitate and sustain conservation efforts at home.
2. Increased meaningful interaction between aquarium staff and the visitor.
3. Development of a conservation ethic among urban children at the pre-school, kindergarten, and elementary level, with encouragement for them to actively engage in specific activities that benefit the environment.
4. Strategies for continued visitor follow-up.
5. For AZA institutions with visitors sympathetic to environmental concerns, the articulation of more explicit conservation messages.

Education through exhibition

Excellent examples of effective educational and interpretive exhibits can be found throughout the modern aquarium community. In 1979, Ocean Park (Hong Kong, China) opened one of the first immersive exhibits, a cross section through a coral reef atoll. The Point Defiance Zoo and Aquarium (Tacoma, Washington, USA) improved on this concept in 1989. Visitors were effectively transported to another place: led through a marine biologist's hut, allowed a peek at the biologist's journal, and given a chance to see sharks and rays in a naturalistic environment. Human curiosity is such that the biologist's journal provided a great opportunity to convey information that may have been ignored on a standard graphics panel.

Touching or interacting with animals leads visitors to experience them as living beings, rather than abstract images (Nardone and Gargiulo, 2001). Interactive exhibits (e.g., ray feeding pools at the Monterey Bay Aquarium, Monterey, California, USA; swim with the sharks program at Discovery Cove, Orlando, Florida, USA; etc.) build important emotional connections between the public, the animals, and the environment. As an example, staff at SeaWorld San Antonio (San Antonio, Texas, USA) teach visitors to snorkel and then invite them to view hammerhead (*Sphyrna* spp.), sand tiger (*Carcharias taurus*), bonnethead (*Sphyrna tiburo*), and zebra (*Stegostoma fasciatum*) sharks from the safety of a cage within the shark exhibit (Figure 38.1). Exit surveys demonstrate 100% success in improving visitor

Table 38.2. Learning characterizations of children, classified by age, showing motor skills, perceptual or cognitive skills, speech and language, and personal and social skills (after Kennedy, pers. com.).

Age	Motor Skills	Perceptual or cognitive skills	Speech and Language	Personal or Social skills
1	Crawls, explores. Walks unassisted. Picks up objects. Throws objects repeatedly. Enjoys pushing and pulling. Stacks objects.	Enjoys hide and seek. Enjoys picture books. Understands functional relationships. Names everyday objects. Shares toys.	Able to produce speech-like patterns. Responds to yes/no questions. Enjoys rhymes and songs.	Asserts independence. Plays alone for short periods. Does not cooperate. Exceedingly curious.
2	Jumps, climbs, rolls, and plays. Throws and retrieves. Good hand and finger coordination. Explores.	Matches similar objects. Able to count. Begins to be creative. Begins to problem solve mentally.	Enjoys stories.	Engages in imaginative play. Possessive. Understands “me”. Begins to play with others.
3	Walks, runs, jumps, gallops, and rides a tricycle. Can catch a large ball. Full of energy and enthusiasm.	Fearful of unfamiliar objects. Curious and asks why? Artistic. Begins to argue.	Sings. Speaks in sentences. Understands words and explanations.	Wants to please or help out. Independent, but family is main interest. Sometimes plays with other children.
4	Bursts of energy. Can throw. High motor drive. Can sit for a longer period of time if occupied.	Has increased self-control. Needs rules and boundaries. Can amuse themselves.	Can talk and eat or dress at the same time. Imaginative. Enjoys made-up words.	Assertive. Can cooperate. Enjoys dramatizations. Likes to dress up and play gown-up.
5-6	Highly developed. Can ride a bike. Likes physical challenges. Big appetites.	Generally calmer. Self-confident. Enjoys routines. Learns quickly.	Needs fresh ideas. Understands cause and effect. Is factual.	Smiles and laughs. Knows and follows rules. Self-centered.
7-9	Good small motor skills. Need wide variety of activities. Need variety of physical challenges.	Curious. Self-centered. Judgmental. Loves to categorize and classify.	Understands complex instructions. Expresses feelings.	Wants to belong. Enjoys one or two friends. Worries about rules.
10-12	Active.	Anxious to grow up. Beginning to think abstractly. Strong opinions. Understands cause and effect. Understands other point of view.	Enjoys talking with adults.	Concerned about social injustices and world problems. Anxious to grow up. Fragile self-image. High sense of fairness.

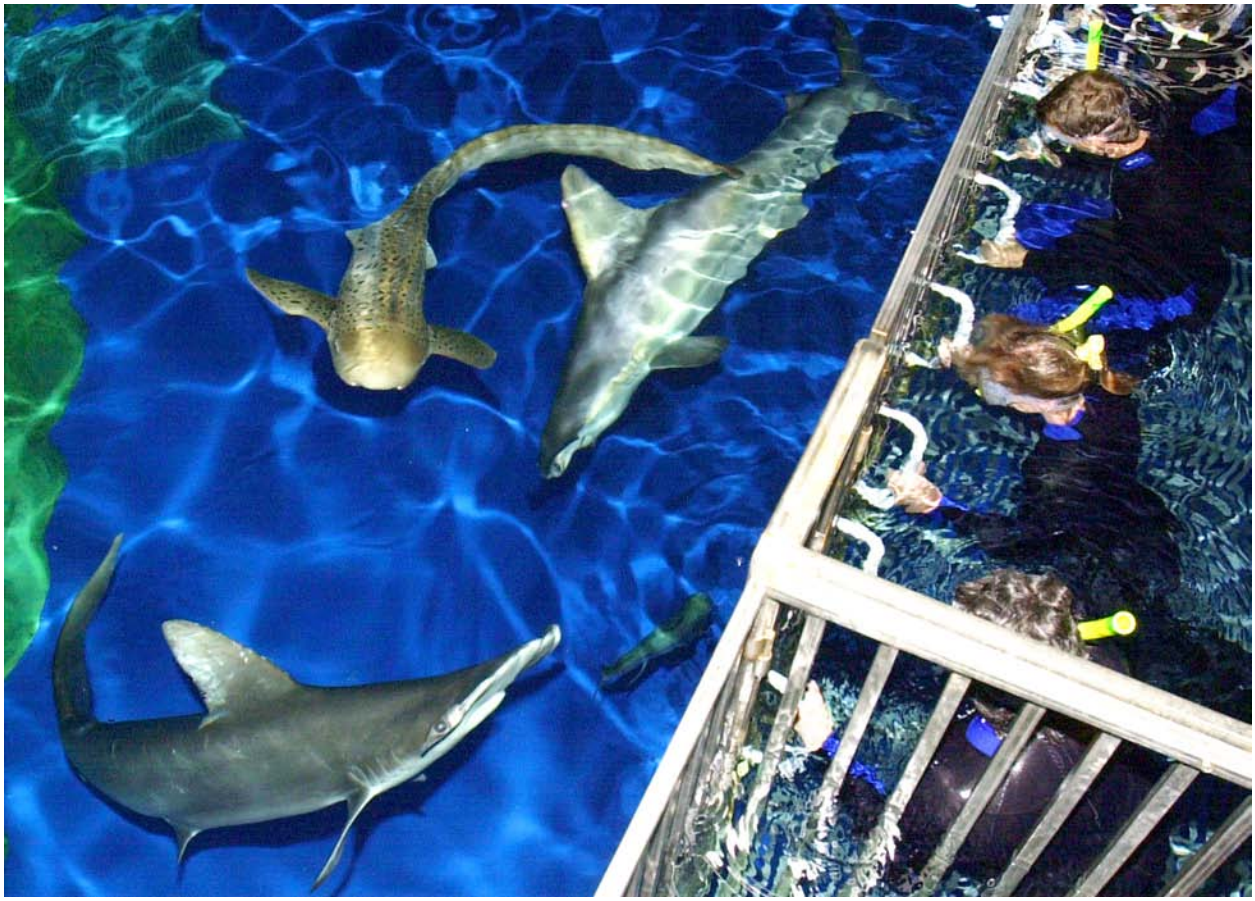


Figure 38.1. The “swim with the sharks” program at SeaWorld San Antonio (San Antonio, Texas, USA), showing participants entranced by the proximity of two hammerhead sharks (*Sphyrna* spp.) and a zebra shark (*Stegostoma fasciatum*) from the safety of a cage.

attitude toward sharks, rays, and the marine environment (Choromanski, pers. com.).

The Florida State Aquarium (Tampa, Florida, USA) found a dramatic way to address the misconception that sharks are frequent killers of human beings. Using a two-story satellite image of Florida, the number of injuries resulting from lightning strike were compared to those inflicted by sharks. The white lightning bolts vastly outnumbered the yellow circles denoting shark attack. Few words were required, but the message was communicated effectively (Yates, pers. com.).

At the Monterey Bay Aquarium an effective display consists of back-lit big skate (*Raja binoculata*) or swell shark (*Cephaloscyllium ventriosum*) egg cases. Acrylic windows are placed in each egg case to allow visitors a clear view of developing embryos. With virtually no graphics these exhibits command attention and visitors leave with an immediate insight into shark reproduction (Powell, pers. com.).

The New England Aquarium (Boston, Massachusetts, USA) has an excellent exhibit consisting of a cart filled with tools that demonstrate the biology, anatomy, and physiology of elasmobranchs (Figure 38.2). Educators at the aquarium recognized that the most effective way to influence visitors was to make the experience personal.

Additional education opportunities

Natural, immersive exhibits, with associated graphic panels and take-home pamphlets, represent a relatively passive means to educate and influence. A good way to build on this foundation is the provision of an extensive library within the aquarium shop. Husbandry staff should periodically review the popular literature and suggest potential additions to their retail departments.

One of the most effective means to capture the attention of the visitor is personal interaction and

interpretation (Parsons, 1995). Visitors enthusiastically "discover" information while listening to keepers, educators, and scientists, in formal classes, informal presentations at exhibits, and behind-the-scenes tours. These forums represent a great opportunity to forge emotional bonds that will ultimately influence the future actions of visitors.

Limited resources will restrict the number of students able to attend formal courses at an aquarium. To reach a larger audience, aquariums have recognized the need to provide teach-the-teachers courses. By teaching the teachers, an aquarium can dramatically increase their student body. In addition, seminars can be arranged so that teachers learn what aquariums have to offer and how best to use their resources. Cooperation with local education departments enable the development of school curricula and professional education courses dedicated to environmental education (Sonnenschein, 2001).

Outreach programs provide an effective means to reach students unable to visit an aquarium and reinforces what other students may have already learned. Example outreach programs include: SeaWorld San Diego's Shamu TV; Vancouver Aquarium's (Vancouver, British Columbia, Canada) distance learning program, a mobile classroom that drives to far-flung communities and schools; and SeaWorld Indonesia's sustainable fisheries and environmental challenges program, communicating directly with local fishing communities.

Figure 38.2. The New England Aquarium's (Boston, Massachusetts, USA) outreach "shark cart", filled with tools that demonstrate the biology, anatomy, and physiology of sharks and rays.

Personal computers and the World Wide Web have dramatically changed the face of education. Most modern aquariums have web sites with environmental information and images, live video feeds or video clips of exhibits, and links to the home pages of other conservation and education groups.

An important initiative by many aquariums has been the development of wallet-sized "seafood watch" cards. These cards indicate sustainable, non-sustainable, or marginal fisheries, and encourage the public to choose their seafood meals from a sustainable fishery.

EDUCATION EVALUATION

How does an aquarium know if its exhibits and programs are effective? Of equal importance to developing key messages, designing exhibits, and developing programs, are the subsequent evaluations conducted by an aquarium. Evaluations should be conducted before program design (to help shape the program), during program development (to fine-tune the program), and following program implementation (to determine if teaching goals are being achieved).

Up-front evaluations are required to effectively design education programs, graphics, and publications. It is important to understand what visitors know, feel, and value, in order to define the problems to be addressed. Goals and measurable objectives can then be set, and appropriate exhibits, programs, publications, and graphics developed. Evaluation during the design phase can be achieved by placing temporary graphics in exhibit halls and allowing staff to question visitors about their effectiveness. Pilot programs can be conducted and participants interviewed.

Once an education program has been completed, a summative evaluation needs to be undertaken. This evaluation should inform staff as to what parts of the program were effective and whether messages were understood. In short, it should determine if identified goals and objectives were met. Importantly, aquariums must determine if they have been effective at positively changing visitors' attitudes and behavior toward the environment. Many studies have evaluated exhibits, the knowledge imparted to visitors, and visitor attitude toward animals, but few studies have examined how effective exhibits have been at shaping attitudes and changing behavior (Dierking, et al., 2001).

CONCLUSIONS

Education has played an important role in aquariums throughout their history. Key messages have evolved as exhibits have become more sophisticated and our approach to conservation changed. The key messages of today include conservation of nature and sustainable use of the earth's resources. These messages are more effectively conveyed when aquariums forge an emotional connection between the visitor and nature.

There are many reasons elasmobranchs are kept in aquariums, yet it must be remembered that the primary reason is conservation. Our strongest tool to reinforce the message of conservation is education. Aquariums are places of learning where we must inspire and motivate our visitors to care about the natural world. Conservation begins at home, moves out into the community, and ultimately spreads globally to help preserve wild places and wild life.

For those seeking more information about conservation education the AZA has an excellent course, introducing background philosophies, techniques for designing programs, techniques to evaluate community needs, techniques to evaluate the effectiveness of programs and graphics, and much more beyond the scope of this chapter.

ACKNOWLEDGEMENTS

I wish to thank Derek Spielman of Ocean Park, Brian Darvell of Hong Kong University, and Alison Davidson of the National Aquarium for their valuable editorial assistance, and Loretta Ho of Ocean Park for her ability to format anything and everything. In addition, I appreciate the guidance and input from many of my colleagues in the field of aquariology and education, who have not only helped shape this chapter, but continually inspire me in this field of work. Special thanks go to JHR for reading, rereading, and kindly commenting on the manuscript from its inception.

REFERENCES

Campbell, N. A., and J. B. Reece. 2001. Biology. Pearson Higher Education Publishers, Upper Saddle River, New Jersey, USA. 1175 p.

Dierking, L. D., K. Burtnyk, K. S. Büchner, and J. H. Falk. 2001. Visitor Learning in Zoos and Aquariums: A Literature Review. Multi-Institutional Visitor Research Project (MIRP), Conservation Education Committee (CEC), American Zoo and Aquarium Association (AZA). American Zoo and Aquarium Association, Silver Spring, Maryland, USA. 33 p.

Fowler, S. L., T. M. Reed, and F. A. Dipper. 2002. Elasmobranch Biodiversity, Conservation and Management. Proceedings of the International Seminar and Workshop, July 1997, Sabah, Malaysia. Occasional Papers of the IUCN Species Survival Commission (SSC) 25. 258 p.

Kisling, V. Jr. 2001. Zoo and Aquarium History: Ancient Animal Collection to Zoological Gardens. CRC Press: Baton Rouge, Louisiana, USA. 440 p.

Levington, J. S. 1982. Marine Ecology. Prentice-Hall Inc., Englewood Cliffs, New Jersey, USA. 526 p.

Nardone, G. and M. L. Gargiulo. 2001. Nautilus project: The handy reef project for a tactile museum of the sea floor. *In:* Proceedings of the Fifth International Aquarium Congress, November 20-25, 2000, Monaco. p. 23-26. Bulletin de l'Institute oceanographique, Monaco, No. spécial 20.

Nightingale, J. 2001. Education: An overview of some big changes in aquariums. *In:* Proceedings of the Fifth International Aquarium Congress, November 20-25, 2000, Monaco. p. 61-74. Bulletin de l'Institute oceanographique, Monaco, No. spécial 20.

Norton, B. G., M. Hutchins, E. F. Stevens, and T. L. Maple. 1996. Ethics on the Ark: Zoos, Animal Welfare and Wildlife Conservation. Smithsonian Institution Press, Washington, D. C., USA. 332 p.

Ohara, K. and N. Genjirou. 2001. Environmental education in aquariums in Japan. *In:* Proceedings of the Fifth International Aquarium Congress, November 20-25, 2000, Monaco. p. 13-22. Bulletin de l'Institute oceanographique, Monaco, No. spécial 20

Parsons, C. 1995. To boldly go beyond school groups: The next generation of aquarium educators, In Proceedings of the third International Aquarium Congress, April 25-29, 1993, Boston, Massachusetts. p. 151-156. New England Aquarium, Boston, USA.

Pauly, D., V. Christensen, J. Dalsgaard, R. Froese, and F. Torres Jr. 1998. Fishing down marine food webs. Science 279: 860-863.

Rodes, B. K., and R. Odell. 1992. A Dictionary of Environmental Quotations. Simon and Schuster, Inc. New York, USA. 335 p.

Rose, D. B. 1996. An Overview of World Trade in Sharks and Other Cartilaginous Fishes. Traffic International, Cambridge, UK. 104 p.

Simard, F. 2001. Thoughts on aquariums in the 21st century. *In:* Proceedings of the Fifth International Aquarium Congress, November 20-25, 2000, Monaco. p. 79-84. Bulletin de l'Institute oceanographique, Monaco, No. spécial 20.

Sobel, D. 1996. Beyond Ecophobia: Reclaiming the Hearts in Nature Education. The Orion Society Press, Great Barrington, Massachusetts, USA. 45 p.

Sonnenschein, L. 2001. Public aquarium education and research responsibilities in the future. *In:* Proceedings of the Fifth International Aquarium Congress, November 20-25, 2000, Monaco. p. 27-35. Bulletin de l'Institute oceanographique, Monaco, No. spécial 20.

Springer, V. G. and J. P. Gold. 1989. Sharks in Question, The Smithsonian Answer Book. Smithsonian Institution Press, Washington, D. C., USA. 192 p.

Taylor, L. 1993. Aquaria: Windows to Nature. Prentice Hall, New York, USA. 168 p.

Vallette, P. 2001. New behavior toward the ocean. *In:* Proceedings of the Fifth International Aquarium Congress, November 20-25, 2000, Monaco. p. 57-62. Bulletin de l'Institute oceanographique, Monaco, No. spécial 20.

Van den Sande, P. and P. Jouk. 2001. Evolution in public aquarium concepts. *In:* Proceedings of the Fifth International Aquarium Congress, November 20-25, 2000, Monaco. p. 85-96. Bulletin de l'Institute oceanographique, Monaco, No. spécial 20.

Würtz, M. 2001. A view into the third millennium aquarium, Are the new aquaria really the future?. *In:* Proceedings of the Fifth International Aquarium Congress, November 20-25, 2000, Monaco. p. 49-56. Bulletin de l'Institute oceanographique, Monaco, No. spécial 20.

PERSONAL COMMUNICATIONS

Burrell, M. 2003. Rotary Club of Hong Kong, Rotary International District 3450, Hong Kong.

Choromanski, J. 1998. Ripley Aquariums, Inc., Orlando, FL 32819, USA.

Darvell, B. 2003. The University of Hong Kong, Hong Kong.

Kennedy, P. 2003. Dallas Zoo, TX 75203, USA.

Mackay, B. 2003. Underwater World Singapore, Sentosa 098969, Singapore.

Powell, D. 2003. Monterey Bay Aquarium, CA 93940, USA.

Yates, K. 2003. The New York State Living Museum, Watertown, NY 13601, USA.

INTERNET RESOURCES

www1 http://www.theoceanproject.org

www2 http://www.aza.org/ConEd/VisitorLearning/

The Elasmobranch Husbandry Manual: Captive Care of Sharks, Rays and their Relatives, pages 533-541.
© 2004 Ohio Biological Survey

Chapter 39
Research on Elasmobranchs in Public Aquariums

MALCOLM J. SMALE

Port Elizabeth Museum,
P.O. Box 13147,
Humewood, 6013, South Africa.
E-Mail: pemmjs@zoo.upe.ac.za

RAYMOND T. JONES

Department of Pathology, University of Maryland,
22 South Greene St.,
Baltimore, MD 21201, USA.
E-mail: rjones@som.umaryland.edu

JOÃO P. CORREIA

Oceanário de Lisboa,
Doca Dos Olivais,
Lisboa, 1990-005, Portugal.
E-Mail: jpcorreia@oceanario.pt

ALAN D. HENNINGSEN

National Aquarium in Baltimore,
Pier 3, 501 E. Pratt Street,
Baltimore, MD 21202, USA.
E-Mail: ahenningsen@aqua.org

GERALD L. CROW

Waikiki Aquarium,
2777 Kalakaua Avenue.
Honolulu, HI 96815-4027, USA.
E-Mail: crow@soest.hawaii.edu

ROD GARNER

58 Carter Road,
Nambour, 4560, Queensland, Australia.
E-Mail: rodgarner@powerup.com.au

Abstract: Public aquariums have contributed to knowledge on elasmobranchs in various fields including diet, age and growth, bioenergetics, physiology, pathology, behavior, captive care, and population dynamics. Benefits of conducting research in public aquariums include: clear water, large tanks, species variety, and knowledge of husbandry. Limitations include: artificial habitats, possible modification of environmental cues (e.g., photoperiods, etc.), and insufficient replicates for adequate hypothesis testing. Although research in aquariums is continuing to increase, it appears to be restricted to relatively few institutions. We actively encourage aquariums to participate in elasmobranch research. We recommend cooperation between aquarists and colleagues at academic organizations to maximize the value of their respective skills. The ultimate aim of each study should be to publish results in peer-reviewed journals or books, ensuring rigorous research practices and knowledge dissemination. Research activities will be of immediate benefit to the aquariums involved and ultimately aid in the conservation of elasmobranchs.

Research and public aquariums may appear not to have a lot of common ground, or areas of common interest, but in this section of the manual we intend to show that research can be and has been achieved in public aquariums, and that there is great benefit in harnessing this potential. We will discuss the benefits and limitations of research conducted in aquariums, and give many examples of successful studies undertaken in various fields. We will sketch the process required to develop and steer research projects through an aquarium administration, and discuss the importance of publishing results.

What is research? According to Webster's New World Dictionary (Nerfeld, 1990) research is defined as:

"…careful, systematic study and investigation in some field of knowledge…"

Basic (or pure) research may be defined as investigating phenomena without specific applications in mind, whereas applied research is intended to gain knowledge or understanding to meet a specific need. Researchers investigate questions (ideas or hypotheses) by testing them to see if they stand up to experimental analyses. Essentially the hypothesis is tested to see if it can be supported or rejected. This process requires multiple repetitions, or replicates, to obtain sufficient information and scientific robustness. Statistical analyses of the data investigate whether the results may be explained by chance alone. The hypothesis may then be modified and tested again. Experimental or observational settings need to be carefully described so that others can replicate the study and achieve consistent results.

Current research on elasmobranchs worldwide is both basic and applied, and in reality the separation into basic and applied may be an artificial division. Most elasmobranch research to date has occurred in academic institutions, affiliated field stations, or in government laboratories. With the proliferation of public aquariums worldwide there is considerable potential for the industry to play a much greater role in research involving elasmobranchs and other aquarium animals.

Applied investigations into improving captive husbandry or meeting the biological needs of specimens on exhibit dominate research generated within public aquariums. This work directly benefits both the institution and wild populations, because the goal is to improve animal health and thereby reduce the number of specimens taken from the wild. Examples of applied research includes studies of nutritional requirements (refer to Chapter 14), hematological studies (refer to Chapters 20 and 23), growth studies (refer to Chapter 15), and species-specific exhibit design (e.g., Chapter 32).

Basic research that addresses a research question or tests a hypothesis, following strict protocols, is relatively rare in aquariums. The availability of experimental control groups, for statistical robustness, is particularly challenging when working with large elasmobranchs. Furthermore, costs in time, space, and personnel have generally restricted the amount of research projects undertaken. However, molecular and cellular studies have often benefited from access to captive specimens, particularly for taxonomic and stock identification purposes. For such studies, one sample is often sufficient.

Cooperation between academic institutions and public aquariums has great potential, as both partners benefit from the relationship. There are already examples of applied and basic research being combined to improve captive care, while also answering a key biological question. Such investigations have been carried out on both bioenergetics (Schmid and Murru, 1994; Henningsen, 1996) and endocrinology (Crow et al., 1998). Partnerships between aquariums and academia have yielded valuable physiology studies (Rasmussen and Murru, 1992; Crow et al., 1998; Henningsen et al., 1999; Henningsen et al., 2000). Such cooperative efforts are ideal as the focus of trained researchers and the unique skills of aquarium staff form effective partnerships in resolving specific research goals.

A model of the process required to develop a research project and steer it through institutional administration is outlined in Figure 39.1.

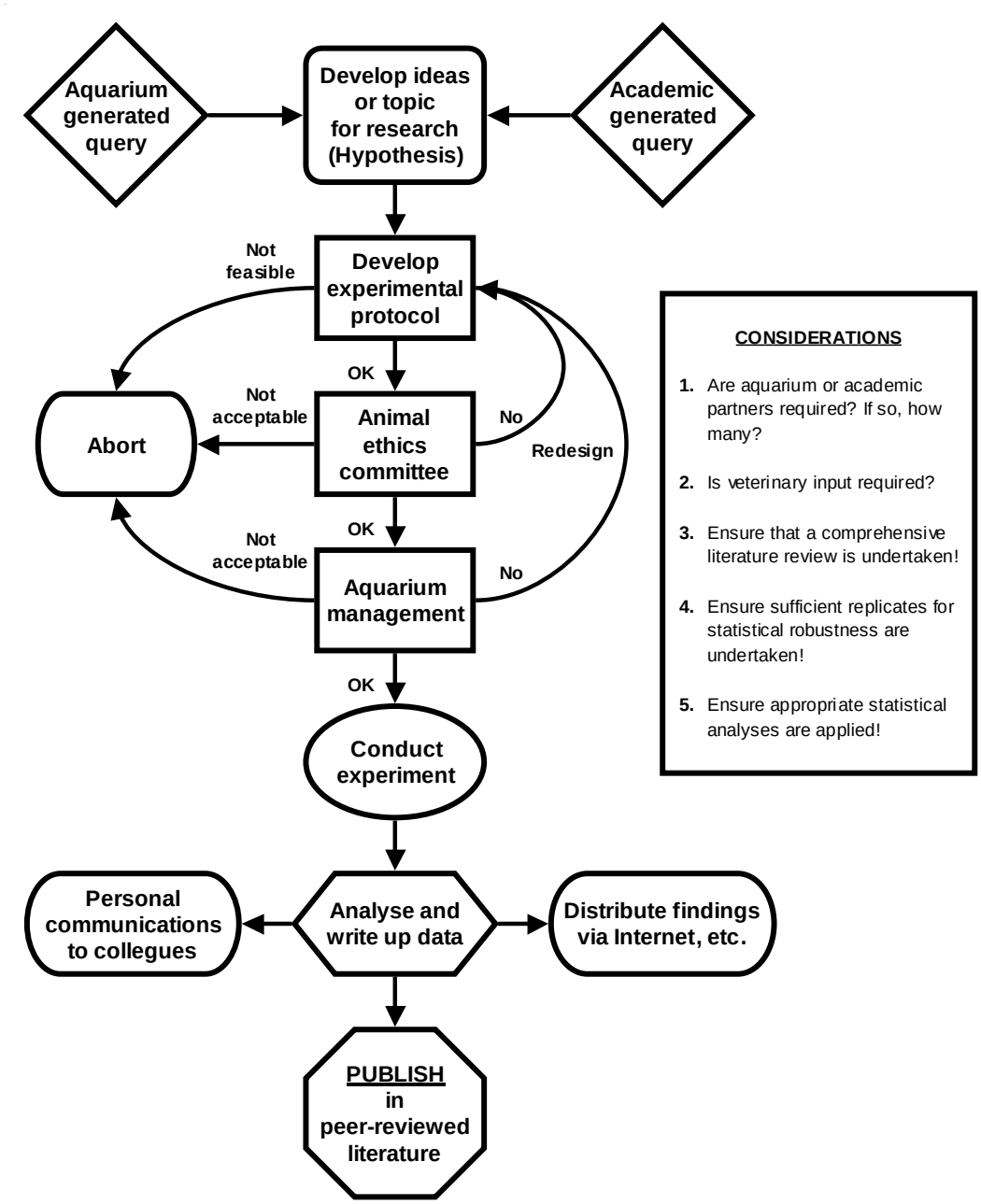


Figure 39.1. A flow diagram illustrating a model for undertaking research in public aquaria. The process may best be seen as an endless loop because the testing of a research hypothesis inevitably results in future research questions that need to be developed and tested.

ADVANTAGES AND LIMITATIONS OF AQUARIUMS

There are both advantages and limitations to research on captive elasmobranchs. Among the advantages are: an availability of specimens belonging to several species, access to captive history (i.e., husbandry and medical records), and knowledge of environmental parameters (e.g., photoperiod, temperature, water chemistry, etc.).

Among the limitations of research on captive elasmobranchs are: small sample sizes, minimal comparability with wild conspecifics, and minimal comparability with conspecifics under different conditions at other institutions. Ideally, information derived from captive elasmobranchs should be verified with data derived from wild conspecifics. For example, steroid titers of captive carcharhinid sharks determined by Rasmussen and Murru (1992) were compared to wild sharks, at comparable stages of maturation and reproductive cycle, and were shown to be similar. Without such comparisons it is possible that results observed are an artifact of the aquarium regimen. All of these limitations should be considered, and accounted for, when constructing a research project.

In captivity the temperature and photoperiod may be modified because aquariums are frequently isolated from the external environment. This isolation limits environmental cues influencing sharks and rays, and may influence their physiology. Older and less sophisticated aquariums can have difficulties keeping seasonal temperature fluctuations within acceptable extremes. This risk may limit the number of species that can be maintained and may also influence the physiology of sharks if extreme temperatures become stressful. The physical limitations of an exhibit may further limit the natural responses of animals, being unable to swim distances possible in the wild and unable to interact at normal distances with conspecifics or other species. These restrictions may constrain social behaviors such as mating and schooling, and artificially influence observations in aquariums.

Research can be expensive, and although many aquariums have funds for investigation allocated in their budgets, they are generally fairly limited. Aquariums generally become involved in research projects that are consistent with their mission statement and will shy away from those that may detract from that statement. Nevertheless, aquariums may undertake excellent and economic research projects, as we will show in the examples below.

ANIMAL WELFARE CONSIDERATIONS

Prior to the initiation of research, an appropriate animal ethics committee should review the proposal to ensure that it is conducted according to internationally-accepted standards of animal welfare. Research must comply with all federal, state, and local laws, and regulations for the humane treatment, care, and use of animals, as well as those covering endangered species. In the USA, institutional animal care and use committees (IACUC) must be established to oversee and evaluate each institution's animal program, as well as the approval of all research involving animals.

AREAS OF RESEARCH

Diet and Nutrition

Research on improving the diet of captive elasmobranchs has been necessitated by the unavailability of natural prey. Usually a narrow selection of frozen fish and invertebrates (normally used for human consumption) is available and experience has shown that elasmobranchs remain healthier if vitamin and mineral supplements are added to their food (Murru, 1990). The introduction of an elasmobranch species not previously kept in aquariums represents a prime candidate for research—accompanied by a literature review, communication with peers, and almost certainly, trial-and-error. Field studies of natural prey (e.g., Randall, 1967; Smale and Compagno, 1997; Smale and Goosen, 1999) should guide the choice of food to be offered. Rigorous and detailed record keeping is essential to allow ultimate knowledge transfer within and between institutions.

Growth and development

Public aquariums offer an opportunity to study the growth and early life stages of unusual elasmobranch species. Such studies may be undertaken as part of standard animal record keeping, although information on feeding rations and temperature ranges should also be maintained. Results should be compared with

results from wild elasmobranchs to assess the influences (if any) of the test environment and feeding regimes. This is undoubtedly one of the areas where aquariology has contributed the most toward elasmobranch research. Species for which growth has been studied in captivity include: nurse sharks, *Ginglymostoma cirratum* (Carrier and Luer, 1990), bull sharks, *Carcharhinus leucas*, sandbar sharks, *Carcharhinus plumbeus*, sand tiger sharks, *Carcharias taurus* (Schmid et al., 1990), broadnose sevengill sharks, *Notorynchus cepedianus* (Van Dykhuizen and Mollet, 1992), and epaulette sharks, *Hemiscyllium ocellatum* (West and Carter, 1990), among others. Coupling length and weight data with nutrition information yields a powerful tool for the assessment of husbandry techniques, allowing an assessment of the adequacy of a given feeding regime.

Bioenergetics

Bioenergetic studies require closed circuits, allowing energy budgets to be calculated under the assumption that the difference between input and output in a system equals growth. It is paramount to conduct such studies under controlled, closed environments and aquariums are ideal for such studies. Many species have been studied in aquariums, including the spiny butterfly ray, *Gymnura altavela* (Henningsen, 1996) and the bull shark (Schmid and Murru, 1994).

Studies of food rations, food retention times, and food passage rates, for ecological studies, have been carried out on lemon sharks, *Negaprion brevirostris*, in laboratory aquariums (Wetherbee et al., 1987; Wetherbee and Gruber, 1990). Such experiments are essential for energetic studies, but are normally restricted to juveniles because of size constraints. Extensions of energetic studies, to include larger individuals, have been achieved in public aquariums. Pole feeding, in combination with detailed record keeping, have provided estimates of daily ration for the broadnose sevengill shark (Van Dykhuizen and Mollet, 1992).

Physiology

Despite the logistical difficulties involved in monitoring biochemical and physiological parameters of large captive elasmobranchs, more and more public aquariums have come to realize the benefits of conducting regular surveys as a preventive rather than corrective measure. Naturally, the procedure of catching and restraining a specimen can bias the results, especially where hormone and serum electrolyte levels are concerned. However, systematic recording of blood parameters (refer to Chapters 20 and 23 of this manual) allows tracking of physiological changes over extended periods of time, provides a valuable tool in identifying and diagnosing potentially pathological situations, and allows comparison of equivalent parameters between institutions. Examples of species studied include: lemon sharks (Murru et al., 1989; Pike et al., 1989; Stoskopf, 1993), sandbar sharks, nurse sharks, and tiger sharks, *Galeocerdo cuvier* (Stoskopf, 1993).

Captive animals are excellent subjects for long-term serum hormone studies (Rasmussen and Crow, 1993). Changes in steroid hormone titers may be monitored over periods of months to better understand fluxes in living animals. The constraints of the aquarium situation need to considered, and care needs to be taken to minimize confounding effects (e.g., circadian rhythms, etc.) that may influence levels in the blood (Rasmussen and Crow, 1993). Although it is important to minimize stress when collecting samples, this artifact may be studied to quantify the effects of long-term stress on captive elasmobranchs.

Tooth-shedding rate, which would be hard to study in the wild, is relatively easy to monitor in captivity. Traditionally, sand tiger sharks have been the focus in this field (Overstrom, 1991; Correia, 1999). Correlation of tooth-shedding rate with environmental variables (e.g. temperature, food intake, etc.) may provide insight into the animal's physiology as well as its adaptability to captivity.

Pathology

Pathology is the study of disease. Despite its obvious negative connotation, the occurrence of disease in captive elasmobranchs necessitates a cure, thereby creating an opportunity for research. Skin scrapes, tissue smears, biopsies, and other procedures often lead to the identification of pathogens and their respective treatments. Stoskopf (1993) provides a review of such cases. Other references include Grimes et al. (1984), Grimes et al. (1986), Noga (1996), and Subra (1998).

The study of elasmobranchs in aquariums has led to the identification of several new species of parasites and also the processes by which they may be eradicated (refer to Chapter 24 of this

manual). A typical example is the description of *Paralebion elongatus* in captive whitetip reef sharks (*Triaenodon obesus*), by Benz et al. (1992).

Histopathological studies of tissues obtained during necropsies can aid in identifying the cause of death of captive elasmobranchs and also provide good research opportunities. Recently Crow et al. (2001) determined, by histological assessment, that elasmobranch and human goiters have a similar pattern of development and etiology. Such studies not only advance the knowledge of human disease, but also facilitate the diagnosis of elasmobranch diseases by other institutions.

Behavior

Clear water and specimen containment allow the observation of behaviors that would otherwise not be possible in the turbid, natural habitat of many elasmobranchs. For example, mating, gestation, and birth can all be recorded, yielding useful information (refer to Chapter 17 of this manual). Examples of such studies include those describing the captive breeding of whitetip reef sharks (Garner and Mackness, 1998a) and the blotched fantail ray, *Taeniura meyeni* (Garner and Mackness (1998b). Uchida et al. (1990) reported details of reproduction in seven species of sharks and seven species of rays held at the Okinawa Expo Aquarium (Okinawa, Japan). Their work expanded knowledge about elasmobranch reproduction. However, successful breeding (which they defined as newborn or hatched pups maintained until they reach maturity and breed themselves) was achieved at the Okinawa Expo Aquarium in only one species, the whitetip reef shark. This suggests that facilities, even in the best public aquariums, are not always suitable for elasmobranch reproduction and the completion of elasmobranch life cycles. As Pratt and Carrier (2001) note in their extensive review of elasmobranch reproduction, the restrictions of aquariums may limit understanding of mating patterns and interpretations may be inaccurate if they are not verified by detailed studies in the wild. Naturally, this is often difficult to achieve. Regardless, there is little doubt that aquariums have advanced our knowledge of reproduction in elasmobranchs, as is evidenced by numerous studies that have produced new information on reproductive behavior (Klimley, 1980; Gordon 1993; Pratt and Carrier, 2001).

Other examples of behavioral studies in captive elasmobranchs include those of Myrberg and Gruber (1974) and Seligson and Weber (1990). The systematic logging of specific behaviors, with the inclusion of pictures or drawings, provides good insight into long-term behavioral changes, when correlated with time, and other variables such as feeding, lighting, introduction of conspecifics, etc. By including data fields on daily record sheets, it is possible to encourage husbandry staff to monitor animal behavior regularly. Although behavior is not always easily described, separation into discreet categories can often provide an adequate compromise. Such categories might include resting, swimming, feeding, mating, etc. The use of video photography may help in recording behaviors that are difficult to describe, and may facilitate communication and comparison between different observers.

Population dynamics

Many elasmobranch population studies have been undertaken by government agencies, such as the National Marine Fisheries Service (Merson and Pratt, 2001), and academic institutions, such as the Virginia Institute of Marine Science (Musick et al., 1993). Since many aquariums collect elasmobranchs from the same locations each year, they can contribute to such studies by keeping accurate field records.

Transport

Public aquariums have been transporting elasmobranchs for decades. Knowledge in this area has increased considerably in recent years (refer to Chapter 8 of this manual), particularly with species traditionally regarded as difficult to transport. Long-duration elasmobranch transport (i.e., >24 hours) has driven aquarium staff to better understand and control elasmobranch physiology and biochemistry, a key factor for transport success. References in this area are numerous and species studied include the sand tiger shark (Smith, 1992), scalloped hammerhead shark, *Sphyrna lewini* (Arai 1997; Young et al., 2002), sandbar shark (Andrews and Jones, 1990; Jones and Andrews, 1990), spiny dogfish, *Squalus acanthias* (Jones et al., 1983), and spotted ratfish, *Hydrolagus colliei* (Correia, 2001).

Dissemination of results

Dissemination of research undertaken in aquariums is vital. Basic ethics dictate that

research results should be shared wherever possible. Inter-institutional dissemination can be as simple as distributing information via e-mail, web sites, or even telephoning colleagues facing similar husbandry challenges. To allow comparisons between studies or localities, the specifics of the study environment need to be clearly described. Ultimately, new and significant findings should be published in books and peer-reviewed journals to maximize global information transfer, and to maintain the highest standards of research. If the decision to publish results is made before the study is initiated, it will help focus research activities and promote experiments with rigorous hypotheses.

Future work

Aquarium studies have contributed significantly to our knowledge of elasmobranchs. However, there are numerous areas suggestive of further research. These include: parasitology, for example the study of un-described parasitic organisms to which elasmobranchs play host (refer to Chapter 24 of this manual); captive breeding, in particular species whose populations are threatened in the wild (refer to Chapter 17 of this manual); and DNA analysis of animals from known sources. Such DNA studies may be achieved with minimal damage to individual specimens and yet would yield important insight into population dynamics, conservation strategies, and associated management plans (refer to Chapter 18 of this manual).

CONCLUSIONS

Historically, research was considered to be outside the focus of public aquariums. Ten years ago it was stated by McCormick-Ray (1993) that:

"…what aquariums generally lack is a coherent approach to the science of aquariology. That is, they lack a research focus that would advance captive animal biology and technology and contribute to existing husbandry, conservation, and educational concerns…"

This chapter seeks to demonstrate that advances have been made to address this criticism.

A recent survey of North American zoos and aquariums reports an increased focus on research (Stoinski et al., 1998). However, it may be more accurate to say that research has been undertaken by a few specific institutions, and is the direct result of the dedication, individual skills, and interests of a handful of employees, as well as their proximity to research professionals outside the aquarium industry. Despite this situation, there appears to be a growing trend of support for scientific investigation in aquariums. Many benefits accrue to institutions that undertake research, and aquarium administrators should be apprised of these rewards and encouraged to support research efforts. Aquariums should initiate research projects and remain receptive to initiatives from outside the institution. A research department should be created and funded so that basic husbandry investigations and field studies are encouraged, structured, supported, undertaken, and ultimately disseminated.

One of the best ways to optimize research potential at a public aquarium is to form partnerships with colleagues in academic institutions. Such partnerships, between animal husbandry experts and trained scientists, will build on the strengths of both parties (e.g., husbandry skills, knowledge of research practices, etc.) and ensure that studies are robust, focused, and of an appropriate academic standard. Communication between aquariums and academic associations that specialize in elasmobranchs is encouraged. These associations include, among others, the IUCN SSG (International Union for the Conservation of Nature and Natural Resources, Species Survival Commission, Shark Specialist Group), AES (American Elasmobranch Society), and the EEA (European Elasmobranch Association).

Research results can be rapidly disseminated using electronic communication, but it is vital that investigations are set up rigorously to allow publication in high quality, peer-reviewed journals. Resultant studies will improve both the husbandry and conservation of elasmobranchs.

REFERENCES

Andrews, J. C. and R. T. Jones. 1990. A method for the transport of sharks for captivity. Journal of Aquariculture and Aquatic Sciences 5: 70-72.

Arai, H. 1997. Collecting, transporting and rearing of the scalloped hammerhead. *In*: Proceedings of the Fourth International Aquarium Congress, June 23-27, 1996, Tokyo, p. 87-89. Tokyo, Japan by the Congress Central Office of IAC '96, Tokyo Sea Life Park. 402 pp.

Benz, G. W., P. J. Mohan, and G. L. Crow. 1992. Developmental stages of *Paralebion elongatus* from aquarium-held reef whitetip sharks (*Triaenodon obesus*) captured in Hawaiian waters. Journal of Parasitology 1992(78): 1027-1035.

Carrier, J. C. and C. A. Luer. 1990. Growth rates in the nurse shark, *Ginglymostoma cirratum*. Copeia 3: 683-692.

Correia, J. P. 1999. Tooth loss rate from two captive sand tiger sharks (*Carcharias taurus*). Zoo Biology 18: 313-317.

Correia, J. P. 2001. Long-term transportation of ratfish, *Hydrolagus colliei*, and tiger rockfish, *Sebastes nigrocinctus*. Zoo Biology 20: 435-441.

Crow, G. L., M. A. Atkinson, B. Ron, S. Atkinson, A. D. K. Skillman, and G. T. F. Wong. 1998. Relationship of water chemistry to serum thyroid hormones in captive sharks with goiters. Aquatic Geochemistry 4: 469-480.

Crow, G. L., W. H. Luer, and J. H. Harshbarger. 2001. Histological assessment of goiters in elasmobranch fishes. Journal of Aquatic Animal Health 13: 1-7.

Garner, R. and B. Mackness. 1998a. Captive breeding of the whitetip reef shark *Triaenodon obesus*. Thylacinus 22(2): 16-17.

Garner, R. and B. Mackness. 1998b. First captive breeding of the blotched fantail ray, *Taeniura meyeni* (Müller and Henle, 1841), in Australia. Thylacinus 22(2): 22-24.

Grimes, D. J., J. Stemmler, H. Hada, E. B. May, D. Maneval, F. M. Hetrick, R. T. Jones, M. Stoskopf, and R. R. Colwell. 1984. Vibrio species associated with mortality of sharks held in captivity. Microbial Ecology 10: 271-282.

Grimes, D. J., P. Brayton, S. H. Gruber, and R. R. Colwell. 1986. Vibrio disease in captive sharks. *In:* Pathology in Marine Aquaculture, p. 231-232. C. P. Vivares, J. R. Bonami, and E. Jaspers (eds.). Proceedings of the first international colloquium Pathology in Marine Aquaculture held from 11 to 14 September 1984 in Montpellier, France. European Aquaculture Society Special Publication No. 9, Oostende, Belgium.

Gordon, I. 1993. Pre-copulatory behavior of captive sand tiger sharks, *Carcharias taurus*. Environmental Biology of Fishes 38: 159-164.

Henningsen, A. D. 1996. Captive husbandry and bioenergetics of the spiny butterfly ray, *Gymnura altavela* (Linnaeus). Zoo Biology 15: 135-142.

Henningsen, A.D., J. M. Trant, and A. R. Place. 1999. Preliminary results on the size of proteins and protein concentration in histotroph from three species of batoids. *In:* Proceedings of the 15th annual Meeting of the American Elasmobranch Society, June 24-30, 1999, Pennsylvania State University, State College, Pennsylvania, USA. Abstract., p. 125.

Henningsen, A.D., J. M. Trant, S. Ijiri, and S. Kumar. 2000. The short term in vitro response of stingray trophonemata to exogenous agents. *In:* Proceedings of the 16th annual Meeting of the American Elasmobranch Society, June 14-20, 2000, Universidad Autonoma de Baja California Sur, la Paz, B.C.S., Mexico. Abstract., p. 190.

Jones, R. T. and J. C. Andrews. 1990. Hematologic and serum chemical effects of simulated transport on sandbar sharks, *Carcharhinus plumbeus* (Nardo). Journal of Aquariculture and Aquatic Sciences 5: 95-100.

Jones, R. T., E. A. Hudson, and J. C. Andrews. 1983. Methods for transport and long-term maintenance of spiny dogfish sharks. Laboratory Animal Science 33: 388-389.

Klimley, A. P. 1980. Observations of courtship and copulation in the nurse shark, *Ginglymostoma cirratum*. Copeia 1980 (4): 878-882.

McCormick-Ray, M. G. 1993. Aquarium science: The substance behind an image. Zoo Biology 12: 413-424.

Merson, R. R. and Pratt, H. L., Jr. 2001. Distribution movement and growth of young sandbar sharks, *Carcharhinus plumbeus*, in the nursery grounds of Delaware Bay. Environmental Biology of Fishes 61: 13-24.

Murru, F. L. 1990. The care and maintenance of elasmobranchs in controlled environments. *In:* Elasmobranchs as Living Resources: Advances in the Biology, Ecology, Systematics, and the Status of the Fisheries, p. 203-209. H. L. Pratt, Jr., S. H. Gruber, and T. Taniuchi (eds.). NOAA Technical Report NMFS 90.

Murru, F. L., M. T. Walsh, B. L. Smith, and J. B. Pangboan. 1989. Whole blood element analysis of captive and wild lemon sharks (*Negaprion brevirostris*) by inductively coupled plasma emission spectroscopy. Journal of Aquariculture and Aquatic Sciences 5(4): 119.

Musick, J. A., S. Branstetter, and J. A. Colvocoresses. 1993. Trends in shark abundance from 1974 to 1991 for the Chesapeake bight region of the U.S. Mid-Atlantic coast. *In:* Conservation Biology of Elasmobranchs. (Branstetter, ed.) NOAA Technical Report NMFS. 115, 1-118.

Myrberg, A. A., Jr. and S. H. Gruber. 1974. The behavior of the bonnethead shark, *Sphyrna tiburo*. Copeia 1974(2): 358-374.

Nerfeld, V. (ed.). 1990. Webster's New World Dictionary. Simon and Schuster, Inc., Riverside, NJ 08075, USA. 1700 p.

Noga, E. J. 1996. Fish disease – Diagnosis and Treatment. Mosby-Year Book Press, St. Louis, Missouri, USA. 367 p.

Overstrom, N. A. 1991. Estimated tooth replacement rate in captive sand tiger sharks (*Carcharias taurus* Rafinesque, 1810). Copeia 1991(2): 525-526.

Pike, III., C. S., S. Charles, and S. H. Gruber. 1989. Preliminary baseline blood parameters for captive juvenile lemon sharks, *Negaprion brevirostris*. Journal of Aquariculture and Aquatic Sciences 5(4): 120.

Pratt, H. L. Jr. and J. C. Carrier. 2001. A review of elasmobranch reproductive behavior with a case study on the nurse shark, *Ginglymostoma cirratum*. Environmental Biology of Fishes 60; 157-188.

Randall, J. E. 1967. Food habits of reef fishes of the West Indies. Studies in Tropical Oceanography 5: 665-847.

Rasmussen, L. E. L. and G. L. Crow. 1993. Serum corticosterone concentrations in immature captive whitetip reef sharks, *Triaenodon obesus*. Journal of Experimental Zoology 267: 283-287.

Rasmussen, L. E. L. and F. L. Murru. 1992. Long-term studies of serum concentrations of reproductively related steroid hormones in individual captive carcharhinids. Australian Journal of Marine and Freshwater Research 43: 273-281.

Schmid, T. H., F. L. Murru, and F. McDonald. 1990. Feeding habits and growth rates of bull (*Carcharhinus leucas* (Valenciennes)), sandbar (*Carcharhinus plumbeus* (Nardo)), sand tiger (*Eugomphodus taurus* (Rafinesque)) and nurse (*Ginglymostoma cirratum* (Bonnaterre)) sharks maintained in captivity. Journal of Aquariculture and Aquatic Sciences 5: 100-105.

Schmid, T. H. and F. L. Murru. 1994. Bioenergetics of the bull shark, *Carcharhinus leucas*, maintained in captivity. Zoo Biology 13: 177-185.

Seligson, S. A. and D. J. Weber. 1990. Alterations in established swimming habits of carcharhinid sharks at the Living Seas Pavilion. Journal of Aquariculture and Aquatic Sciences 5: 105-111.

Smale, M. J. and L. J. V. Compagno. 1997. Life history and diet of two Southern African smooth-hound sharks, *Mustelus mustelus* (Linnaeus, 1752) and *Mustelus palumbes* Smith, 1957 (Pisces: Triakidae). South African Journal of Marine Science 18: 229-248.

Smale, M. J. and A. J. J. Goosen. 1999. Reproduction and feeding of spotted gully shark, *Triakis megalopterus*, off the Eastern Cape, South Africa. U. S. Fisheries Bulletin 97: 987-998.

Smith, M. F. L. 1992. Capture and transportation of elasmobranchs, with emphasis on the gray nurse shark (*Carcharias taurus*). Australian Journal of Marine and

Freshwater Research (Sharks: Biology and Fisheries) 43: 325-343.

Stoinski, T.S., K.E. Lukas, and T. L. Maple. 1998. A survey of research in North American zoos and aquariums. Zoo Biology 17: 167-180.

Stoskopf, M. K. 1993. Clinical pathology of sharks, skates and rays. *In:* Fish Medicine, p. 754-757. M. K. Stoskopf (ed.). W. B. Saunders Company, Philadelphia, Pennsylvania, USA.

Subra, S. 1998. Conservation and Pathologies of Sharks Living in Captivity in French Aquariums. Ecole Nationale Veterinaire, Lyon, France. 241 p.

Uchida, S., M. Toda, and Y. Kamei. 1990. Reproduction of elasmobranchs in captivity. *In:* Elasmobranchs as Living Resources: Advances in the Biology, Ecology, Systematics, and the Status of the Fisheries, p. 211-237. H. L. Pratt, Jr., S. H. Gruber, and T. Taniuchi (eds.). NOAA Technical Report NMFS, 90.

Van Dykhuizen, G. and H. F. Mollet. 1992. Growth, age estimation and feeding of captive sevengill sharks, *Notorynchus cepedianus*, at the Monterey Bay Aquarium. *In:* Sharks: Biology and Fisheries. J. G. Pepperell (ed.). Australian Journal of Marine and Freshwater Research 43: 297-318.

West, J. G. and S. Carter. 1990. Observations on the development and growth of the epaulette shark *Hemiscyllium ocellatum* (Bonnaterre) in captivity. Journal of Aquariculture and Aquatic Sciences 5: 111-117.

Wetherbee, B. M., S. H. Gruber, A. L. and Ramsey. 1987. X-radiographic observations of food passage through digestive tracts of lemon sharks. Transactions of the American Fisheries Society 116: 763-767.

Wetherbee, B.M. and S. H. Gruber. 1990. The effects of ration level on food retention time in juvenile lemon sharks, *Negaprion brevirostris*. Environmental Biology of Fishes 29: 59-65.

Young, F. A., S. M. Kajiura, G. J. Visser, J. P. S. Correia, and M. F. L. Smith. 2002. Notes on the long-term transportation of the scalloped hammerhead shark, *Sphyrna lewini*. Zoo Biology 21 (3): 242-251.

Appendix 1. Elasmobranchs cited in Elasmobranch Husbandry Manual (sorted by scientific name).

Scientific Name	Common Name	Scientific Name	Common Name
Aetobatus narinari	spotted eagle ray	*Dasyatis americana*	southern stingray
Aetomylaeus niehofii	banded eagle ray	*Dasyatis brevicaudata*	short-tail stingray
Alopias superciliosus	bigeye thresher shark	*Dasyatis brevis*	whiptail stingray
Alopias vulpinus	thintail thresher shark	*Dasyatis centroura*	roughtail stingray
Amblyraja radiata	thorny skate	*Dasyatis chrysonata*	blue stingray
Anoxypristis cuspidata	knifetooth sawfish	*Dasyatis fluviorum*	estuary stingray
Apristurus brunneus	brown cat shark	*Dasyatis garouaensis*	smooth freshwater stingray
Aptychotrema bougainvillii	short-snouted shovelnose ray	*Dasyatis izuensis*	Izu stingray
Aptychotrema rostrata	eastern shovelnose ray	*Dasyatis laosensis*	Mekong stingray
Asymbolus analis	Australian spotted catshark	*Dasyatis lata*	brown stingray
Atelomycterus macleayi	Australian marbled cat shark	*Dasyatis marmorata*	marbled stingray
Atelomycterus marmoratus	coral cat shark	*Dasyatis matsubarai*	pitted stingray
Bathyraja abyssicola	deepsea skate	*Dasyatis microps*	smalleye stingray
Bathyraja aleutica	Aleutian skate	*Dasyatis pastinaca*	common stingray
Bathyraja interrupta	sandpaper skate	*Dasyatis sabina*	Atllantic stingray
Brachaelurus waddi	blind shark	*Dasyatis say*	bluntnose stingray
Callorhinchus callorynchus	cockfish	*Dipturus batis*	skate
Callorhinchus capensis	cape elephantfish	*Dipturus laevis*	barndoor skate
Callorhinchus milii	ghost shark	*Dipturus nasutus*	rough skate
Carcharhinus acronotus	blacknose shark	*Dipturus oxyrinchus*	longnosed skate
Carcharhinus altimus	bignose shark	*Echinorhinus cookei*	prickly shark
Carcharhinus amblyrhynchoides	graceful shark	*Etmopterus lucifer*	blackbelly lantern shark
Carcharhinus amblyrhynchos	gray reef shark	*Etmopterus spinax*	velvet belly
Carcharhinus amboinensis	pigeye shark	*Eucrossorhinus dasypogon*	tasseled wobbegong
Carcharhinus borneensis	Borneo shark	*Furgaleus macki*	whiskery shark
Carcharhinus brachyurus	copper shark	*Galeocerdo cuvier*	tiger shark
Carcharhinus brevipinna	spinner shark	*Galeorhinus galeus*	tope shark
Carcharhinus dussumieri	whitecheek	*Galeus melastomus*	blackmouth catshark
Carcharhinus falciformis	silky shark	*Ginglymostoma brevicaudatum*	short-tailed nurse shark
Carcharhinus galapagensis	Galapagos shark	*Ginglymostoma cirratum*	nurse shark
Carcharhinus hemiodon	Pondicherry shark	*Glyphis gangeticus*	Ganges shark
Carcharhinus isodon	finetooth shark	*Glyphis glyphis (species A)*	speartooth shark
Carcharhinus leiodon	smalltooth shark	*Glyphis sp. (species C)*	northern river shark
Carcharhinus leucas	bull shark	*Gymnura altavela*	spiny butterfly ray
Carcharhinus limbatus	blacktip shark	*Gymnura japonica*	Japanese butterfly ray
Carcharhinus longimanus	oceanic whitetip shark	*Gymnura marmorata*	California butterfly ray
Carcharhinus macloti	hardnose shark	*Gymnura micrura*	smooth butterfly ray
Carcharhinus melanopterus	blacktip reef shark	*Haploblepharus edwardsii*	puffadder shyshark
Carcharhinus obscurus	dusky shark	*Haploblepharus fuscus*	brown shyshark
Carcharhinus perezi	Caribbean reef shark	*Haploblepharus pictus*	dark shy shark
Carcharhinus plumbeus	sandbar shark	*Hemigaleus microstoma*	sicklefin weasel shark
Carcharhinus porosus	smalltail shark	*Hemiscyllium hallstromi*	Papauan epaulette shark
Carcharhinus signatus	night shark	*Hemiscyllium ocellatum*	epaulette shark
Carcharhinus sorrah	spottail shark	*Hemitriakis leucoperiptera*	whitefin topeshark
Carcharhinus tilstoni	Australian blacktip shark	*Heterodontus francisci*	horn shark
Carcharias taurus	sand tiger shark	*Heterodontus galeatus*	crested bullhead shark
Carcharodon carcharias	white shark	*Heterodontus japonicus*	Japanese bullhead shark
Centrophorus granulosus	gulper shark	*Heterodontus mexicanus*	Mexican horn shark
Centrophorus harrissoni	dumb gulper shark	*Heterodontus portusjacksoni*	Port Jackson shark
Centrophorus uyato	little gulper shark	*Heteroscyllium colcloughi*	bluegray carpetshark
Centroscyllium fabricii	black dogfish	*Hexanchus griseus*	bluntnose sixgill shark
Cephaloscyllium laticeps	Australian swell shark	*Hexanchus nakamurai*	bigeye sixgill shark
Cephaloscyllium umbratile	Japanese swell shark	*Himantura bleekeri*	Bleeker's whipray
Cephaloscyllium ventriosum	swell shark	*Himantura chaophraya*	freshwater stingray
Cetorhinus maximus	basking shark	*Himantura fai*	pink whipray
Chiloscyllium arabicum	Arabian carpet shark	*Himantura fluviatilis*	Ganges stingray
Chiloscyllium griseum	gray bamboo shark	*Himantura gerrardi*	sharpnose stingray
Chiloscyllium indicum	slender bambooshark	*Himantura imbricata*	scaly whipray
Chiloscyllium plagiosum	whitespotted bamboo shark	*Himantura oxyrhynchus*	marbled whipray
Chiloscyllium punctatum	brownbanded bamboo shark	*Himantura schmardae*	chupare stingray
Chimaera monstrosa	rabbit fish	*Himantura signifer*	white-rimmed whipray
Chimaera phantasma	silver chimaera	*Himantura uarnak*	honeycomb stingray
Chlamydoselachus anguineus	frilled shark	*Himantura undulata*	leopard whipray
Dalatias licha	kitefin shark	*Hydrolagus colliei*	spotted ratfish
Dasyatis akajei	red stingray	*Hydrolagus ogilbyi*	Ogilby's ghostshark

Appendix 1 (continued). Elasmobranchs cited in Elasmobranch Husbandry Manual (sorted by scientific name).

Scientific Name	Common Name	Scientific Name	Common Name
Hypnos monopterygium	Australian numbfish	*Pristis clavata*	dwarf sawfish
Hypogaleus hyugaensis	blacktip topeshark	*Pristis microdon*	largetooth sawfish
Isistius brasiliensis	cookiecutter shark	*Pristis pectinata*	smalltooth sawfish
Isurus oxyrinchus	shortfin mako	*Pristis perotteti*	large-tooth sawfish
Isurus paucus	longfin mako	*Pristis pristis*	common sawfish
Lamna ditropis	salmon shark	*Pristis zijsron*	longcomb sawfish
Lamna nasus	porbeagle	*Pseudocarcharias kamoharai*	crocodile shark
Leptocharias smithii	barbeled houndshark	*Pseudotriakis microdon*	false cat shark
Leucoraja erinacea	little skate	*Pteroplatytrygon violacea*	pelagic stingray
Leucoraja naevus	Cuckoo ray	*Raja asterias*	starry ray
Leucoraja ocellata	winter skate	*Raja binoculata*	big skate
Malacoraja senta	smooth skate	*Raja brachyura*	blonde ray
Manta birostris	giant manta	*Raja clavata*	thornback ray
Megachasma pelagios	megamouth shark	*Raja eglanteria*	clearnose skate
Mobula diabola	devil ray	*Raja inornata*	California ray
Mobula mobular	devil fish	*Raja microocellata*	small-eyed ray
Mobula munkiana	Munk's devil ray	*Raja miraletus*	brown ray
Mustelus antarcticus	gummy shark	*Raja montagui*	spotted skate
Mustelus asterias	starry smooth-hound	*Raja rhina*	longnose skate
Mustelus californicus	grey smooth-hound	*Raja sp. L*	Maugaen skate
Mustelus canis	dusky smooth-hound	*Raja stellulata*	starry skate
Mustelus henlei	brown smooth-hound	*Raja texana*	Roundel skate
Mustelus lenticulatus	spotted estuary smooth-hound	*Raja undulata*	undulate ray
Mustelus manazo	star-spotted smooth-hound	*Rhina ancyclostoma*	bowmouth guitarfish
Mustelus mustelus	smooth-hound	*Rhina percellens*	Chola guitarfish
Mustelus norrisi	Florida smooth-hound	*Rhincodon typus*	whale shark
Myliobatis aquila	common eagle ray	*Rhinobatos annulatus*	lesser sandshark
Myliobatis australis	Australian bull ray	*Rhinobatos cemiculus*	blackchin guitarfish
Myliobatis californica	bat eagle ray	*Rhinobatos granulatus*	sharpnose guitarfish
Myliobatis freminvillii	bullnose eagle ray	*Rhinobatos horkeli*	Brazilian guitarfish
Narcine brasiliensis	Brazilian electric ray	*Rhinobatos hynnicephalus*	ringstraked guitarfish
Narcine entemedor	electric ray	*Rhinobatos lentiginosus*	Atlantic guitarfish
Nebrius ferrugineus	tawny nurse shark	*Rhinobatos productus*	shovelnose guitarfish
Negaprion acutidens	sicklefin lemon shark	*Rhinobatos rhinobatos*	common guitarfish
Negaprion brevirostris	lemon shark	*Rhinobatos typus*	giant shovelnose ray
Notorynchus cepedianus	broadnose sevengill shark	*Rhinoptera bonasus*	cownose ray
Odontaspis ferox	smalltooth sand tiger shark	*Rhinoptera javanica*	Javanese cownose ray
Odontaspis noronhai	bigeye sand tiger shark	*Rhinoptera neglecta*	Australian cownose ray
Okamejei kenojei	spiny rasp skate	*Rhizoprionodon acutes*	milk shark
Orectolobus japonicus	Japanese wobbegong	*Rhizoprionodon longurio*	Pacific sharpnose shark
Orectolobus maculatus	spotted wobbegong	*Rhizoprionodon porosus*	Caribbean sharpnose shark
Orectolobus ornatus	ornate wobbegong	*Rhizoprionodon taylori*	Australian sharpnose shark
Oxynotus centrina	angular roughshark	*Rhizoprionodon terraenovae*	Atlantic sharpnose shark
Paragaleus randalli	Slender weasel shark	*Rhynchobatus djiddensis*	giant guitarfish
Paratrygon aiereba	ceja stingray	*Schroederichthys bivius*	narrowmouthed catshark
Paratrygon leopoldi	white-blotched stingray	*Schroederichthys chilensis*	redspotted catshark
Parmaturus xaniurus	filetail cat shark	*Scoliodon laticaudus*	spadenose catshark
Pastinachus sephen	cowtail stingray	*Scyliorhinus canicula*	smallspotted catshark
Platyrhinoidis triseriata	thornback guitarfish	*Scyliorhinus capensis*	yellowspotted catshark
Plesiotrygon iwamae	long-tailed river stingray	*Scyliorhinus retifer*	chain dogfish
Poroderma africanum	striped cat shark	*Scyliorhinus stellaris*	nursehound
Poroderma pantherinum	leopard cat shark	*Scyliorhinus tokubee*	Izu cat shark
Potamotrygon brachyura	short-tailed river stingray	*Scyliorhinus torazame*	cloudy cat shark
Potamotrygon falkneri	largespot river stingray	*Scylliogaleus quecketti*	flapnose houndshark
Potamotrygon henlei	bigtooth river stingray	*Somniosus microcephalus*	Greenland shark
Potamotrygon histrix	porcupine river stingray	*Somniosus pacificus*	Pacific sleeper shark
Potamotrygon leopoldi	white-blotched river stingray	*Sphyrna lewini*	scalloped hammerhead
Potamotrygon magdalenae	Magdalena river stingray	*Sphyrna mokarran*	great hammerhead
Potamotrygon motoro	ocellate river stingray	*Sphyrna tiburo*	bonnethead
Potamotrygon ocellata	red-blotched river stingray	*Sphyrna tudes*	smalleye hammerhead
Potamotrygon orbignyi	smooth back river stingray	*Sphyrna zygaena*	smooth hammerhead
Potamotrygon reticulatus	spotted freshwater ray	*Squalus acanthias*	spiny dogfish
Potamotrygon schroederi	rosette river stingray	*Squalus megalops*	shortnose spurdog
Prionace glauca	blue shark	*Squatina argentina*	Argentine angelshark
Pristiophorus cirratus	longnose sawshark	*Squatina australis*	Australian angelshark

Appendix 1 (continued). Elasmobranchs cited in Elasmobranch Husbandry Manual (sorted by scientific name).

Scientific Name	Common Name	Scientific Name	Common Name
Squatina californica	Pacific angel shark		
Squatina dumeril	sand devil		
Squatina guggenheim	angular angelshark		
Squatina japonica	Japanese angel shark		
Squatina occulta	hidden angelshark		
Squatina squatina	angelshark		
Stegostoma fasciatum	zebra shark		
Taeniura lymma	bluespotted ribbontail ray		
Taeniura meyeni	blotched fantail ray		
Torpedo californica	Pacific electric ray		
Torpedo marmorata	marbled electric ray		
Torpedo nobiliana	electric ray		
Torpedo panthera	panther electric ray		
Torpedo torpedo	common torpedo		
Triaenodon obesus	whitetip reef shark		
Triakis acutipinna	sharpfin houndshark		
Triakis megalopterus	sharptooth houndshark		
Triakis scyllium	banded houndshark		
Triakis semifasciata	leopard shark		
Trygonnorhina sp. A (undescribed)	eastern fiddler ray		
Trygonorrhina fasciata	southern fiddler ray		
Urobatis halleri	round stingray		
Urobatis jamaicensis	yellow stingray		
Urogymnus asperrimus	porcupine ray		
Urogymnus ukpam	thorny freshwater stingray		
Urolophus aurantiacus	sepia stingray		
Urolophus halleri	Haller's round ray		
Urolophus sufflavus	yellowback stingaree		
Zapteryx exasperata	banded guitarfish		

Appendix 2. Elasmobranchs cited in Elasmobranch Husbandry Manual (sorted by common name).

Common Name	Scientific Name	Common Name	Common Name
Aleutian skate	*Bathyraja aleutica*	cape elephantfish	*Callorhinchus capensis*
angelshark	*Squatina squatina*	Caribbean reef shark	*Carcharhinus perezi*
angular angelshark	*Squatina guggenheim*	Caribbean sharpnose shark	*Rhizoprionodon porosus*
angular roughshark	*Oxynotus centrina*	ceja stingray	*Paratrygon aiereba*
Arabian carpet shark	*Chiloscyllium arabicum*	chain dogfish	*Scyliorhinus retifer*
Argentine angelshark	*Squatina argentina*	Chola guitarfish	*Rhina percellens*
Atlantic guitarfish	*Rhinobatos lentiginosus*	chupare stingray	*Himantura schmardae*
Atlantic sharpnose shark	*Rhizoprionodon terraenovae*	clearnose skate	*Raja eglanteria*
Atllantic stingray	*Dasyatis sabina*	cloudy cat shark	*Scyliorhinus torazame*
Australian angelshark	*Squatina australis*	cockfish	*Callorhinchus callorynchus*
Australian blacktip shark	*Carcharhinus tilstoni*	common eagle ray	*Myliobatis aquila*
Australian bull ray	*Myliobatis australis*	common guitarfish	*Rhinobatos rhinobatos*
Australian cownose ray	*Rhinoptera neglecta*	common sawfish	*Pristis pristis*
Australian marbled cat shark	*Atelomycterus macleayi*	common stingray	*Dasyatis pastinaca*
Australian numbfish	*Hypnos monopterygium*	common torpedo	*Torpedo torpedo*
Australian sharpnose shark	*Rhizoprionodon taylori*	cookiecutter shark	*Isistius brasiliensis*
Australian spotted catshark	*Asymbolus analis*	copper shark	*Carcharhinus brachyurus*
Australian swell shark	*Cephaloscyllium laticeps*	coral cat shark	*Atelomycterus marmoratus*
banded eagle ray	*Aetomylaeus niehofii*	cownose ray	*Rhinoptera bonasus*
banded guitarfish	*Zapteryx exasperata*	cowtail stingray	*Pastinachus sephen*
banded houndshark	*Triakis scyllium*	crested bullhead shark	*Heterodontus galeatus*
barbeled houndshark	*Leptocharias smithii*	crocodile shark	*Pseudocarcharias kamoharai*
barndoor skate	*Dipturus laevis*	Cuckoo ray	*Leucoraja naevus*
basking shark	*Cetorhinus maximus*	dark shy shark	*Haploblepharus pictus*
bat eagle ray	*Myliobatis californica*	deepsea skate	*Bathyraja abyssicola*
big skate	*Raja binoculata*	devil fish	*Mobula mobular*
bigeye sand tiger shark	*Odontaspis noronhai*	devil ray	*Mobula diabola*
bigeye sixgill shark	*Hexanchus nakamurai*	dumb gulper shark	*Centrophorus harrissoni*
bigeye thresher shark	*Alopias superciliosus*	dusky shark	*Carcharhinus obscurus*
bignose shark	*Carcharhinus altimus*	dusky smooth-hound	*Mustelus canis*
bigtooth river stingray	*Potamotrygon henlei*	dwarf sawfish	*Pristis clavata*
black dogfish	*Centroscyllium fabricii*	eastern fiddler ray	*Trygonnorhina sp. A (undescribed)*
blackbelly lantern shark	*Etmopterus lucifer*	eastern shovelnose ray	*Aptychotrema rostrata*
blackchin guitarfish	*Rhinobatos cemiculus*	electric ray	*Narcine entemedor*
blackmouth catshark	*Galeus melastomus*	electric ray	*Torpedo nobiliana*
blacknose shark	*Carcharhinus acronotus*	epaulette shark	*Hemiscyllium ocellatum*
blacktip reef shark	*Carcharhinus melanopterus*	estuary stingray	*Dasyatis fluviorum*
blacktip shark	*Carcharhinus limbatus*	false cat shark	*Pseudotriakis microdon*
blacktip topeshark	*Hypogaleus hyugaensis*	filetail cat shark	*Parmaturus xaniurus*
Bleeker's whipray	*Himantura bleekeri*	finetooth shark	*Carcharhinus isodon*
blind shark	*Brachaelurus waddi*	flapnose houndshark	*Scylliogaleus quecketti*
blonde ray	*Raja brachyura*	Florida smooth-hound	*Mustelus norrisi*
blotched fantail ray	*Taeniura meyeni*	freshwater stingray	*Himantura chaophraya*
blue shark	*Prionace glauca*	frilled shark	*Chlamydoselachus anguineus*
blue stingray	*Dasyatis chrysonata*	Galapagos shark	*Carcharhinus galapagensis*
bluegray carpetshark	*Heteroscyllium colcloughi*	Ganges shark	*Glyphis gangeticus*
bluespotted ribbontail ray	*Taeniura lymma*	Ganges stingray	*Himantura fluviatilis*
bluntnose sixgill shark	*Hexanchus griseus*	ghost shark	*Callorhinchus milii*
bluntnose stingray	*Dasyatis say*	giant guitarfish	*Rhynchobatus djiddensis*
bonnethead	*Sphyrna tiburo*	giant manta	*Manta birostris*
Borneo shark	*Carcharhinus borneensis*	giant shovelnose ray	*Rhinobatos typus*
bowmouth guitarfish	*Rhina ancyclostoma*	graceful shark	*Carcharhinus amblyrhynchoides*
Brazilian electric ray	*Narcine brasiliensis*	gray bamboo shark	*Chiloscyllium griseum*
Brazilian guitarfish	*Rhinobatos horkeli*	gray reef shark	*Carcharhinus amblyrhynchos*
broadnose sevengill shark	*Notorynchus cepedianus*	great hammerhead	*Sphyrna mokarran*
brown cat shark	*Apristurus brunneus*	Greenland shark	*Somniosus microcephalus*
brown ray	*Raja miraletus*	grey smooth-hound	*Mustelus californicus*
brown shyshark	*Haploblepharus fuscus*	gulper shark	*Centrophorus granulosus*
brown smooth-hound	*Mustelus henlei*	gummy shark	*Mustelus antarcticus*
brown stingray	*Dasyatis lata*	Haller's round ray	*Urolophus halleri*
brownbanded bamboo shark	*Chiloscyllium punctatum*	hardnose shark	*Carcharhinus macloti*
bull shark	*Carcharhinus leucas*	hidden angelshark	*Squatina occulta*
bullnose eagle ray	*Myliobatis freminvillii*	honeycomb stingray	*Himantura uarnak*
California butterfly ray	*Gymnura marmorata*	horn shark	*Heterodontus francisci*
California ray	*Raja inornata*	Izu cat shark	*Scyliorhinus tokubee*

Appendix 2 (continued). Elasmobranchs cited in Elasmobranch Husbandry Manual (sorted by common name).

Common Name	Scientific Name	Common Name	Common Name
Izu stingray	*Dasyatis izuensis*	ringstraked guitarfish	*Rhinobatos hynnicephalus*
Japanese angel shark	*Squatina japonica*	rosette river stingray	*Potamotrygon schroederi*
Japanese bullhead shark	*Heterodontus japonicus*	rough skate	*Dipturus nasutus*
Japanese butterfly ray	*Gymnura japonica*	roughtail stingray	*Dasyatis centroura*
Japanese swell shark	*Cephaloscyllium umbratile*	round stingray	*Urobatis halleri*
Japanese wobbegong	*Orectolobus japonicus*	Roundel skate	*Raja texana*
Javanese cownose ray	*Rhinoptera javanica*	salmon shark	*Lamna ditropis*
kitefin shark	*Dalatias licha*	sand devil	*Squatina dumeril*
knifetooth sawfish	*Anoxypristis cuspidata*	sand tiger shark	*Carcharias taurus*
largespot river stingray	*Potamotrygon falkneri*	sandbar shark	*Carcharhinus plumbeus*
largetooth sawfish	*Pristis microdon*	sandpaper skate	*Bathyraja interrupta*
large-tooth sawfish	*Pristis perotteti*	scalloped hammerhead	*Sphyrna lewini*
lemon shark	*Negaprion brevirostris*	scaly whipray	*Himantura imbricata*
leopard cat shark	*Poroderma pantherinum*	sepia stingray	*Urolophus aurantiacus*
leopard shark	*Triakis semifasciata*	sharpfin houndshark	*Triakis acutipinna*
leopard whipray	*Himantura undulata*	sharpnose guitarfish	*Rhinobatos granulatus*
lesser sandshark	*Rhinobatos annulatus*	sharpnose stingray	*Himantura gerrardi*
little gulper shark	*Centrophorus uyato*	sharptooth houndshark	*Triakis megalopterus*
little skate	*Leucoraja erinacea*	shortfin mako	*Isurus oxyrinchus*
longcomb sawfish	*Pristis zijsron*	shortnose spurdog	*Squalus megalops*
longfin mako	*Isurus paucus*	short-snouted shovelnose ray	*Aptychotrema bougainvillii*
longnose sawshark	*Pristiophorus cirratus*	short-tail stingray	*Dasyatis brevicaudata*
longnose skate	*Raja rhina*	short-tailed nurse shark	*Ginglymostoma brevicaudatum*
longnosed skate	*Dipturus oxyrinchus*	short-tailed river stingray	*Potamotrygon brachyura*
long-tailed river stingray	*Plesiotrygon iwamae*	shovelnose guitarfish	*Rhinobatos productus*
Magdalena river stingray	*Potamotrygon magdalenae*	sicklefin lemon shark	*Negaprion acutidens*
marbled electric ray	*Torpedo marmorata*	sicklefin weasel shark	*Hemigaleus microstoma*
marbled stingray	*Dasyatis marmorata*	silky shark	*Carcharhinus falciformis*
marbled whipray	*Himantura oxyrhynchus*	silver chimaera	*Chimaera phantasma*
Maugaen skate	*Raja sp. L*	skate	*Dipturus batis*
megamouth shark	*Megachasma pelagios*	slender bambooshark	*Chiloscyllium indicum*
Mekong stingray	*Dasyatis laosensis*	Slender weasel shark	*Paragaleus randalli*
Mexican horn shark	*Heterodontus mexicanus*	smalleye hammerhead	*Sphyrna tudes*
milk shark	*Rhizoprionodon acutes*	smalleye stingray	*Dasyatis microps*
Munk's devil ray	*Mobula munkiana*	small-eyed ray	*Raja microocellata*
narrowmouthed catshark	*Schroederichthys bivius*	smallspotted catshark	*Scyliorhinus canicula*
night shark	*Carcharhinus signatus*	smalltail shark	*Carcharhinus porosus*
northern river shark	*Glyphis sp. (species C)*	smalltooth sand tiger shark	*Odontaspis ferox*
nurse shark	*Ginglymostoma cirratum*	smalltooth sawfish	*Pristis pectinata*
nursehound	*Scyliorhinus stellaris*	smalltooth shark	*Carcharhinus leiodon*
oceanic whitetip shark	*Carcharhinus longimanus*	smooth back river stingray	*Potamotrygon orbignyi*
ocellate river stingray	*Potamotrygon motoro*	smooth butterfly ray	*Gymnura micrura*
Ogilby's ghostshark	*Hydrolagus ogilbyi*	smooth freshwater stingray	*Dasyatis garouaensis*
ornate wobbegong	*Orectolobus ornatus*	smooth hammerhead	*Sphyrna zygaena*
Pacific angel shark	*Squatina californica*	smooth skate	*Malacoraja senta*
Pacific electric ray	*Torpedo californica*	smooth-hound	*Mustelus mustelus*
Pacific sharpnose shark	*Rhizoprionodon longurio*	southern fiddler ray	*Trygonorrhina fasciata*
Pacific sleeper shark	*Somniosus pacificus*	southern stingray	*Dasyatis americana*
panther electric ray	*Torpedo panthera*	spadenose catshark	*Scoliodon laticaudus*
Papauan epaulette shark	*Hemiscyllium hallstromi*	speartooth shark	*Glyphis glyphis (species A)*
pelagic stingray	*Pteroplatytrygon violacea*	spinner shark	*Carcharhinus brevipinna*
pigeye shark	*Carcharhinus amboinensis*	spiny butterfly ray	*Gymnura altavela*
pink whipray	*Himantura fai*	spiny dogfish	*Squalus acanthias*
pitted stingray	*Dasyatis matsubarai*	spiny rasp skate	*Okamejei kenojei*
Pondicherry shark	*Carcharhinus hemiodon*	spottail shark	*Carcharhinus sorrah*
porbeagle	*Lamna nasus*	spotted eagle ray	*Aetobatus narinari*
porcupine ray	*Urogymnus asperrimus*	spotted estuary smooth-hound	*Mustelus lenticulatus*
porcupine river stingray	*Potamotrygon histrix*	spotted freshwater ray	*Potamotrygon reticulatus*
Port Jackson shark	*Heterodontus portusjacksoni*	spotted ratfish	*Hydrolagus colliei*
prickly shark	*Echinorhinus cookei*	spotted skate	*Raja montagui*
puffadder shyshark	*Haploblepharus edwardsii*	spotted wobbegong	*Orectolobus maculatus*
rabbit fish	*Chimaera monstrosa*	starry ray	*Raja asterias*
red stingray	*Dasyatis akajei*	starry skate	*Raja stellulata*
red-blotched river stingray	*Potamotrygon ocellata*	starry smooth-hound	*Mustelus asterias*
redspotted catshark	*Schroederichthys chilensis*	star-spotted smooth-hound	*Mustelus manazo*

Appendix 2 (continued). Elasmobranchs cited in Elasmobranch Husbandry Manual (sorted by common name).

Common Name	Scientific Name	Common Name	Common Name
swell shark	*Cephaloscyllium ventriosum*		
tasseled wobbegong	*Eucrossorhinus dasypogon*		
tawny nurse shark	*Nebrius ferrugineus*		
thintail thresher shark	*Alopias vulpinus*		
thornback guitarfish	*Platyrhinoidis triseriata*		
thornback ray	*Raja clavata*		
thorny freshwater stingray	*Urogymnus ukpam*		
thorny skate	*Amblyraja radiata*		
tiger shark	*Galeocerdo cuvier*		
tope shark	*Galeorhinus galeus*		
undulate ray	*Raja undulata*		
velvet belly	*Etmopterus spinax*		
whale shark	*Rhincodon typus*		
whiptail stingray	*Dasyatis brevis*		
whiskery shark	*Furgaleus macki*		
white shark	*Carcharodon carcharias*		
white-blotched river stingray	*Potamotrygon leopoldi*		
white-blotched stingray	*Paratrygon leopoldi*		
whitecheek	*Carcharhinus dussumieri*		
whitefin topeshark	*Hemitriakis leucoperiptera*		
white-rimmed whipray	*Himantura signifer*		
whitespotted bamboo shark	*Chiloscyllium plagiosum*		
whitetip reef shark	*Triaenodon obesus*		
winter skate	*Leucoraja ocellata*		
yellow stingray	*Urobatis jamaicensis*		
yellowback stingaree	*Urolophus sufflavus*		
yellowspotted catshark	*Scyliorhinus capensis*		
zebra shark	*Stegostoma fasciatum*		

Appendix 3. Checklist of elasmobranchs (sorted by scientific name).

Scientific name	Common name	Scientific name	Common name
Aculeola nigra	hooktooth dogfish	*Atelomycterus fasciatus*	banded sand catshark
Aetobatus flagellum	plain eagle ray	*Atelomycterus macleayi*	Australian marbled catshark
Aetobatus narinari	spotted eagle ray	*Atelomycterus marmoratus*	coral catshark
Aetobatus ocellatus		*Atlantoraja castelnaui*	
Aetomylaeus maculatus	mottled eagle ray	*Atlantoraja cyclophora*	
Aetomylaeus milvus		*Atlantoraja platana*	
Aetomylaeus nichofii	banded eagle ray	*Aulohalaelurus kanakorum*	New Caledonia catshark
Aetomylaeus vespertilio	ornate eagle ray	*Aulohalaelurus labiosus*	Australian blackspotted catshark
Alopias pelagicus	pelagic thresher	*Bathyraja abyssicola*	deepsea skate
Alopias superciliosus	bigeye thresher	*Bathyraja aguja*	
Alopias vulpinus	thintail thresher	*Bathyraja albomaculata*	
Amblyraja badia	broad skate	*Bathyraja aleutica*	Aleutian skate
Amblyraja doellojuradoi		*Bathyraja andriashevi*	
Amblyraja frerichsi		*Bathyraja bergi*	
Amblyraja georgiana		*Bathyraja brachyurops*	
Amblyraja hyperborea	Arctic skate	*Bathyraja caeluronigricans*	
Amblyraja jenseni	Jensen's skate	*Bathyraja diplotaenia*	
Amblyraja radiata	thorny skate	*Bathyraja eatonii*	
Amblyraja reversa		*Bathyraja fedorovi*	
Amblyraja robertsi	bigmouth skate	*Bathyraja griseocauda*	
Amblyraja taaf		*Bathyraja hesperafricana*	
Anacanthobatis americanus		*Bathyraja interrupta*	sandpaper skate
Anacanthobatis borneensis		*Bathyraja irrasa*	
Anacanthobatis donghaiensis		*Bathyraja isotrachys*	
Anacanthobatis folirostris		*Bathyraja lindbergi*	
Anacanthobatis longirostris		*Bathyraja longicauda*	
Anacanthobatis marmoratus	spotted legskate	*Bathyraja maccaini*	
Anacanthobatis melanosoma		*Bathyraja macloviana*	
Anacanthobatis nanhaiensis		*Bathyraja maculata*	
Anacanthobatis ori	black legskate	*Bathyraja magellanica*	
Anacanthobatis stenosomus		*Bathyraja matsubarai*	
Anoxypristis cuspidata	knifetooth sawfish	*Bathyraja meridionalis*	
Apristurus acanutus		*Bathyraja minispinosa*	whitebrow skate
Apristurus aphyodes		*Bathyraja multispinis*	
Apristurus atlanticus	Atlantic ghost catshark	*Bathyraja murrayi*	
Apristurus brunneus	brown catshark	*Bathyraja notoroensis*	
Apristurus canutus	hoary catshark	*Bathyraja pallida*	pale ray
Apristurus gibbosus		*Bathyraja papilionifera*	
Apristurus herklotsi	longfin catshark	*Bathyraja parmifera*	Alaska skate
Apristurus indicus	smallbelly catshark	*Bathyraja peruana*	
Apristurus investigatoris	broadnose catshark	*Bathyraja pseudoisotrachys*	bottom skate
Apristurus japonicus	Japanese catshark	*Bathyraja richardsoni*	richardson's ray
Apristurus kampae	longnose catshark	*Bathyraja scaphiops*	
Apristurus laurussonii	Iceland catshark	*Bathyraja schroederi*	
Apristurus longicephalus	longhead catshark	*Bathyraja shuntovi*	longnose deepsea skate
Apristurus macrorhynchus	flathead catshark	*Bathyraja simoterus*	
Apristurus macrostomus		*Bathyraja smirnovi*	
Apristurus manis	ghost catshark	*Bathyraja smithii*	African softnose skate
Apristurus microps	smalleye catshark	*Bathyraja spinicauda*	spinetail ray
Apristurus micropterygeus		*Bathyraja spinosissima*	white skate
Apristurus nasutus	largenose catshark	*Bathyraja trachouros*	
Apristurus parvipinnis	smallfin catshark	*Bathyraja trachura*	roughtail skate
Apristurus platyrhynchus	spatulasnout catshark	*Bathyraja tzinovskii*	
Apristurus profundorum	deepwater catshark	*Bathyraja violacea*	Okhotsk skate
Apristurus riveri	broadgill catshark	*Benthobatis marcida*	blind torpedo
Apristurus saldanha	Saldanha catshark	*Benthobatis moresbyi*	
Apristurus sibogae	pale catshark	*Brachaelurus waddi*	blind shark
Apristurus sinensis	South China catshark	*Breviraja claramaculata*	
Apristurus spongiceps	spongehead catshark	*Breviraja colesi*	
Apristurus stenseni	Panama ghost catshark	*Breviraja marklei*	
Apristurus verweyi	Borneo catshark	*Breviraja mouldi*	
Aptychotrema bougainvillii	short-snouted shovelnose ray	*Breviraja nigriventralis*	
Aptychotrema rostrata	eastern shovelnose ray	*Breviraja spinosa*	
Aptychotrema vincentiana	western shovelnose ray	*Callorhinchus callorynchus*	plownose chimaera (unesco)
Arhynchobatis asperrimus	longtail skate	*Callorhinchus capensis*	Cape elephantfish
Asymbolus analis	Australian spotted catshark	*Callorhinchus milii*	ghost shark
Asymbolus vincenti	gulf catshark	*Carcharhinus acronotus*	blacknose shark
		Carcharhinus albimarginatus	silvertip shark

Appendix 3 (continued). Checklist of elasmobranchs (sorted by scientific name).

Scientific name	Common name	Scientific name	Common name
Carcharhinus altimus	bignose shark	*Chiloscyllium confusum*	
Carcharhinus amblyrhynchoides	graceful shark	*Chiloscyllium griseum*	grey bambooshark
Carcharhinus amblyrhynchos	grey reef shark	*Chiloscyllium hasselti*	Hasselt's bambooshark
Carcharhinus amboinensis	pigeye shark	*Chiloscyllium indicum*	slender bambooshark
Carcharhinus borneensis	Borneo shark	*Chiloscyllium plagiosum*	whitespotted bambooshark
Carcharhinus brachyurus	copper shark	*Chiloscyllium punctatum*	brownbanded bambooshark
Carcharhinus brevipinna	spinner shark	*Chimaera cubana*	chimaera
Carcharhinus cautus	nervous shark	*Chimaera jordani*	
Carcharhinus dussumieri	whitecheek shark	*Chimaera monstrosa*	rabbit fish
Carcharhinus falciformis	silky shark	*Chimaera owstoni*	
Carcharhinus fitzroyensis	creek whaler	*Chimaera panthera*	
Carcharhinus galapagensis	Galapagos shark	*Chimaera phantasma*	silver chimaera
Carcharhinus hemiodon	pondicherry shark	*Chlamydoselachus anguineus*	frilled shark
Carcharhinus isodon	finetooth shark	*Cirrhigaleus asper*	roughskin spurdog
Carcharhinus leiodon	smoothtooth shark (unesco)	*Cirrhigaleus barbifer*	mandarin dogfish
Carcharhinus leucas	bull shark	*Cirrhoscyllium expolitum*	barbelthroat carpetshark
Carcharhinus limbatus	blacktip shark	*Cirrhoscyllium formosanum*	Taiwan saddled carpetshark
Carcharhinus longimanus	oceanic whitetip shark	*Cirrhoscyllium japonicum*	saddle carpetshark
Carcharhinus macloti	hardnose shark	*Crassinarke dormitor*	
Carcharhinus melanopterus	blacktip reef shark	*Cruriraja andamanica*	
Carcharhinus obscurus	dusky shark	*Cruriraja atlantis*	Cuban legskate
Carcharhinus perezi	Caribbean reef shark	*Cruriraja cadenati*	
Carcharhinus plumbeus	sandbar shark	*Cruriraja durbanensis*	smoothnose legskate
Carcharhinus porosus	smalltail shark	*Cruriraja parcomaculata*	roughnose legskate
Carcharhinus sealei	blackspot shark	*Cruriraja poeyi*	
Carcharhinus signatus	night shark	*Cruriraja rugosa*	
Carcharhinus sorrah	spottail shark	*Cruriraja triangularis*	triangular legskate
Carcharhinus tilstoni	Australian blacktip shark	*Ctenacis fehlmanni*	harlequin catshark
Carcharias taurus	sand tiger shark	*Dactylobatus armatus*	
Carcharias tricuspidatus	Indian sand tiger	*Dactylobatus clarki*	
Carcharodon carcharias	great white shark	*Dalatias licha*	kitefin shark
Centrophorus acus	needle dogfish	*Dasyatis acutirostra*	
Centrophorus atromarginatus	blackfin gulper shark	*Dasyatis akajei*	red stingray
Centrophorus granulosus	gulper shark	*Dasyatis americana*	southern stingray
Centrophorus harrissoni	dumb gulper shark	*Dasyatis annotata*	plain maskray
Centrophorus isodon		*Dasyatis bennetti*	Bennett's stingray
Centrophorus lusitanicus	lowfin gulper shark	*Dasyatis brevicaudata*	short-tail stingray
Centrophorus moluccensis	smallfin gulper shark	*Dasyatis brevis*	whiptail stingray
Centrophorus niaukang	Taiwan gulper shark	*Dasyatis centroura*	roughtail stingray
Centrophorus squamosus	leafscale gulper shark	*Dasyatis chrysonota*	
Centrophorus tessellatus	mosaic gulper shark	*Dasyatis dipterura*	diamond stingray
Centrophorus uyato	little gulper shark	*Dasyatis fluviorum*	estuary stingray
Centroscyllium excelsum		*Dasyatis garouaensis*	
Centroscyllium fabricii	black dogfish	*Dasyatis geijskesi*	sharpsnout stingray
Centroscyllium granulatum	granular dogfish	*Dasyatis giganteus*	
Centroscyllium kamoharai	bareskin dogfish	*Dasyatis guttata*	longnose stingray
Centroscyllium nigrum	combtooth dogfish	*Dasyatis izuensis*	
Centroscyllium ornatum	ornate dogfish	*Dasyatis kuhlii*	bluespotted stingray
Centroscyllium ritteri	whitefin dogfish	*Dasyatis laevigatus*	
Centroscymnus coelolepis	Portuguese dogfish	*Dasyatis laosensis*	Mekong stingray
Centroscymnus crepidater	longnose velvet dogfish	*Dasyatis lata*	brown stingray
Centroscymnus cryptacanthus	shortnose velvet dogfish	*Dasyatis leylandi*	painted maskray
Centroscymnus owstoni	roughskin dogfish	*Dasyatis longus*	longtail stingray
Centroscymnus plunketi	plunket shark	*Dasyatis margarita*	daisy stingray
Cephaloscyllium fasciatum	reticulated swellshark	*Dasyatis margaritella*	
Cephaloscyllium isabellum	draughtsboard shark	*Dasyatis marmorata*	marbled stingray
Cephaloscyllium laticeps	Australian swellshark	*Dasyatis matsubarai*	
Cephaloscyllium nascione	whitefinned swellshark	*Dasyatis microphthalmus*	
Cephaloscyllium silasi	Indian swellshark	*Dasyatis microps*	smalleye stingray
Cephaloscyllium sufflans	balloon shark	*Dasyatis navarrae*	
Cephaloscyllium umbratile	blotchy swell shark	*Dasyatis pastinaca*	common stingray
Cephaloscyllium ventriosum	swellshark	*Dasyatis rudis*	
Cephalurus cephalus	lollipop catshark	*Dasyatis sabina*	Atlantic stingray
Cetorhinus maximus	basking shark	*Dasyatis say*	bluntnose stingray
Chaenogaleus macrostoma	hooktooth shark	*Dasyatis sinensis*	
Chiloscyllium arabicum	Arabian carpetshark	*Dasyatis thetidis*	thorntail stingray
Chiloscyllium burmensis		*Dasyatis tortonesei*	tortonese's stingray
Chiloscyllium caerulopunctatum	bluespotted bambooshark	*Dasyatis ukpam*	

Appendix 3 (continued). Checklist of elasmobranchs (sorted by scientific name).

Scientific name	Common name	Scientific name	Common name
Dasyatis ushiei		*Etmopterus unicolor*	brown lanternshark
Dasyatis zugei	pale-edged stingray	*Etmopterus villosus*	Hawaiian lanternshark
Deania calcea	birdbeak dogfish	*Etmopterus virens*	green lanternshark
Deania histricosa	rough longnose dogfish	*Eucrossorhinus dasypogon*	tasselled wobbegong
Deania profundorum	arrowhead dogfish	*Euprotomicroides zantedeschia*	taillight shark
Deania quadrispinosa	longsnout dogfish	*Euprotomicrus bispinatus*	pygmy shark
Diplobatis colombiensis		*Eusphyra blochii*	winghead shark
Diplobatis guamachensis		*Fenestraja atripinna*	
Diplobatis ommata	ocellated electric ray	*Fenestraja cubensis*	
Diplobatis picta		*Fenestraja ishiyamai*	
Dipturus batis	skate	*Fenestraja maceachrani*	
Dipturus bullisi	bullis skate	*Fenestraja mamillidens*	
Dipturus campbelli	blackspot skate	*Fenestraja plutonia*	
Dipturus chilensis	barn-door skate	*Fenestraja sibogae*	
Dipturus crosnieri		*Fenestraja sinusmexicanus*	
Dipturus doutrei	violet skate	*Furgaleus macki*	whiskery shark
Dipturus ecuadoriensis		*Galeocerdo cuvier*	tiger shark
Dipturus garricki		*Galeorhinus galeus*	tope shark
Dipturus gigas		*Galeus antillensis*	
Dipturus gudgeri	greenback skate	*Galeus arae*	roughtail catshark
Dipturus innominatus		*Galeus atlanticus*	Atlantic sawtail catshark
Dipturus johannisdavisi		*Galeus boardmani*	Australian sawtail catshark
Dipturus kwangtungensis	Kwangtung skate	*Galeus cadenati*	
Dipturus laevis	barndoor skate	*Galeus eastmani*	gecko catshark
Dipturus lanceorostratus	rattail skate	*Galeus gracilis*	slender sawtail catshark
Dipturus leptocauda		*Galeus longirostris*	
Dipturus linteus	sailray	*Galeus melastomus*	blackmouth catshark
Dipturus macrocauda		*Galeus murinus*	mouse catshark
Dipturus nasutus	rough skate	*Galeus nipponensis*	broadfin sawtail catshark
Dipturus nidarosiensis	Norwegian skate	*Galeus piperatus*	peppered catshark
Dipturus olseni	spreadfin skate	*Galeus polli*	African sawtail catshark
Dipturus oregoni		*Galeus sauteri*	blacktip sawtail catshark
Dipturus oxyrinchus	longnosed skate	*Galeus schultzi*	dwarf sawtail catshark
Dipturus pullopunctatus	slime skate	*Galeus springeri*	
Dipturus springeri	roughbelly skate	*Ginglymostoma brevicaudatum*	short-tail nurse shark
Dipturus stenorhynchus	prow-nose skate	*Ginglymostoma cirratum*	nurse shark
Dipturus teevani	prickly brown ray	*Glyphis gangeticus*	Ganges shark
Dipturus tengu		*Glyphis glyphis*	speartooth shark
Dipturus trachyderma		*Gogolia filewoodi*	sailback houndshark
Discopyge tschudii		*Gollum attenuatus*	slender smooth-hound
Echinorhinus brucus	bramble shark	*Gurgesiella atlantica*	
Echinorhinus cookei	prickly shark	*Gurgesiella dorsalifera*	
Eridacnis barbouri	Cuban ribbontail catshark	*Gurgesiella furvescens*	southern false skate
Eridacnis radcliffei	pygmy ribbontail catshark	*Gymnura altavela*	spiny butterfly ray
Eridacnis sinuans	African ribbontail catshark	*Gymnura australis*	Australian butterfly ray
Etmopterus baxteri	New Zealand lanternshark	*Gymnura bimaculata*	
Etmopterus bigelowi		*Gymnura crebripunctata*	longsnout butterfly ray
Etmopterus brachyurus	shorttail lanternshark	*Gymnura crooki*	
Etmopterus bullisi	lined lanternshark	*Gymnura hirundo*	
Etmopterus carteri		*Gymnura japonica*	Japanese butterflyray
Etmopterus compagnoi		*Gymnura marmorata*	California butterfly ray
Etmopterus decacuspidatus	combtooth lanternshark	*Gymnura micrura*	smooth butterfly ray
Etmopterus gracilispinis	broadbanded lanternshark	*Gymnura natalensis*	backwater butterfly ray
Etmopterus granulosus	southern lanternshark	*Gymnura poecilura*	longtail butterfly ray
Etmopterus hillianus	Caribbean lanternshark	*Gymnura tentaculata*	
Etmopterus litvinovi		*Gymnura zonura*	zonetail butterfly ray
Etmopterus lucifer	blackbelly lanternshark	*Halaelurus alcocki*	Arabian catshark
Etmopterus molleri	slendertail lanternshark	*Halaelurus boesemani*	speckled catshark
Etmopterus perryi		*Halaelurus buergeri*	blackspotted catshark
Etmopterus polli	African lanternshark	*Halaelurus canescens*	dusky catshark
Etmopterus princeps	great lanternshark	*Halaelurus clevai*	
Etmopterus pusillus	smooth lanternshark	*Halaelurus dawsoni*	New Zealand catshark
Etmopterus pycnolepis		*Halaelurus hispidus*	bristly catshark
Etmopterus robinsi		*Halaelurus immaculatus*	spotless catshark
Etmopterus schultzi	fringfin lanternshark	*Halaelurus lineatus*	lined catshark
Etmopterus sentosus	thorny lanternshark	*Halaelurus lutarius*	mud catshark
Etmopterus spinax	velvet belly	*Halaelurus natalensis*	tiger catshark
Etmopterus splendidus	splendid lanternshark	*Halaelurus quagga*	quagga catshark

Appendix 3 (continued). Checklist of elasmobranchs (sorted by scientific name).

Scientific name	Common name	Scientific name	Common name
Haploblepharus edwardsii	puffadder shyshark	*Hydrolagus mitsukurii*	spookfish
Haploblepharus fuscus	brown shyshark	*Hydrolagus novaezealandiae*	dark ghost shark
Haploblepharus pictus	dark shyshark	*Hydrolagus ogilbyi*	Ogilby's ghostshark
Harriotta haeckeli	smallspine spookfish	*Hydrolagus pallidus*	
Harriotta raleighana	narrownose chimaera	*Hydrolagus purpurescens*	purple chimaera
Hemigaleus microstoma	sicklefin weasel shark	*Hypnos monopterygium*	Australian numbfish
Hemipristis elongata	snaggletooth shark	*Hypogaleus hyugaensis*	blacktip tope
Hemiscyllium freycineti	Indonesia speckled carpetshark	*Irolita waitii*	southern round skate
Hemiscyllium hallstromi	Papuan epaulette shark	*Isistius brasiliensis*	cookiecutter shark
Hemiscyllium ocellatum	epaulette shark	*Isistius plutodus*	largetooth cookiecutter shark
Hemiscyllium strahani	hooded carpetshark	*Isogomphodon oxyrhynchus*	daggernose shark
Hemiscyllium trispeculare	speckled carpetshark	*Isurus oxyrinchus*	shortfin mako
Hemitriakis abdita		*Isurus paucus*	longfin mako
Hemitriakis falcata		*Lago garricki*	longnose houndshark
Hemitriakis japanica	Japanese topeshark	*Lago omanensis*	bigeye houndshark
Hemitriakis leucoperiptera	whitefin topeshark	*Lamiopsis temmincki*	broadfin shark
Heptranchias perlo	sharpnose sevengill shark	*Lamna ditropis*	salmon shark
Heterodontus francisci	horn shark	*Lamna nasus*	porbeagle
Heterodontus galeatus	crested bullhead shark	*Leptocharias smithii*	barbeled houndshark
Heterodontus japonicus	Japanese bullhead shark	*Leucoraja circularis*	sandy ray
Heterodontus mexicanus	Mexican hornshark	*Leucoraja compagnoi*	
Heterodontus portusjacksoni	Port Jackson shark	*Leucoraja erinacea*	little skate
Heterodontus quoyi	Galapagos bullhead shark	*Leucoraja fullonica*	shagreen ray
Heterodontus ramalheira	whitespotted bullhead shark	*Leucoraja garmani*	freckled skate
Heterodontus zebra	zebra bullhead shark	*Leucoraja lentiginosa*	
Heteronarce bentuviai		*Leucoraja leucosticta*	
Heteronarce garmani	natal electric ray	*Leucoraja melitensis*	Maltese ray
Heteronarce mollis		*Leucoraja naevus*	cuckoo ray
Heteronarce prabhui		*Leucoraja ocellata*	winter skate
Heteroscyllium colcloughi	bluegray carpetshark	*Leucoraja wallacei*	yellowspotted skate
Heteroscymnoides marleyi	longnose pygmy shark	*Leucoraja yucatanensis*	
Hexanchus griseus	bluntnose sixgill shark	*Loxodon macrorhinus*	sliteye shark
Hexanchus nakamurai	bigeyed sixgill shark	*Malacoraja kreffti*	Krefft's ray
Hexatrygon bickelli	sixgill stingray	*Malacoraja senta*	smooth skate
Hexatrygon longirostra		*Malacoraja spinacidermis*	roughskin skate
Hexatrygon taiwanensis		*Manta birostris*	giant manta
Hexatrygon yangi		*Manta ehrenbergii*	
Himantura alcockii		*Manta raya*	
Himantura bleekeri	Bleeker's whipray	*Megachasma pelagios*	megamouth shark
Himantura chaophraya	freshwater whipray	*Miroscyllium sheikoi*	
Himantura draco	dragon stingray	*Mitsukurina owstoni*	goblin shark
Himantura fai	pink whipray	*Mobula coilloti*	
Himantura fluviatilis		*Mobula diabola*	devil ray
Himantura gerrardi	sharpnose stingray	*Mobula eregoodootenkee*	pygmy devil ray
Himantura granulata	mangrove whipray	*Mobula hypostoma*	lesser devil ray
Himantura imbricata	scaly whipray	*Mobula japanica*	spinetail mobula
Himantura jenkinsii	pointed-nose stingray	*Mobula kuhlii*	shortfin devilray
Himantura krempfi		*Mobula mobular*	devil fish
Himantura marginata	blackedge whipray	*Mobula munkiana*	munk's devil ray
Himantura oxyrhynchus	marbled whipray	*Mobula rochebrunei*	
Himantura pacifica	Pacific chupare	*Mobula tarapacana*	Chilean devil ray
Himantura schmardae	chupare stingray	*Mobula thurstoni*	smoothtail mobula
Himantura signifer	white-rimmed stingray	*Mollisquama parini*	
Himantura toshi	black-spotted whipray	*Mustelus antarcticus*	gummy shark
Himantura uarnak	honeycomb stingray	*Mustelus asterias*	starry smooth-hound
Himantura undulata	leopard whipray	*Mustelus californicus*	grey smooth-hound
Himantura walga	dwarf whipray	*Mustelus canis*	dusky smooth-hound
Holohalaelurus punctatus	African spotted catshark	*Mustelus dorsalis*	sharptooth smooth-hound
Holohalaelurus regani	izak catshark	*Mustelus fasciatus*	striped smooth-hound
Hydrolagus affinis	smalleyed rabbitfish	*Mustelus griseus*	spotless smooth-hound
Hydrolagus africanus	African chimaera	*Mustelus henlei*	brown smooth-hound
Hydrolagus alberti		*Mustelus higmani*	smalleye smooth-hound
Hydrolagus barbouri		*Mustelus lenticulatus*	spotted estuary smooth-hound
Hydrolagus colliei	spotted ratfish	*Mustelus lunulatus*	sicklefin smooth-hound
Hydrolagus deani	Philippine chimaera	*Mustelus manazo*	starspotted smooth-hound
Hydrolagus eidolon		*Mustelus mento*	speckled smooth-hound
Hydrolagus lemures	blackfin ghostshark	*Mustelus minicanis*	
Hydrolagus mirabilis	large-eyed rabbitfish	*Mustelus mosis*	Arabian smooth-hound

Appendix 3 (continued). Checklist of elasmobranchs (sorted by scientific name).

Scientific name	Common name	Scientific name	Common name
Mustelus mustelus	smooth-hound	*Orectolobus maculatus*	spotted wobbegong
Mustelus norrisi	narrowfin smooth-hound	*Orectolobus ornatus*	ornate wobbegong
Mustelus palumbes	whitespotted smooth-hound	*Orectolobus wardi*	northern wobbegong
Mustelus punctulatus	blackspotted smooth-hound	*Oxynotus bruniensis*	prickly dogfish
Mustelus schmitti	narrownose smooth-hound	*Oxynotus caribbaeus*	Caribbean roughshark
Mustelus sinusmexicanus		*Oxynotus centrina*	angular roughshark
Mustelus whitneyi	humpback smooth-hound	*Oxynotus japonicus*	
Myliobatis aquila	common eagle ray	*Oxynotus paradoxus*	sailfin roughshark
Myliobatis australis	Australian bull ray	*Paragaleus leucolomatus*	whitetip weasel shark
Myliobatis californica	bat eagle ray	*Paragaleus pectoralis*	Atlantic weasel shark
Myliobatis chilensis		*Paragaleus randalli*	slender weasel shark
Myliobatis freminvillii	bullnose eagle ray	*Paragaleus tengi*	straight-tooth weasel shark
Myliobatis goodei	southern eagle ray	*Parascyllium collare*	collared carpetshark
Myliobatis hamlyni	purple eagle ray	*Parascyllium ferrugineum*	rusty carpetshark
Myliobatis longirostris	snouted eagle ray	*Parascyllium variolatum*	necklace carpetshark
Myliobatis peruvianus		*Paratrygon aiereba*	
Myliobatis tenuicaudatus	eagle ray	*Parmaturus campechiensis*	campeche catshark
Myliobatis tobijei	Japanese eagle ray	*Parmaturus macmillani*	Mcmillan's cat shark
Narcine bancrofti		*Parmaturus melanobranchius*	blackgill catshark
Narcine brasiliensis	Brazilian electric ray	*Parmaturus pilosus*	salamander shark
Narcine brevilabiata		*Parmaturus xaniurus*	filetail catshark
Narcine brunnea	brown numbfish	*Pastinachus sephen*	cowtail stingray
Narcine entemedor	giant electric ray	*Pavoraja alleni*	Allen's skate
Narcine indica		*Pavoraja nitida*	peacock skate
Narcine lingula		*Pentanchus profundicolus*	onefin catshark
Narcine maculata		*Platyrhina limboonkengi*	
Narcine prodorsalis		*Platyrhina sinensis*	
Narcine rierai	slender electric ray	*Platyrhinoidis triseriata*	thornback guitarfish
Narcine tasmaniensis	Tasmanian numbfish	*Plesiobatis daviesi*	deepwater stingray
Narcine timlei	spotted numbfish	*Pliotrema warreni*	sixgill sawshark
Narcine vermiculatus	vermiculate electric ray	*Poroderma africanum*	striped catshark
Narcine westraliensis	banded numbfish	*Poroderma pantherinum*	leopard catshark
Narke capensis	Cape numbfish	*Potamotrygon brachyura*	
Narke dipterygia	numbray	*Potamotrygon castexi*	
Narke japonica	electric numb ray	*Potamotrygon constellata*	
Nasolamia velox	whitenose shark	*Potamotrygon dumerilii*	
Nebrius ferrugineus	tawny nurse shark	*Potamotrygon falkneri*	
Negaprion acutidens	sicklefin lemon shark	*Potamotrygon henlei*	
Negaprion brevirostris	lemon shark	*Potamotrygon humerosa*	
Neoharriotta carri		*Potamotrygon hystrix*	
Neoharriotta pinnata	sicklefin chimaera	*Potamotrygon laticeps*	freshwater stingray
Neoharriotta pumila		*Potamotrygon leopoldi*	
Neoraja africana		*Potamotrygon magdalenae*	
Neoraja caerulea	blue ray	*Potamotrygon motoro*	ocellate river stingray
Neoraja carolinensis		*Potamotrygon orbignyi*	
Neoraja stehmanni	African pygmy skate	*Potamotrygon reticulatus*	
Notoraja asperula	smooth deepsea skate	*Potamotrygon schroederi*	
Notoraja laxipella		*Potamotrygon schuemacheri*	
Notoraja ochroderma		*Potamotrygon scobina*	
Notoraja spinifera	prickly deepsea skate	*Potamotrygon signata*	
Notoraja subtilispinosa		*Potamotrygon yepezi*	
Notoraja tobitukai		*Prionace glauca*	blue shark
Notorynchus cepedianus	broadnose sevengill shark	*Pristiophorus cirratus*	longnose sawshark
Odontaspis ferox	smalltooth sand tiger	*Pristiophorus japonicus*	Japanese sawshark
Odontaspis noronhai	bigeye sand tiger	*Pristiophorus nudipinnis*	shortnose sawshark
Okamejei acutispina		*Pristiophorus schroederi*	Bahamas sawshark
Okamejei australis	Sydney skate	*Pristis clavata*	dwarf sawfish
Okamejei boesemani		*Pristis microdon*	largetooth sawfish
Okamejei cerva	white-spotted skate	*Pristis pectinata*	smalltooth sawfish
Okamejei heemstrai		*Pristis perotteti*	large-tooth sawfish
Okamejei hollandi		*Pristis pristis*	common sawfish
Okamejei kenojei		*Pristis zijsron*	longcomb sawfish
Okamejei lemprieri	thornback skate	*Proscyllium habereri*	graceful catshark
Okamejei meerdervoortii		*Psammobatis bergi*	
Okamejei pita		*Psammobatis extenta*	
Okamejei powelli		*Psammobatis lentiginosa*	
Okamejei schmidti		*Psammobatis normani*	
Orectolobus japonicus	Japanese wobbegong	*Psammobatis parvacauda*	

Appendix 3 (continued). Checklist of elasmobranchs (sorted by scientific name).

Scientific name	Common name	Scientific name	Common name
Psammobatis rudis		*Rhinobatos halavi*	Halavi's guitarfish
Psammobatis rutrum		*Rhinobatos holcorhynchus*	slender guitarfish
Psammobatis scobina		*Rhinobatos horkeli*	Brazilian guitarfish
Pseudocarcharias kamoharai	crocodile shark	*Rhinobatos hynnicephalus*	angel fish
Pseudoraja fischeri		*Rhinobatos irvinei*	
Pseudotriakis microdon	false catshark	*Rhinobatos lentiginosus*	Atlantic guitarfish
Pteromylaeus asperrimus	rough eagle ray	*Rhinobatos leucorhynchus*	whitesnout guitarfish
Pteromylaeus bovinus	bull ray	*Rhinobatos leucospilus*	grayspottted guitarfish
Pteroplatytrygon violacea	pelagic stingray	*Rhinobatos lionotus*	
Raja ackleyi	ocellate skate	*Rhinobatos obtusus*	
Raja africana	African ray	*Rhinobatos ocellatus*	
Raja asterias	starry ray	*Rhinobatos percellens*	fiddlerfish
Raja bahamensis		*Rhinobatos planiceps*	Pacific guitarfish
Raja binoculata	big skate	*Rhinobatos prahli*	
Raja brachyura	blonde ray	*Rhinobatos productus*	shovelnose guitarfish
Raja cervigoni	finspot ray	*Rhinobatos punctifer*	
Raja clavata	thornback ray	*Rhinobatos rhinobatos*	common guitarfish
Raja confundens	bigthorn skate	*Rhinobatos salalah*	
Raja cortezensis	Cortez' ray	*Rhinobatos schlegelii*	yellow guitarfish
Raja eglanteria	clearnose skate	*Rhinobatos spinosus*	
Raja equatorialis	Ecuatorial ray	*Rhinobatos thouin*	thouin ray
Raja flavirostris		*Rhinobatos typus*	giant shovelnose ray
Raja herwigi		*Rhinochimaera africana*	
Raja inornata	California ray	*Rhinochimaera atlantica*	spearnose chimaera
Raja koreana		*Rhinochimaera pacifica*	Pacific spookfish
Raja maderensis	Madeiran ray	*Rhinoptera adspersa*	rough cownose ray
Raja microocellata	small-eyed ray	*Rhinoptera bonasus*	cownose ray
Raja miraletus	brown ray	*Rhinoptera brasiliensis*	ticon cownose ray
Raja montagui	spotted ray	*Rhinoptera javanica*	Javanese cownose ray
Raja polyommata	argus skate	*Rhinoptera jayakari*	Oman cownose ray
Raja polystigma	speckled ray	*Rhinoptera marginata*	Lusitanian cownose ray
Raja pulchra		*Rhinoptera neglecta*	Australian cownose ray
Raja radula	rough ray	*Rhinoptera steindachneri*	Pacific cownose ray
Raja rhina	longnose skate	*Rhinoraja kujiensis*	
Raja rondeleti	Rondelet's ray	*Rhinoraja longi*	
Raja rouxi		*Rhinoraja longicauda*	
Raja stellulata	starry skate	*Rhinoraja odai*	
Raja straeleni	biscuit skate	*Rhinoraja taranetzi*	
Raja texana	roundel skate	*Rhizoprionodon acutus*	milk shark
Raja undulata	undulate ray	*Rhizoprionodon lalandii*	Brazilian sharpnose shark
Raja velezi	velez ray	*Rhizoprionodon longurio*	Pacific sharpnose shark
Raja whitleyi	wedgenose skate	*Rhizoprionodon oligolinx*	grey sharpnose shark
Rajella annandalei		*Rhizoprionodon porosus*	Caribbean sharpnose shark
Rajella barnardi		*Rhizoprionodon taylori*	Australian sharpnose shark
Rajella bathyphila	deepwater ray	*Rhizoprionodon terraenovae*	Atlantic sharpnose shark
Rajella bigelowi	Bigelow's ray	*Rhynchobatus australiae*	
Rajella caudaspinosa	munchkin skate	*Rhynchobatus djiddensis*	giant guitarfish
Rajella dissimilis	ghost skate	*Rhynchobatus luebberti*	African wedgefish
Rajella eisenhardti		*Rioraja agassizii*	
Rajella fuliginea		*Rostroraja alba*	white skate
Rajella fyllae	round ray	*Schroederichthys bivius*	narrowmouthed catshark
Rajella kukujevi		*Schroederichthys chilensis*	redspotted catshark
Rajella leopardus	leopard skate	*Schroederichthys maculatus*	narrowtail catshark
Rajella nigerrima		*Schroederichthys tenuis*	slender catshark
Rajella purpuriventralis		*Scoliodon laticaudus*	spadenose shark
Rajella ravidula	smoothback skate	*Scyliorhinus besnardi*	polkadot catshark
Rajella sadowskii		*Scyliorhinus boa*	boa catshark
Rhina ancylostoma	bowmouth guitarfish	*Scyliorhinus canicula*	smallspotted catshark
Rhincodon typus	whale shark	*Scyliorhinus capensis*	yellowspotted catshark
Rhinobatos albomaculatus		*Scyliorhinus cervigoni*	West African catshark
Rhinobatos annandalei	Annandale's guitarfish	*Scyliorhinus comoroensis*	
Rhinobatos annulatus	lesser sandshark	*Scyliorhinus garmani*	brownspotted catshark
Rhinobatos batillum		*Scyliorhinus haeckelii*	freckled catshark
Rhinobatos blochii	bluntnose guitarfish	*Scyliorhinus hesperius*	whitesaddled catshark
Rhinobatos cemiculus	blackchin guitarfish	*Scyliorhinus meadi*	blotched catshark
Rhinobatos formosensis		*Scyliorhinus retifer*	chain catshark
Rhinobatos glaucostigma	speckled guitarfish	*Scyliorhinus stellaris*	nursehound
Rhinobatos granulatus	sharpnose guitarfish	*Scyliorhinus tokubee*	

Appendix 3 (continued). Checklist of elasmobranchs (sorted by scientific name).

Scientific name	Common name	Scientific name	Common name
Scyliorhinus torazame	cloudy catshark	*Torpedo microdiscus*	
Scyliorhinus torrei	dwarf catshark	*Torpedo nobiliana*	electric ray
Scylliogaleus quecketti	flapnose houndshark	*Torpedo panthera*	panther electric ray
Scymnodalatias albicauda	whitetail dogfish	*Torpedo peruana*	
Scymnodalatias garricki		*Torpedo semipelagica*	
Scymnodalatias oligodon		*Torpedo sinuspersici*	marbled electric ray
Scymnodalatias sherwoodi	sherwood dogfish	*Torpedo tokionis*	
Scymnodon ichiharai		*Torpedo torpedo*	common torpedo
Scymnodon macracanthus	largespine velvet dogfish	*Torpedo tremens*	torpedo
Scymnodon obscurus	smallmouth velvet dogfish	*Triaenodon obesus*	whitetip reef shark
Scymnodon ringens	knifetooth dogfish	*Triakis acutipinna*	sharpfin houndshark
Scymnodon squamulosus	velvet dogfish	*Triakis maculata*	spotted houndshark
Somniosus antarcticus		*Triakis megalopterus*	sharptooth houndshark
Somniosus microcephalus	Greenland shark	*Triakis scyllium*	banded houndshark
Somniosus pacificus	Pacific sleeper shark	*Triakis semifasciata*	leopard shark
Somniosus rostratus	little sleeper shark	*Trigonognathus kabeyai*	
Sphyrna corona	scalloped bonnethead	*Trygonoptera javanica*	
Sphyrna couardi	whitefin hammerhead	*Trygonoptera kaiana*	
Sphyrna lewini	scalloped hammerhead	*Trygonoptera mucosa*	western shovelnose stingaree
Sphyrna media	scoophead	*Trygonoptera ovalis*	striped stingaree
Sphyrna mokarran	great hammerhead	*Trygonoptera personata*	masked stingaree
Sphyrna tiburo	bonnethead	*Trygonorrhina fasciata*	southern fiddler
Sphyrna tudes	smalleye hammerhead	*Trygonorrhina guaneria*	
Sphyrna zygaena	smooth hammerhead	*Trygonorrhina melaleuca*	magpie fiddler ray
Squaliolus aliae	smalleye pygmy shark	*Typhlonarke aysoni*	blind electric ray
Squaliolus laticaudus	spined pygmy shark	*Typhlonarke tarakea*	oval electric ray
Squalus acanthias	spiny dogfish	*Urobatis halleri*	Haller's round ray
Squalus acutirostris		*Urogymnus asperrimus*	porcupine ray
Squalus blainville	longnose spurdog	*Urogymnus natalensis*	backwater butterfly ray
Squalus cubensis	Cuban dogfish	*Urogymnus poecilura*	longtail butterfly ray
Squalus japonicus	Japanese spurdog	*Urogymnus ukpam*	thorny freshwater stingray
Squalus megalops	shortnose spurdog	*Urolophus armatus*	
Squalus melanurus	blacktailed spurdog	*Urolophus aurantiacus*	
Squalus mitsukurii	shortspine spurdog	*Urolophus bucculentus*	sandyback stingaree
Squalus rancureli	cyrano spurdog	*Urolophus circularis*	circular stingaree
Squatina aculeata	sawback angelshark	*Urolophus concentricus*	spot-on-spot round ray
Squatina africana	African angelshark	*Urolophus cruciatus*	crossback stingaree
Squatina argentina	Argentine angelshark	*Urolophus expansus*	wide stingaree
Squatina australis	Australian angelshark	*Urolophus flavomosaicus*	patchwork stingaree
Squatina californica	Pacific angelshark	*Urolophus gigas*	spotted stingaree
Squatina dumeril	sand devil	*Urolophus jamaicensis*	yellow stingray
Squatina formosa	Taiwan angelshark	*Urolophus lobatus*	lobed stingaree
Squatina guggenheim		*Urolophus maculatus*	spotted round ray
Squatina japonica	Japanese angelshark	*Urolophus mitosis*	mitotic stingaree
Squatina nebulosa	clouded angelshark	*Urolophus orarius*	coastal stingaree
Squatina occulta		*Urolophus paucimaculatus*	sparsely-spotted stingaree
Squatina oculata	smoothback angelshark	*Urolophus sufflavus*	yellowback stingaree
Squatina squatina	angelshark	*Urolophus testaceus*	common stingaree
Squatina tergocellata	ornate angelshark	*Urolophus viridis*	greenback stingaree
Squatina tergocellatoides	ocellated angelshark	*Urolophus westraliensis*	brown stingaree
Stegostoma fasciatum	zebra shark	*Urotrygon aspidura*	spiny-tail round ray
Sutorectus tentaculatus	cobbler wobbegong	*Urotrygon chilensis*	Chilean round ray
Sympterygia acuta		*Urotrygon microphthalmum*	
Sympterygia bonapartii		*Urotrygon munda*	munda round ray
Sympterygia brevicaudata		*Urotrygon nana*	dwarf round ray
Sympterygia lima		*Urotrygon reticulata*	reticulate round ray
Taeniura grabata	round stingray	*Urotrygon rogersi*	Rogers' round ray
Taeniura lymma	bluespotted ribbontail ray	*Urotrygon serrula*	stingray
Taeniura meyeni	blotched fantail ray	*Urotrygon simulatrix*	fake round ray
Temera hardwickii		*Urotrygon venezuelae*	
Torpedo andersoni		*Zanobatus schoenleinii*	
Torpedo bauchotae		*Zapteryx brevirostris*	lesser guitarfish
Torpedo californica	Pacific electric ray	*Zapteryx exasperata*	banded guitarfish
Torpedo fairchildi		*Zapteryx xyster*	
Torpedo fuscomaculata	blackspotted electric ray		
Torpedo mackayana			
Torpedo macneilli	short-tail torpedo ray		
Torpedo marmorata	marbled electric ray		

Appendix 4. Checklist of elasmobranchs (sorted by common name).

Common name	Scientific name	Common name	Scientific name
African angelshark	*Squatina africana*	birdbeak dogfish	*Deania calcea*
African chimaera	*Hydrolagus africanus*	biscuit skate	*Raja straeleni*
African lanternshark	*Etmopterus polli*	black dogfish	*Centroscyllium fabricii*
African pygmy skate	*Neoraja stehmanni*	black legskate	*Anacanthobatis ori*
African ray	*Raja africana*	blackbelly lanternshark	*Etmopterus lucifer*
African ribbontail catshark	*Eridacnis sinuans*	blackchin guitarfish	*Rhinobatos cemiculus*
African sawtail catshark	*Galeus polli*	blackedge whipray	*Himantura marginata*
African softnose skate	*Bathyraja smithii*	blackfin ghostshark	*Hydrolagus lemures*
African spotted catshark	*Holohalaelurus punctatus*	blackfin gulper shark	*Centrophorus atromarginatus*
African wedgefish	*Rhynchobatus luebberti*	blackgill catshark	*Parmaturus melanobranchius*
Alaska skate	*Bathyraja parmifera*	blackmouth catshark	*Galeus melastomus*
Aleutian skate	*Bathyraja aleutica*	blacknose shark	*Carcharhinus acronotus*
Allen's skate	*Pavoraja alleni*	blackspot shark	*Carcharhinus sealei*
angel fish	*Rhinobatos hynnicephalus*	blackspot skate	*Dipturus campbelli*
angelshark	*Squatina squatina*	blackspotted catshark	*Halaelurus buergeri*
angular roughshark	*Oxynotus centrina*	blackspotted electric ray	*Torpedo fuscomaculata*
Annandale's guitarfish	*Rhinobatos annandalei*	blackspotted smooth-hound	*Mustelus punctulatus*
Arabian carpetshark	*Chiloscyllium arabicum*	black-spotted whipray	*Himantura toshi*
Arabian catshark	*Halaelurus alcocki*	blacktailed spurdog	*Squalus melanurus*
Arabian smooth-hound	*Mustelus mosis*	blacktip reef shark	*Carcharhinus melanopterus*
Arctic skate	*Amblyraja hyperborea*	blacktip sawtail catshark	*Galeus sauteri*
Argentine angelshark	*Squatina argentina*	blacktip shark	*Carcharhinus limbatus*
argus skate	*Raja polyommata*	blacktip tope	*Hypogaleus hyugaensis*
arrowhead dogfish	*Deania profundorum*	Bleeker's whipray	*Himantura bleekeri*
Atlantic ghost catshark	*Apristurus atlanticus*	blind electric ray	*Typhlonarke aysoni*
Atlantic guitarfish	*Rhinobatos lentiginosus*	blind shark	*Brachaelurus waddi*
Atlantic sawtail catshark	*Galeus atlanticus*	blind torpedo	*Benthobatis marcida*
Atlantic sharpnose shark	*Rhizoprionodon terraenovae*	blonde ray	*Raja brachyura*
Atlantic stingray	*Dasyatis sabina*	blotched catshark	*Scyliorhinus meadi*
Atlantic weasel shark	*Paragaleus pectoralis*	blotched fantail ray	*Taeniura meyeni*
Australian angelshark	*Squatina australis*	blotchy swell shark	*Cephaloscyllium umbratile*
Australian blackspotted catshark	*Aulohalaelurus labiosus*	blue ray	*Neoraja caerulea*
Australian blacktip shark	*Carcharhinus tilstoni*	blue shark	*Prionace glauca*
Australian bull ray	*Myliobatis australis*	bluegray carpetshark	*Heteroscyllium colcloughi*
Australian butterfly ray	*Gymnura australis*	bluespotted bambooshark	*Chiloscyllium caerulopunctatum*
Australian cownose ray	*Rhinoptera neglecta*	bluespotted ribbontail ray	*Taeniura lymma*
Australian marbled catshark	*Atelomycterus macleayi*	bluespotted stingray	*Dasyatis kuhlii*
Australian numbfish	*Hypnos monopterygium*	bluntnose guitarfish	*Rhinobatos blochii*
Australian sawtail catshark	*Galeus boardmani*	bluntnose sixgill shark	*Hexanchus griseus*
Australian sharpnose shark	*Rhizoprionodon taylori*	bluntnose stingray	*Dasyatis say*
Australian spotted catshark	*Asymbolus analis*	boa catshark	*Scyliorhinus boa*
Australian swellshark	*Cephaloscyllium laticeps*	bonnethead	*Sphyrna tiburo*
backwater butterfly ray	*Gymnura natalensis*	Borneo catshark	*Apristurus verweyi*
backwater butterfly ray	*Urogymnus natalensis*	Borneo shark	*Carcharhinus borneensis*
Bahamas sawshark	*Pristiophorus schroederi*	bottom skate	*Bathyraja pseudoisotrachys*
balloon shark	*Cephaloscyllium sufflans*	bowmouth guitarfish	*Rhina ancylostoma*
banded eagle ray	*Aetomylaeus nichofii*	bramble shark	*Echinorhinus brucus*
banded guitarfish	*Zapteryx exasperata*	Brazilian electric ray	*Narcine brasiliensis*
banded houndshark	*Triakis scyllium*	Brazilian guitarfish	*Rhinobatos horkeli*
banded numbfish	*Narcine westraliensis*	Brazilian sharpnose shark	*Rhizoprionodon lalandii*
banded sand catshark	*Atelomycterus fasciatus*	bristly catshark	*Halaelurus hispidus*
barbeled houndshark	*Leptocharias smithii*	broad skate	*Amblyraja badia*
barbelthroat carpetshark	*Cirrhoscyllium expolitum*	broadbanded lanternshark	*Etmopterus gracilispinis*
bareskin dogfish	*Centroscyllium kamoharai*	broadfin sawtail catshark	*Galeus nipponensis*
barndoor skate	*Dipturus laevis*	broadfin shark	*Lamiopsis temmincki*
barn-door skate	*Dipturus chilensis*	broadgill catshark	*Apristurus riveri*
basking shark	*Cetorhinus maximus*	broadnose catshark	*Apristurus investigatoris*
bat eagle ray	*Myliobatis californica*	broadnose sevengill shark	*Notorynchus cepedianus*
Bennett's stingray	*Dasyatis bennetti*	brown catshark	*Apristurus brunneus*
big skate	*Raja binoculata*	brown lanternshark	*Etmopterus unicolor*
Bigelow's ray	*Rajella bigelowi*	brown numbfish	*Narcine brunnea*
bigeye houndshark	*Lago omanensis*	brown ray	*Raja miraletus*
bigeye sand tiger	*Odontaspis noronhai*	brown shyshark	*Haploblepharus fuscus*
bigeye thresher	*Alopias superciliosus*	brown smooth-hound	*Mustelus henlei*
bigeyed sixgill shark	*Hexanchus nakamurai*	brown stingaree	*Urolophus westraliensis*
bigmouth skate	*Amblyraja robertsi*	brown stingray	*Dasyatis lata*
bignose shark	*Carcharhinus altimus*	brownbanded bambooshark	*Chiloscyllium punctatum*
bigthorn skate	*Raja confundens*	brownspotted catshark	*Scyliorhinus garmani*

Appendix 4 (continued). Checklist of elasmobranchs (sorted by common name).

Common name	Scientific name	Common name	Scientific name
bull ray	*Pteromylaeus bovinus*	dwarf sawtail catshark	*Galeus schultzi*
bull shark	*Carcharhinus leucas*	dwarf whipray	*Himantura walga*
bullis skate	*Dipturus bullisi*	eagle ray	*Myliobatis tenuicaudatus*
bullnose eagle ray	*Myliobatis freminvillii*	eastern shovelnose ray	*Aptychotrema rostrata*
California butterfly ray	*Gymnura marmorata*	Ecuatorial ray	*Raja equatorialis*
California ray	*Raja inornata*	electric numb ray	*Narke japonica*
campeche catshark	*Parmaturus campechiensis*	electric ray	*Torpedo nobiliana*
Cape elephantfish	*Callorhinchus capensis*	epaulette shark	*Hemiscyllium ocellatum*
Cape numbfish	*Narke capensis*	estuary stingray	*Dasyatis fluviorum*
Caribbean lanternshark	*Etmopterus hillianus*	fake round ray	*Urotrygon simulatrix*
Caribbean reef shark	*Carcharhinus perezi*	false catshark	*Pseudotriakis microdon*
Caribbean roughshark	*Oxynotus caribbaeus*	fiddlerfish	*Rhinobatos percellens*
Caribbean sharpnose shark	*Rhizoprionodon porosus*	filetail catshark	*Parmaturus xaniurus*
chain catshark	*Scyliorhinus retifer*	finetooth shark	*Carcharhinus isodon*
Chilean devil ray	*Mobula tarapacana*	finspot ray	*Raja cervigoni*
Chilean round ray	*Urotrygon chilensis*	flapnose houndshark	*Scylliogaleus quecketti*
chimaera	*Chimaera cubana*	flathead catshark	*Apristurus macrorhynchus*
chupare stingray	*Himantura schmardae*	freckled catshark	*Scyliorhinus haeckelii*
circular stingaree	*Urolophus circularis*	freckled skate	*Leucoraja garmani*
clearnose skate	*Raja eglanteria*	freshwater stingray	*Potamotrygon laticeps*
clouded angelshark	*Squatina nebulosa*	freshwater whipray	*Himantura chaophraya*
cloudy catshark	*Scyliorhinus torazame*	frilled shark	*Chlamydoselachus anguineus*
coastal stingaree	*Urolophus orarius*	fringfin lanternshark	*Etmopterus schultzi*
cobbler wobbegong	*Sutorectus tentaculatus*	Galapagos bullhead shark	*Heterodontus quoyi*
collared carpetshark	*Parascyllium collare*	Galapagos shark	*Carcharhinus galapagensis*
combtooth dogfish	*Centroscyllium nigrum*	Ganges shark	*Glyphis gangeticus*
combtooth lanternshark	*Etmopterus decacuspidatus*	gecko catshark	*Galeus eastmani*
common eagle ray	*Myliobatis aquila*	ghost catshark	*Apristurus manis*
common guitarfish	*Rhinobatos rhinobatos*	ghost shark	*Callorhinchus milii*
common sawfish	*Pristis pristis*	ghost skate	*Rajella dissimilis*
common stingaree	*Urolophus testaceus*	giant electric ray	*Narcine entemedor*
common stingray	*Dasyatis pastinaca*	giant guitarfish	*Rhynchobatus djiddensis*
common torpedo	*Torpedo torpedo*	giant manta	*Manta birostris*
cookiecutter shark	*Isistius brasiliensis*	giant shovelnose ray	*Rhinobatos typus*
copper shark	*Carcharhinus brachyurus*	goblin shark	*Mitsukurina owstoni*
coral catshark	*Atelomycterus marmoratus*	graceful catshark	*Proscyllium habereri*
Cortez' ray	*Raja cortezensis*	graceful shark	*Carcharhinus amblyrhynchoides*
cownose ray	*Rhinoptera bonasus*	granular dogfish	*Centroscyllium granulatum*
cowtail stingray	*Pastinachus sephen*	grayspottted guitarfish	*Rhinobatos leucospilus*
creek whaler	*Carcharhinus fitzroyensis*	great hammerhead	*Sphyrna mokarran*
crested bullhead shark	*Heterodontus galeatus*	great lanternshark	*Etmopterus princeps*
crocodile shark	*Pseudocarcharias kamoharai*	great white shark	*Carcharodon carcharias*
crossback stingaree	*Urolophus cruciatus*	green lanternshark	*Etmopterus virens*
Cuban dogfish	*Squalus cubensis*	greenback skate	*Dipturus gudgeri*
Cuban legskate	*Cruriraja atlantis*	greenback stingaree	*Urolophus viridis*
Cuban ribbontail catshark	*Eridacnis barbouri*	Greenland shark	*Somniosus microcephalus*
cuckoo ray	*Leucoraja naevus*	grey bambooshark	*Chiloscyllium griseum*
cyrano spurdog	*Squalus rancureli*	grey reef shark	*Carcharhinus amblyrhynchos*
daggernose shark	*Isogomphodon oxyrhynchus*	grey sharpnose shark	*Rhizoprionodon oligolinx*
daisy stingray	*Dasyatis margarita*	grey smooth-hound	*Mustelus californicus*
dark ghost shark	*Hydrolagus novaezealandiae*	gulf catshark	*Asymbolus vincenti*
dark shyshark	*Haploblepharus pictus*	gulper shark	*Centrophorus granulosus*
deepsea skate	*Bathyraja abyssicola*	gummy shark	*Mustelus antarcticus*
deepwater catshark	*Apristurus profundorum*	Halavi's guitarfish	*Rhinobatos halavi*
deepwater ray	*Rajella bathyphila*	Haller's round ray	*Urobatis halleri*
deepwater stingray	*Plesiobatis daviesi*	hardnose shark	*Carcharhinus macloti*
devil fish	*Mobula mobular*	harlequin catshark	*Ctenacis fehlmanni*
devil ray	*Mobula diabola*	Hasselt's bambooshark	*Chiloscyllium hasselti*
diamond stingray	*Dasyatis dipterura*	Hawaiian lanternshark	*Etmopterus villosus*
dragon stingray	*Himantura draco*	hoary catshark	*Apristurus canutus*
draughtsboard shark	*Cephaloscyllium isabellum*	honeycomb stingray	*Himantura uarnak*
dumb gulper shark	*Centrophorus harrissoni*	hooded carpetshark	*Hemiscyllium strahani*
dusky catshark	*Halaelurus canescens*	hooktooth dogfish	*Aculeola nigra*
dusky shark	*Carcharhinus obscurus*	hooktooth shark	*Chaenogaleus macrostoma*
dusky smooth-hound	*Mustelus canis*	horn shark	*Heterodontus francisci*
dwarf catshark	*Scyliorhinus torrei*	humpback smooth-hound	*Mustelus whitneyi*
dwarf round ray	*Urotrygon nana*	Iceland catshark	*Apristurus laurussonii*
dwarf sawfish	*Pristis clavata*	Indian sand tiger	*Carcharias tricuspidatus*

Appendix 4 (continued). Checklist of elasmobranchs (sorted by common name).

Common name	Scientific name	Common name	Scientific name
Indian swellshark	*Cephaloscyllium silasi*	marbled electric ray	*Torpedo marmorata*
Indonesia speckled carpetshark	*Hemiscyllium freycineti*	marbled electric ray	*Torpedo sinuspersici*
izak catshark	*Holohalaelurus regani*	marbled stingray	*Dasyatis marmorata*
Japanese angelshark	*Squatina japonica*	marbled whipray	*Himantura oxyrhynchus*
Japanese bullhead shark	*Heterodontus japonicus*	masked stingaree	*Trygonoptera personata*
Japanese butterflyray	*Gymnura japonica*	Mcmillan's cat shark	*Parmaturus macmillani*
Japanese catshark	*Apristurus japonicus*	megamouth shark	*Megachasma pelagios*
Japanese eagle ray	*Myliobatis tobijei*	Mekong stingray	*Dasyatis laosensis*
Japanese sawshark	*Pristiophorus japonicus*	Mexican hornshark	*Heterodontus mexicanus*
Japanese spurdog	*Squalus japonicus*	milk shark	*Rhizoprionodon acutus*
Japanese topeshark	*Hemitriakis japanica*	mitotic stingaree	*Urolophus mitosis*
Japanese wobbegong	*Orectolobus japonicus*	mosaic gulper shark	*Centrophorus tessellatus*
Javanese cownose ray	*Rhinoptera javanica*	mottled eagle ray	*Aetomylaeus maculatus*
Jensen's skate	*Amblyraja jenseni*	mouse catshark	*Galeus murinus*
kitefin shark	*Dalatias licha*	mud catshark	*Halaelurus lutarius*
knifetooth dogfish	*Scymnodon ringens*	munchkin skate	*Rajella caudaspinosa*
knifetooth sawfish	*Anoxypristis cuspidata*	munda round ray	*Urotrygon munda*
Krefft's ray	*Malacoraja kreffti*	munk's devil ray	*Mobula munkiana*
Kwangtung skate	*Dipturus kwangtungensis*	narrowfin smooth-hound	*Mustelus norrisi*
large-eyed rabbitfish	*Hydrolagus mirabilis*	narrowmouthed catshark	*Schroederichthys bivius*
largenose catshark	*Apristurus nasutus*	narrownose chimaera	*Harriotta raleighana*
largespine velvet dogfish	*Scymnodon macracanthus*	narrownose smooth-hound	*Mustelus schmitti*
largetooth cookiecutter shark	*Isistius plutodus*	narrowtail catshark	*Schroederichthys maculatus*
largetooth sawfish	*Pristis microdon*	natal electric ray	*Heteronarce garmani*
large-tooth sawfish	*Pristis perotteti*	necklace carpetshark	*Parascyllium variolatum*
leafscale gulper shark	*Centrophorus squamosus*	needle dogfish	*Centrophorus acus*
lemon shark	*Negaprion brevirostris*	nervous shark	*Carcharhinus cautus*
leopard catshark	*Poroderma pantherinum*	New Caledonia catshark	*Aulohalaelurus kanakorum*
leopard shark	*Triakis semifasciata*	New Zealand catshark	*Halaelurus dawsoni*
leopard skate	*Rajella leopardus*	New Zealand lanternshark	*Etmopterus baxteri*
leopard whipray	*Himantura undulata*	night shark	*Carcharhinus signatus*
lesser devil ray	*Mobula hypostoma*	northern wobbegong	*Orectolobus wardi*
lesser guitarfish	*Zapteryx brevirostris*	Norwegian skate	*Dipturus nidarosiensis*
lesser sandshark	*Rhinobatos annulatus*	numbray	*Narke dipterygia*
lined catshark	*Halaelurus lineatus*	nurse shark	*Ginglymostoma cirratum*
lined lanternshark	*Etmopterus bullisi*	nursehound	*Scyliorhinus stellaris*
little gulper shark	*Centrophorus uyato*	oceanic whitetip shark	*Carcharhinus longimanus*
little skate	*Leucoraja erinacea*	ocellate skate	*Raja ackleyi*
little sleeper shark	*Somniosus rostratus*	ocellated angelshark	*Squatina tergocellatoides*
lobed stingaree	*Urolophus lobatus*	ocellated electric ray	*Diplobatis ommata*
lollipop catshark	*Cephalurus cephalus*	ocellate river stingray	*Potamotrygon motoro*
longcomb sawfish	*Pristis zijsron*	Ogilby's ghostshark	*Hydrolagus ogilbyi*
longfin catshark	*Apristurus herklotsi*	Okhotsk skate	*Bathyraja violacea*
longfin mako	*Isurus paucus*	Oman cownose ray	*Rhinoptera jayakari*
longhead catshark	*Apristurus longicephalus*	onefin catshark	*Pentanchus profundicolus*
longnose catshark	*Apristurus kampae*	ornate angelshark	*Squatina tergocellata*
longnose deepsea skate	*Bathyraja shuntovi*	ornate dogfish	*Centroscyllium ornatum*
longnose houndshark	*Lago garricki*	ornate eagle ray	*Aetomylaeus vespertilio*
longnose pygmy shark	*Heteroscymnoides marleyi*	ornate wobbegong	*Orectolobus ornatus*
longnose sawshark	*Pristiophorus cirratus*	oval electric ray	*Typhlonarke tarakea*
longnose skate	*Raja rhina*	Pacific angelshark	*Squatina californica*
longnose spurdog	*Squalus blainville*	Pacific chupare	*Himantura pacifica*
longnose stingray	*Dasyatis guttata*	Pacific cownose ray	*Rhinoptera steindachneri*
longnose velvet dogfish	*Centroscymnus crepidater*	Pacific electric ray	*Torpedo californica*
longnosed skate	*Dipturus oxyrinchus*	Pacific guitarfish	*Rhinobatos planiceps*
longsnout butterfly ray	*Gymnura crebripunctata*	Pacific sharpnose shark	*Rhizoprionodon longurio*
longsnout dogfish	*Deania quadrispinosa*	Pacific sleeper shark	*Somniosus pacificus*
longtail butterfly ray	*Gymnura poecilura*	Pacific spookfish	*Rhinochimaera pacifica*
longtail butterfly ray	*Urogymnus poecilura*	painted maskray	*Dasyatis leylandi*
longtail skate	*Arhynchobatis asperrimus*	pale catshark	*Apristurus sibogae*
longtail stingray	*Dasyatis longus*	pale ray	*Bathyraja pallida*
lowfin gulper shark	*Centrophorus lusitanicus*	pale-edged stingray	*Dasyatis zugei*
Lusitanian cownose ray	*Rhinoptera marginata*	Panama ghost catshark	*Apristurus stenseni*
Madeiran ray	*Raja maderensis*	panther electric ray	*Torpedo panthera*
magpie fiddler ray	*Trygonorrhina melaleuca*	Papuan epaulette shark	*Hemiscyllium hallstromi*
Maltese ray	*Leucoraja melitensis*	patchwork stingaree	*Urolophus flavomosaicus*
mandarin dogfish	*Cirrhigaleus barbifer*	peacock skate	*Pavoraja nitida*
mangrove whipray	*Himantura granulata*	pelagic stingray	*Pteroplatytrygon violacea*

Appendix 4 (continued). Checklist of elasmobranchs (sorted by common name).

Common name	Scientific name	Common name	Scientific name
pelagic thresher	*Alopias pelagicus*	scalloped bonnethead	*Sphyrna corona*
peppered catshark	*Galeus piperatus*	scalloped hammerhead	*Sphyrna lewini*
Philippine chimaera	*Hydrolagus deani*	scaly whipray	*Himantura imbricata*
pigeye shark	*Carcharhinus amboinensis*	scoophead	*Sphyrna media*
pink whipray	*Himantura fai*	shagreen ray	*Leucoraja fullonica*
plain eagle ray	*Aetobatus flagellum*	sharpfin houndshark	*Triakis acutipinna*
plain maskray	*Dasyatis annotata*	sharpnose guitarfish	*Rhinobatos granulatus*
plownose chimaera (unesco)	*Callorhinchus callorynchus*	sharpnose sevengill shark	*Heptranchias perlo*
plunket shark	*Centroscymnus plunketi*	sharpnose stingray	*Himantura gerrardi*
pointed-nose stingray	*Himantura jenkinsii*	sharpsnout stingray	*Dasyatis geijskesi*
polkadot catshark	*Scyliorhinus besnardi*	sharptooth houndshark	*Triakis megalopterus*
pondicherry shark	*Carcharhinus hemiodon*	sharptooth smooth-hound	*Mustelus dorsalis*
porbeagle	*Lamna nasus*	sherwood dogfish	*Scymnodalatias sherwoodi*
porcupine ray	*Urogymnus asperrimus*	shortfin devilray	*Mobula kuhlii*
Port Jackson shark	*Heterodontus portusjacksoni*	shortfin mako	*Isurus oxyrinchus*
Portuguese dogfish	*Centroscymnus coelolepis*	shortnose sawshark	*Pristiophorus nudipinnis*
prickly brown ray	*Dipturus teevani*	shortnose spurdog	*Squalus megalops*
prickly deepsea skate	*Notoraja spinifera*	shortnose velvet dogfish	*Centroscymnus cryptacanthus*
prickly dogfish	*Oxynotus bruniensis*	short-snouted shovelnose ray	*Aptychotrema bougainvillii*
prickly shark	*Echinorhinus cookei*	shortspine spurdog	*Squalus mitsukurii*
prow-nose skate	*Dipturus stenorhynchus*	shorttail lanternshark	*Etmopterus brachyurus*
puffadder shyshark	*Haploblepharus edwardsii*	short-tail nurse shark	*Ginglymostoma brevicaudatum*
purple chimaera	*Hydrolagus purpurescens*	short-tail stingray	*Dasyatis brevicaudata*
purple eagle ray	*Myliobatis hamlyni*	short-tail torpedo ray	*Torpedo macneilli*
pygmy devil ray	*Mobula eregoodootenkee*	shovelnose guitarfish	*Rhinobatos productus*
pygmy ribbontail catshark	*Eridacnis radcliffei*	sicklefin chimaera	*Neoharriotta pinnata*
pygmy shark	*Euprotomicrus bispinatus*	sicklefin lemon shark	*Negaprion acutidens*
quagga catshark	*Halaelurus quagga*	sicklefin smooth-hound	*Mustelus lunulatus*
rabbit fish	*Chimaera monstrosa*	sicklefin weasel shark	*Hemigaleus microstoma*
rattail skate	*Dipturus lanceorostratus*	silky shark	*Carcharhinus falciformis*
red stingray	*Dasyatis akajei*	silver chimaera	*Chimaera phantasma*
redspotted catshark	*Schroederichthys chilensis*	silvertip shark	*Carcharhinus albimarginatus*
reticulate round ray	*Urotrygon reticulata*	sixgill sawshark	*Pliotrema warreni*
reticulated swellshark	*Cephaloscyllium fasciatum*	sixgill stingray	*Hexatrygon bickelli*
richardson's ray	*Bathyraja richardsoni*	skate	*Dipturus batis*
Rogers' round ray	*Urotrygon rogersi*	slender bambooshark	*Chiloscyllium indicum*
Rondelet's ray	*Raja rondeleti*	slender catshark	*Schroederichthys tenuis*
rough cownose ray	*Rhinoptera adspersa*	slender electric ray	*Narcine rierai*
rough eagle ray	*Pteromylaeus asperrimus*	slender guitarfish	*Rhinobatos holcorhynchus*
rough longnose dogfish	*Deania histricosa*	slender sawtail catshark	*Galeus gracilis*
rough ray	*Raja radula*	slender smooth-hound	*Gollum attenuatus*
rough skate	*Dipturus nasutus*	slender weasel shark	*Paragaleus randalli*
roughbelly skate	*Dipturus springeri*	slendertail lanternshark	*Etmopterus molleri*
roughnose legskate	*Cruriraja parcomaculata*	slime skate	*Dipturus pullopunctatus*
roughskin dogfish	*Centroscymnus owstoni*	sliteye shark	*Loxodon macrorhinus*
roughskin skate	*Malacoraja spinacidermis*	smallbelly catshark	*Apristurus indicus*
roughskin spurdog	*Cirrhigaleus asper*	smalleye catshark	*Apristurus microps*
roughtail catshark	*Galeus arae*	smalleye hammerhead	*Sphyrna tudes*
roughtail skate	*Bathyraja trachura*	smalleye pygmy shark	*Squaliolus aliae*
roughtail stingray	*Dasyatis centroura*	smalleye smooth-hound	*Mustelus higmani*
round ray	*Rajella fyllae*	smalleye stingray	*Dasyatis microps*
round stingray	*Taeniura grabata*	smalleyed rabbitfish	*Hydrolagus affinis*
roundel skate	*Raja texana*	small-eyed ray	*Raja microocellata*
rusty carpetshark	*Parascyllium ferrugineum*	smallfin catshark	*Apristurus parvipinnis*
saddle carpetshark	*Cirrhoscyllium japonicum*	smallfin gulper shark	*Centrophorus moluccensis*
sailback houndshark	*Gogolia filewoodi*	smallmouth velvet dogfish	*Scymnodon obscurus*
sailfin roughshark	*Oxynotus paradoxus*	smallspine spookfish	*Harriotta haeckeli*
sailray	*Dipturus linteus*	smallspotted catshark	*Scyliorhinus canicula*
salamander shark	*Parmaturus pilosus*	smalltail shark	*Carcharhinus porosus*
Saldanha catshark	*Apristurus saldanha*	smalltooth sand tiger	*Odontaspis ferox*
salmon shark	*Lamna ditropis*	smalltooth sawfish	*Pristis pectinata*
sand devil	*Squatina dumeril*	smooth butterfly ray	*Gymnura micrura*
sand tiger shark	*Carcharias taurus*	smooth deepsea skate	*Notoraja asperula*
sandbar shark	*Carcharhinus plumbeus*	smooth hammerhead	*Sphyrna zygaena*
sandpaper skate	*Bathyraja interrupta*	smooth lanternshark	*Etmopterus pusillus*
sandy ray	*Leucoraja circularis*	smooth skate	*Malacoraja senta*
sandyback stingaree	*Urolophus bucculentus*	smoothback angelshark	*Squatina oculata*
sawback angelshark	*Squatina aculeata*	smoothback skate	*Rajella ravidula*

Appendix 4 (continued). Checklist of elasmobranchs (sorted by common name).

Common name	Scientific name	Common name	Scientific name
smooth-hound	*Mustelus mustelus*	thornback ray	*Raja clavata*
smoothnose legskate	*Cruriraja durbanensis*	thornback skate	*Okamejei lemprieri*
smoothtail mobula	*Mobula thurstoni*	thorntail stingray	*Dasyatis thetidis*
smoothtooth shark (unesco)	*Carcharhinus leiodon*	thorny freshwater stingray	*Urogymnus ukpam*
snaggletooth shark	*Hemipristis elongata*	thorny lanternshark	*Etmopterus sentosus*
snouted eagle ray	*Myliobatis longirostris*	thorny skate	*Amblyraja radiata*
South China catshark	*Apristurus sinensis*	thouin ray	*Rhinobatos thouin*
southern eagle ray	*Myliobatis goodei*	ticon cownose ray	*Rhinoptera brasiliensis*
southern false skate	*Gurgesiella furvescens*	tiger catshark	*Halaelurus natalensis*
southern fiddler	*Trygonorrhina fasciata*	tiger shark	*Galeocerdo cuvier*
southern lanternshark	*Etmopterus granulosus*	tope shark	*Galeorhinus galeus*
southern round skate	*Irolita waitii*	torpedo	*Torpedo tremens*
southern stingray	*Dasyatis americana*	tortonese's stingray	*Dasyatis tortonesei*
spadenose shark	*Scoliodon laticaudus*	triangular legskate	*Cruriraja triangularis*
sparsely-spotted stingaree	*Urolophus paucimaculatus*	undulate ray	*Raja undulata*
spatulasnout catshark	*Apristurus platyrhynchus*	velez ray	*Raja velezi*
spearnose chimaera	*Rhinochimaera atlantica*	velvet belly	*Etmopterus spinax*
speartooth shark	*Glyphis glyphis*	velvet dogfish	*Scymnodon squamulosus*
speckled carpetshark	*Hemiscyllium trispeculare*	vermiculate electric ray	*Narcine vermiculatus*
speckled catshark	*Halaelurus boesemani*	violet skate	*Dipturus doutrei*
speckled guitarfish	*Rhinobatos glaucostigma*	wedgenose skate	*Raja whitleyi*
speckled ray	*Raja polystigma*	West African catshark	*Scyliorhinus cervigoni*
speckled smooth-hound	*Mustelus mento*	western shovelnose ray	*Aptychotrema vincentiana*
spined pygmy shark	*Squaliolus laticaudus*	western shovelnose stingaree	*Trygonoptera mucosa*
spinetail mobula	*Mobula japanica*	whale shark	*Rhincodon typus*
spinetail ray	*Bathyraja spinicauda*	whiptail stingray	*Dasyatis brevis*
spinner shark	*Carcharhinus brevipinna*	whiskery shark	*Furgaleus macki*
spiny butterfly ray	*Gymnura altavela*	white skate	*Bathyraja spinosissima*
spiny dogfish	*Squalus acanthias*	white skate	*Rostroraja alba*
spiny-tail round ray	*Urotrygon aspidura*	whitebrow skate	*Bathyraja minispinosa*
splendid lanternshark	*Etmopterus splendidus*	whitecheek shark	*Carcharhinus dussumieri*
spongehead catshark	*Apristurus spongiceps*	whitefin dogfish	*Centroscyllium ritteri*
spookfish	*Hydrolagus mitsukurii*	whitefin hammerhead	*Sphyrna couardi*
spotless catshark	*Halaelurus immaculatus*	whitefin topeshark	*Hemitriakis leucoperiptera*
spotless smooth-hound	*Mustelus griseus*	whitefinned swellshark	*Cephaloscyllium nascione*
spot-on-spot round ray	*Urolophus concentricus*	whitenose shark	*Nasolamia velox*
spottail shark	*Carcharhinus sorrah*	white-rimmed stingray	*Himantura signifer*
spotted eagle ray	*Aetobatus narinari*	whitesaddled catshark	*Scyliorhinus hesperius*
spotted estuary smooth-hound	*Mustelus lenticulatus*	whitesnout guitarfish	*Rhinobatos leucorhynchus*
spotted houndshark	*Triakis maculata*	whitespotted bambooshark	*Chiloscyllium plagiosum*
spotted legskate	*Anacanthobatis marmoratus*	whitespotted bullhead shark	*Heterodontus ramalheira*
spotted numbfish	*Narcine timlei*	white-spotted skate	*Okamejei cerva*
spotted ratfish	*Hydrolagus colliei*	whitespotted smooth-hound	*Mustelus palumbes*
spotted ray	*Raja montagui*	whitetail dogfish	*Scymnodalatias albicauda*
spotted round ray	*Urolophus maculatus*	whitetip reef shark	*Triaenodon obesus*
spotted stingaree	*Urolophus gigas*	whitetip weasel shark	*Paragaleus leucolomatus*
spotted wobbegong	*Orectolobus maculatus*	wide stingaree	*Urolophus expansus*
spreadfin skate	*Dipturus olseni*	winghead shark	*Eusphyra blochii*
starry ray	*Raja asterias*	winter skate	*Leucoraja ocellata*
starry skate	*Raja stellulata*	yellow guitarfish	*Rhinobatos schlegelii*
starry smooth-hound	*Mustelus asterias*	yellow stingray	*Urolophus jamaicensis*
starspotted smooth-hound	*Mustelus manazo*	yellowback stingaree	*Urolophus sufflavus*
stingray	*Urotrygon serrula*	yellowspotted catshark	*Scyliorhinus capensis*
straight-tooth weasel shark	*Paragaleus tengi*	yellowspotted skate	*Leucoraja wallacei*
striped catshark	*Poroderma africanum*	zebra bullhead shark	*Heterodontus zebra*
striped smooth-hound	*Mustelus fasciatus*	zebra shark	*Stegostoma fasciatum*
striped stingaree	*Trygonoptera ovalis*	zonetail butterfly ray	*Gymnura zonura*
swellshark	*Cephaloscyllium ventriosum*		
Sydney skate	*Okamejei australis*		
taillight shark	*Euprotomicroides zantedeschia*		
Taiwan angelshark	*Squatina formosa*		
Taiwan gulper shark	*Centrophorus niaukang*		
Taiwan saddled carpetshark	*Cirrhoscyllium formosanum*		
Tasmanian numbfish	*Narcine tasmaniensis*		
tasselled wobbegong	*Eucrossorhinus dasypogon*		
tawny nurse shark	*Nebrius ferrugineus*		
thintail thresher	*Alopias vulpinus*		
thornback guitarfish	*Platyrhinoidis triseriata*		

Elasmobranch Scientific Names Index

Elasmobranch Common Names Index