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Elasmobranch Husbandry Manual II:

Recent Advances in the Care of Sharks, Rays and their Relatives

Mark Smith, Doug Warmolts, Dennis Thoney, Robert Hueter, Michael Murray, and Juan Ezcurra (Editors)

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The Elasmobranch Husbandry Manual II:

Recent Advances in the Care of Sharks, Rays and their Relatives

Editors

Mark Smith
Doug Warmolts
Dennis Thoney
Robert Hueter
Michael Murray
Juan Ezcurra

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INTRODUCTION

It has been over a decade since the publication of the first **Elasmobranch Husbandry Manual**. In the intervening years progress in the field of elasmobranch husbandry has been impressive, with noteworthy advances in applied clinical techniques, diagnostic imaging, nutrition, behavioral conditioning, reproduction within aquariums, and collaborative field research. Researchers frequently cite the **Manual** as an invaluable resource that has aided the pursuit of these endeavors.

The announcement of a 2nd International Elasmobranch Husbandry Symposium and the publication of a second manual sparked immediate interest and anticipation. Hosted by the Monterey Bay Aquarium from 11 to 13 November 2013, the Symposium attracted 230 attendees from 24 countries. This volume contains core contributions from the Symposium and presents the material as the **Elasmobranch Husbandry Manual II: Recent Advances in the Care of Sharks, Rays and their Relatives**. The intent of this volume is to build on the foundation established by the first **Manual**, and to further advance the ethical management and welfare of elasmobranchs in human care.

As is the case for the first **Manual**, an electronic version of the **Elasmobranch Husbandry Manual II** can be downloaded free-of-charge from the Elasmobranch Husbandry website (elasmobranchhusbandry.org). Additional material presented at the Symposium has been compiled by Peter J. Mohan and published electronically as a special edition of Drum and Croaker, which also may be accessed free-of-charge through either the Elasmobranch Husbandry or Drum & Croaker (drumandcroaker.org) websites. Every presentation given at the Symposium has been captured in video format and can be accessed at the Animal Professionals website (animalprofessionals.com), providing an invaluable historical archive of the meeting.

Despite advances in elasmobranch conservation, many of the anthropogenic threats to wild populations prevail and the important role of public aquariums in communicating these challenges to the community persists. In addition, public aquariums continue to play a key role in adding to the body of knowledge about elasmobranchs by supporting *in situ* and *ex situ* conservation and research efforts.

The **Elasmobranch Husbandry Manual II** is deliberately inclusive of contributions from a broad spectrum of professionals, comprising dedicated and well-published academicians to individuals working in the field with little formal scientific training. This active choice was recognition of the urgent need for meaningful partnerships among laboratory researchers, field biologists and cultural institutions, such as public aquariums, to further advance elasmobranch conservation. We encourage the reader to embrace the **Elasmobranch Husbandry Manual II** in this cooperative spirit.

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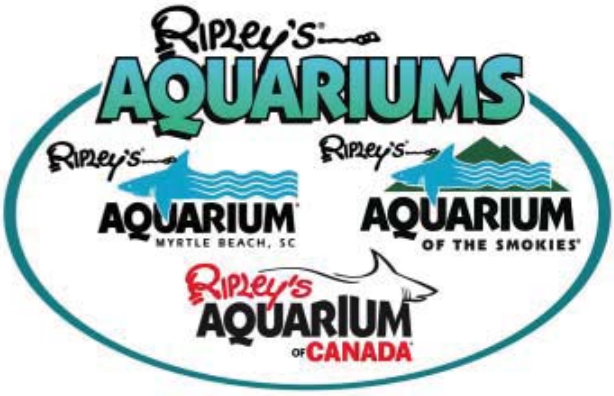

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DISCLAIMER

The Elasmobranch Husbandry Manual II: Recent Advances in the Care of Sharks, Rays, and their Relatives *is intended to present the current scientific and experimental understanding of the captive care of elasmobranchs in aquariums or research 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 anecdotal experience of working professionals, because relevant scientific literature is scant or non-existent. The contributors and editors can not 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.*

TABLE OF CONTENTS

	Introduction Mark Smith, Doug Warmolts, Dennis A. Thoney, Robert Hueter, Michael J. Murray, and Juan M Ezcurra	iii
Chapter 1	**Biology of the White Shark (*Carcharodon carcharias*) in Aquaria** Juan M. Ezcurra, Christopher G. Lowe, Henry F. Mollet, Lara A. Ferry, Michael J. Murray and John B. O'Sullivan	1
Chapter 2	**Notes on Husbandry of Whale Sharks, *Rhincodon typus*, in Aquaria** Rui Matsumoto, Minoru Toda, Yosuke Matsumoto, Keiichi Ueda, Miyuki Nakazato, Keiichi Sato, and Senzo Uchida	15
Chapter 3	**Husbandry of the Tiger Shark, *Galeocerdo cuvier*, at the Acuario de Veracruz, México** Raul Marin-Osorno, Juan M. Ezcurra, and John B. O'Sullivan	23
Chapter 4	**Collection, transport and handling of the Greenland shark, *Somniosus microcephalus*** Joseph M. Choromanski, Francis E. Bulman, Timothy H. Handsel, Robert H. George, John H. Batt, Chris Harvey-Clark, and Jeffrey J. Gallant	33
Chapter 5	**Collection, transport and husbandry of the blue shark, *Prionace glauca*** Núria Baylina, Nuno Pereira, Hugo Batista and João Correia	43
Chapter 6	**Capture, Transport and Husbandry of Silvertip Sharks, *Carcharhinus albimarginatus*** Paul Lötter and Lyle Squire, Jnr.	53
Chapter 7	**Notes on the husbandry of Manta Rays** Christopher Coco and Christian Schreiber	59
Chapter 8	**Husbandry of Bowmouth Guitarfish, *Rhina ancylostoma*** Mark J. Dvornak, Jolene Hanna, Jen Hazeres, and Scott Brehob	67
Chapter 9	**Husbandry of sawfishes** Stacia White, Katy Duke, and Lyle Squire, Jnr.	75
Chapter 10	**Husbandry of Whale Sharks** Christian Schreiber and Christopher Coco	87
Chapter 11	**Husbandry of freshwater stingrays** Jennifer Reynolds, Erica Hornbrook, George Stettner, and Richard Terrell	99
Chapter 12	**Elasmobranch quarantine** Catherine A. Hadfield and Leigh A. Clayton	113
Chapter 13	**Elasmobranch Mineral and Vitamin Requirements** Lisa A. Hoopes	135
Chapter 14	**Development of a body condition scoring tool for the spotted eagle ray, *Aetobatus narinari*** Tanya Y. Kamerman, Lisa Davis and Jane Capobianco	147
Chapter 15	**Preliminary evidence for a biennial feeding strategy related to reproduction in female sand tiger sharks, *Carcharias taurus* (Rafinesque, 1810)** Rob Townsend and Sam Gilchrist	153
Chapter 16	**Effects of noise and vibration on the behavior and feeding activity of whale sharks, *Rhincodon typus* (Smith, 1828), in Osaka Aquarium Kaiyukan** Takaomi Ito, Kiyoko Onda, and Kiyonori Nishida	159
Chapter 17	**Preliminary investigation of electricity in aquaria with elasmobranchs** Mary McCarthy and Julie Levans	169
Chapter 18	**Elasmobranch touch pools** Beth Firchau	177
Chapter 19	**Elasmobranchs and guest-immersive programs** Beth Firchau	191

Chapter 20 **Diving with and handling Elasmobranchs**
Allan Marshall, Beth Marshall, and Mark Smith 197

Chapter 21 **Training and conditioning of elasmobranchs in aquaria**
Jennie D. Janssen, Ashley Kidd, Ana Ferreira, and Skylar Snowden 209

Chapter 22 **Husbandry training of striped catshark, *Poroderma africanum* (Gmelin, 1789)**
Pamela Montbach and Jarrod Willis 223

Chapter 23 **Rescue, rehabilitation and release of a whale shark, *Rhincodon typus*, in the Arabian Gulf**
R. Bennett, S. Kaiser, R. Selvan, R. Hueter, J. Tyminski, and P. Lötter 229

Chapter 24 **Elasmobranch Deaccession**
Michael J. Murray 237

Chapter 25 **Shark Health Management**
Michael K. Stoskopf 245

Chapter 26 **Physical examination, blood sampling, and sedation of large elasmobranchs**
Keiichi Ueda, Makio Yanagisawa, Kiyomi Murakumo, Yosuke Matsumoto, Keiichi Sato, and Senzo Uchida 255

Chapter 27 **Emerging diseases of elasmobranchs in aquaria**
Alistair D. M. Dove, Tonya M. Clauss, David P. Marancik, and Alvin C. Camus 263

Chapter 28 **A review of pathologic findings in elasmobranchs: a retrospective case series**
Mark F. Stidworthy, Sue M. Thornton, and Robin James 277

Chapter 29 **Pharmacology of elasmobranchs: updates and techniques**
Natalie D. Mylniczenko and Tonya Clauss 289

Chapter 30 **Diagnostic imaging of elasmobranchs: updates and case examples**
Natalie D. Mylniczenko, Erin E. Culpepper, and Tonya Clauss 303

Chapter 31 **Ultrasound assessment of pregnant ribbontail stingrays, *Taeniura lymma* (Forsskål, 1775)**
Nuno Pereira, Hugo Batista and Núria Baylina 325

Chapter 32 **Chemical immobilization of elasmobranchs at uShaka Sea World, Durban, South Africa**
M. R. Penning, D. B. Vaughan, K. Fivaz and T. McEwan 331

Chapter 33 **Anesthetic trials using various species of elasmobranch at Nausicaá Aquarium**
Alexis Lécu, Renaud Herbert, Ludwig Coulier, Denis Tirmarche, and Stéphane Hénard 339

Chapter 34 **Removal of an intracoelomic hook via laparotomy in a Sandbar Shark (*Carcharhinus plumbeus*)**
Alexis Lécu, Renaud Herbert, Ludwig Coulier, and Michael J. Murray 349

Chapter 35 **Diagnosis and treatment of common reproductive problems in elasmobranchs**
Robert H. George, James Steeil, and Katherine Baine 357

Chapter 36 **The use of reproductive technologies in breeding programs for elasmobranchs in aquaria**
Jonathan Daly and Robert Jones 363

Chapter 37 **Reproduction of sand tiger sharks, *Carcharias taurus*, in aquaria: a framework for a managed breeding program**
Alan Henningsen, Elizabeth Claus, David Littlehale, Joseph Choromanski, Ian Gordon, and Kate Willson 375

Chapter 38 **Reproduction of the sand tiger shark, *Carcharias taurus* (Rafinesque, 1810), at UnderWater World SEA LIFE Mooloolaba from 1992 – 2012**
Kate Willson and Mark Smith 391

Chapter 39 **Small-scale elasmobranch husbandry and life support systems for research environments**
Blake K. Chapman and Clint A. Chapman 403

Chapter 40 **Aquarium reproduction, growth and husbandry of the Pacific angelshark, *Squatina californica***
Michael Grassmann, Christina J. Slager, and Melissa Schouest 411

Chapter 41 **Reproduction and husbandry of zebra sharks, *Stegostoma fasciatum*, in aquaria**
Lise Watson and Max Janse 421

Chapter 42 **Reproduction of spotted eagle rays, *Aetobatus narinari*, in aquaria**
Denise A. Swider, Allison L. Corwin, Tanya Y. Kamerman, Shannon L. Zimmerman, Gary C. Violetta, Jane Davis, and Max Janse 433

Chapter 43 **Blacktip reef shark reproduction and neonate survivorship in public aquaria**
Jean-Denis Hibbitt, Emma Rees, and Chris Brown 443

Chapter 44 **Fecundity, egg capsule size and neonate morphometrics of big skate, *Beringraja binoculata* (Girard, 1855)**
Michael J. Howard 451

Chapter 45 **Annually recurring parthenogenesis in a zebra shark, *Stegostoma fasciatum***
D. P. Robinson, W. Baverstock, A. Al-Jaru, K. Hyland, and K. A. Khazanehdari 459

General Index 465

Elasmobranch Husbandry Manual II: Recent Advances in the Care of Sharks, Rays and their Relatives, pages 1-14.

Chapter 1

Biology of the White Shark (*Carcharodon carcharias*) in Aquaria

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Abstract: Since 2004, the Monterey Bay Aquarium, California, has displayed six juvenile white sharks, *Carcharodon carcharias* (Linnaeus, 1758), in the 3,800 m^3 Outer Bay exhibit. Upon capture, the sharks (132 - 164 cm total length (TL) and 19.6 - 47.0 kg body mass (BM)) were held in a 13,800 m^3 ocean pen to initiate feeding prior to transport. Oxygen consumption rates of free-swimming *C. carcharias* during transports were analyzed, yielding one of the highest reported mass-specific muscle oxygen consumptions (MO_2) for any shark species (246 ± 13 mg O_2/kg/h). While on display (70 - 198 days), four of the *C. carcharias* fed consistently at a daily ration of 747 ± 46 g, or 1.62 ± 0.15% BM/day. One shark did not feed and was released after 11 days; another shark fed intermittently and was released after 55 days, but died immediately post-release. Mean mass growth rate for *C. carcharias* at the Monterey Bay Aquarium was 71.6 ± 8.2 kg/yr, with a corresponding mean dietary gross conversion efficiency of 27.1 ± 3.8%. The mean length growth rate (64.9 ± 8.5 cm/yr), was

approximately twice the rate estimated from a published von Bertalanffy growth function. All *C. carcharias* were fitted with pop-up archival satellite tags upon release, which provided evidence of post-release survivorship.

INTRODUCTION

Although the white shark, *Carcharodon carcharias* (Linnaeus, 1758), is the focus of much interest from both the research community and the public, the display and study of a living specimen has been difficult to achieve. The white shark is a large, top-level predator (Mollet et al., 1996) and has a cosmopolitan distribution in temperate and tropical seas (Compagno, 1984). It is a member of the family Lamnidae and is a regional endotherm, using vascular countercurrent heat exchangers (*retia mirabilia*) to maintain elevated tissue temperatures (Carey and Teal, 1969; Carey et al., 1981; Carey et al., 1982; Block and Carey, 1985; Carey et al., 1985; Goldman et al., 1996; Goldman, 1997; Bernal et al., 2001b; Bernal et al., 2005). Many studies have focused on the predatory behavior (Anderson et al., 1996; Long et al., 1996; Klimley et al., 1996; Klimley et al., 2001), reproductive biology (Pratt, 1996; Uchida et al., 1996; Francis, 1996; Saidi et al., 2005), age and growth (Cailliet et al., 1985; Hamady et al., 2014), and more recently, on large scale migrations of adult *C. carcharias* (Boustany et al., 2002; Bonfil et al., 2005; Bruce et al., 2006; Weng et al., 2007a; Domeier and Nasby-Lucas, 2008; Jorgensen et al., 2009). However, because of the difficulty in obtaining large specimens, researchers have not been able to study the metabolic demands and feeding rates of *C. carcharias*, which were theorized to be very high due to their elevated tissue temperatures and increased activity levels (Lowe and Goldman, 2001; Carlson et al., 2004). Similarly, until recent decades (Klimley, 1985; Lowe et al., 2012; Lyons et al., 2013), very little was known about neonates and juveniles of this species in the northeast Pacific Ocean.

Historically, the long-term display of a living *C. carcharias* has been attempted by many public aquaria with little success, due to the difficulty of acquiring healthy specimens and the challenges of transport (Hewitt, 1984). However, since 2004, the Monterey Bay Aquarium has displayed six juvenile *C. carcharias*. Furthermore, the advent of satellite archival tag technology has enabled the study of swimming behavior and the thermal niche of young-of-the-year (YOY) and juvenile *C. carcharias* in the Southern California Bight (Dewar et al., 2004; Weng et al., 2007b). This information facilitated the development of a program to further study juvenile *C. carcharias* in the wild and to place living specimens on display at the Aquarium where they could be viewed by millions of visitors. The Monterey Bay Aquarium white shark program consisted of a multi-year, incremental approach to the study of YOY in the Southern California Bight nursery area, which yielded important recommendations for the conservation of this species (Weng et al., 2007b; Lyons et al., 2013). In addition, the opportunity to handle live YOY *C. carcharias* displayed in the 3,800 m^3 Outer Bay exhibit, allowed aquarium staff to record oxygen consumption rates during transport, record feeding and growth, and determine energy budgets for this species. As appropriate, mean values are reported with (±) standard error.

CAPTURE AND HOLDING

YOY *C. carcharias* were captured in the Southern California Bight between August 2004 and August 2011, with the intent to place them on public display. After capture, *C. carcharias* were transported to a 40 m (diameter), 13,800 m^3 ocean pen (Figure 1) anchored along the coast of Malibu, California, to allow the sharks to recover from capture stress and begin feeding prior to transport to the Aquarium. These sharks, ranging in size from 132 - 164 cm total length (TL) and 19.6 - 47.0 kg body mass (BM) (Table 1), were bycatch in commercial gill nets, or specifically targeted using hook and line or commercial purse seine fishing methods. While *C. carcharias* were in the ocean pen (10 - 25 days), aquarium staff offered food items (e.g., chub mackerel, *Scomber japonicus* (Houttuyn, 1782); white croaker, *Genyonemus lineatus* (Ayres, 1855); Eastern pacific bonito, *Sarda chiliensis* (Cuvier, 1832); and Chinook salmon, *Oncorhynchus tshawytscha* (Walbaum, 1792)) by suspending them with monofilament from two lines intersecting the top of the enclosure, in an attempt to initiate feeding by the sharks. After a feeding event, a team of divers verified whether missing food items had broken free from the monofilament or had been bitten and then rejected by the shark, which occasionally occurred. Observations on the condition of the shark, and the evasive behavior of the shark towards the divers, also helped to

Table 1. Total length (m TL) and body mass (kg BM), upon introduction to the Outer Bay exhibit and upon release, of six YOY white sharks, *Carcharodon carcharias* (Linnaeus, 1758), displayed at the Monterey Bay Aquarium. Duration in captivity (days), growth in TL (cm/yr) and BM (kg/yr), and gross conversion efficiency (K_1) on a wet weight (WW) and energetic equivalent (EE) basis are included.

Carcharodon carcharias ID	Duration in aquarium (days)	Initial / final total length TL (m)	Initial / final body mass BM (kg)	Growth in length (cm/yr)	Growth in mass (kg/yr)	K_1 wet weight K_1 WW (%)	K_1 energetic equivalent K_1 EE (%)
#04-01	198	1.41 / 1.84	28.0 / 73.4	80.8	83.7	30.9	29.7
#06-01	138	1.64 / 1.87	47.0 / 77.6	56.3	80.9	28.2	26.1
#07-01	161	1.43 / 1.76	30.6 /63.6	76.2	74.8	33.3	31.4
#08-01	11	1.37 / 1.37	25.2 / 22.6	0	-86.3	—	—
#09-01	70	1.57 / 1.66	36.2 / 45.4	45.2	48	16	24.3
#11-01	55	1.32 / 1.38	19.6 / 23.6	33.8	26.6	18.2	20.3

determine if a shark was alert and ready for transport.

Transport and Oxygen Consumption Rates

When a *C. carcharias* was deemed a candidate for display, the ocean pen was 'pursed up' by a team of commercial fishers to allow aquarium staff to re-capture the shark and commence transport. Sharks scheduled for transport were not fed for at least 24 h prior to being removed from the ocean pen. After being captured with a hoop-net, the sharks were placed unrestrained in a 250 L vinyl shark box containing oxygenated seawater (~125% saturation) at 16°C. The shark box was

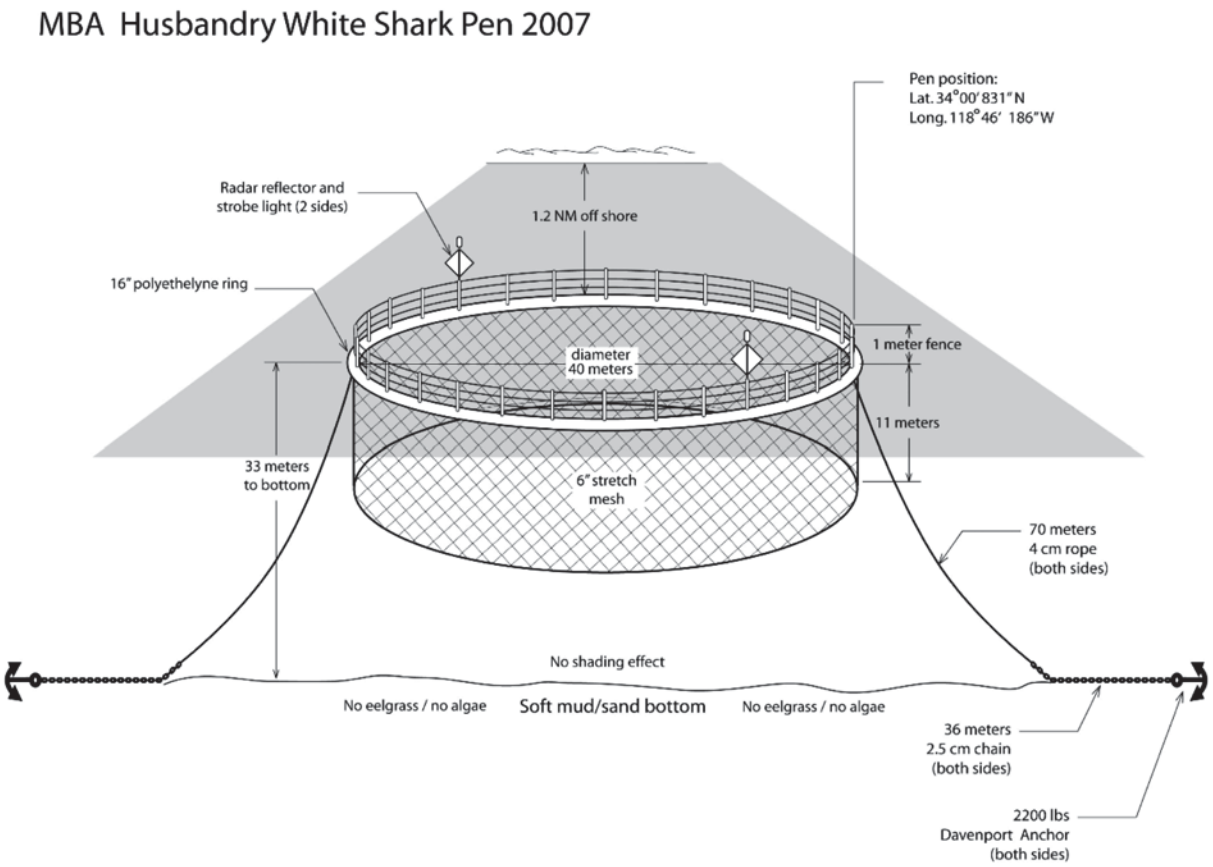

Figure 1. The ocean net-pen (40 m diameter x 11 m depth) used to hold YOY white sharks, *Carcharodon carcharias* (Linnaeus, 1758), after capture in the Southern California Bight. Sharks were allowed to recover from capture stress and begin feeding, prior to transport to the Monterey Bay Aquarium for public display.

equipped with a recirculating submersible pump (Model 4164 L/h Rule Industries, Massachsetts, USA), which provided ventilation during the 30 - 90 min transport, via boat, to the shore (a distance of 15 - 30 km). The sharks were then transferred to an 11.36 m^3 pelagic fish transport tank (Figure 2) mounted on the trailer of a commercial tractor for the drive to the aquarium (a distance of 524 km). Upon placement in the transport tank sharks were able to swim unimpeded, although at times they would rest on the bottom of the tank for periods of 30 - 90 sec during the ~6 h trip to Monterey. Only one shark was ever transported at a time.

Four of the six sharks were ultimately released directly from Monterey, due to their large size resulting from high growth rates in human care. The two smaller sharks (#08-01 and #11-01) that did not feed, or fed inconsistently, were transported back to southern California for release in the nursery area, to minimize potential interaction with predators.

Oxygen consumption rate data, muscle oxygen consumption (MO_2), was obtained for four YOY *C. carcharias* during transport from southern California to the Monterey Bay Aquarium between 14 September 2004 and 26 August 2009 (Ezcurra et al., 2012a). Oxygen levels in the transport tank were allowed to drop between 8 - 11 mg/L to quantify the MO_2 of the sharks. Oxygen administration to the transport tank was accomplished by delivering pure oxygen from a cylinder and regulator through flexible airline tubing to venturi injectors in the filtration piping. Water flow in the transport tank was driven through a filter loop by a ¾ HP Metric pool pump (Hayward Industries, Inc. New Jersey, USA), with the intent to induce the shark to swim constantly and, therefore, minimize stress caused by reduced circulatory efficiency and acidosis (Smith et al., 2004). Water temperature, pH, and oxygen concentration data were logged with a YSI Model 556 Multi Probe (YSI Incorporated, Ohio, USA), which sampled water off the main filtration loop. Water temperature in the transport tank was kept cooler (15.2 - 17.9°C; mean = 17.1 ± 0.3°C) than the ambient seawater surface temperature at the ocean pen (~20°C) to minimize thermal stress. Transport water pH declined (maximum decrease was 0.4 pH units) from the production of CO_2 by the shark during transport; however, pH always remained above 7.4 due to the large water volume in the transport tank.

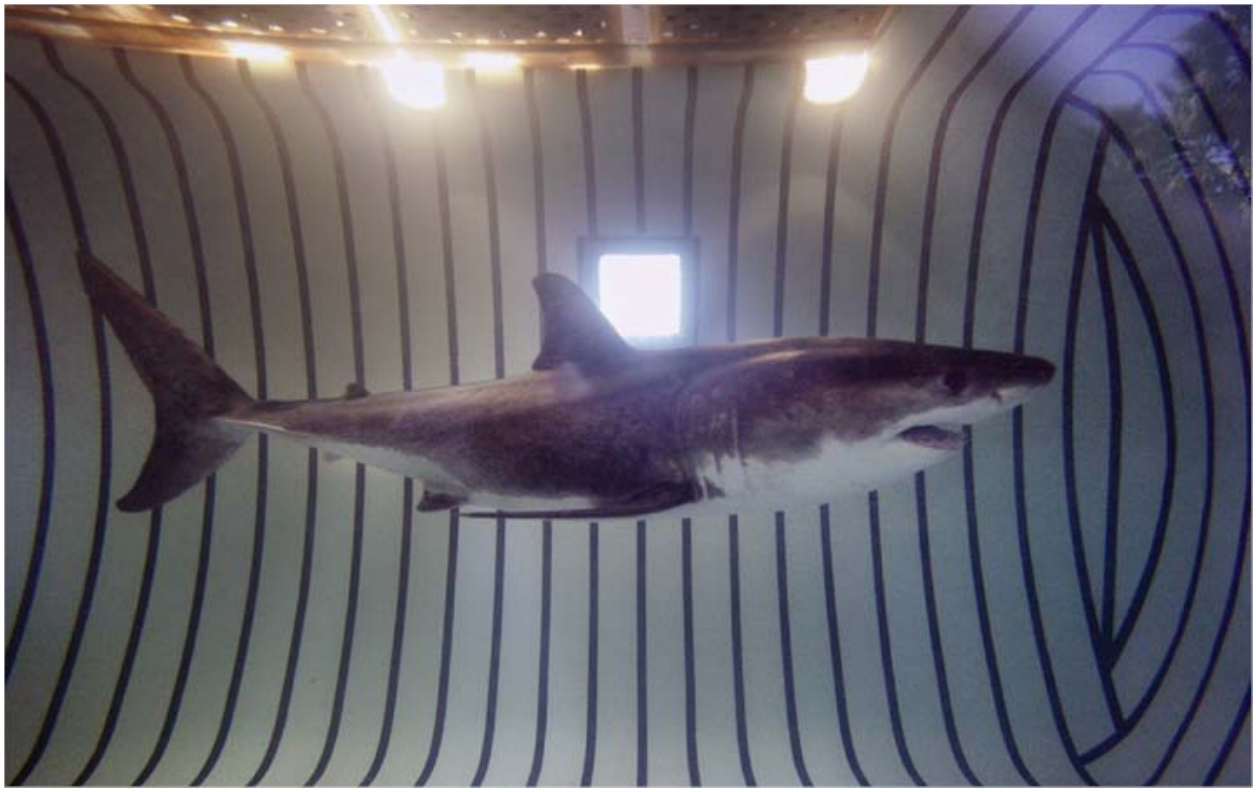

Figure 2. A YOY white shark, *Carcharodon carcharias* (Linnaeus, 1758), swimming in the pelagic fish transport tank (volume 11.36 m^3), on transit to the Monterey Bay Aquarium (see also Figure 2. from Murray, this volume).

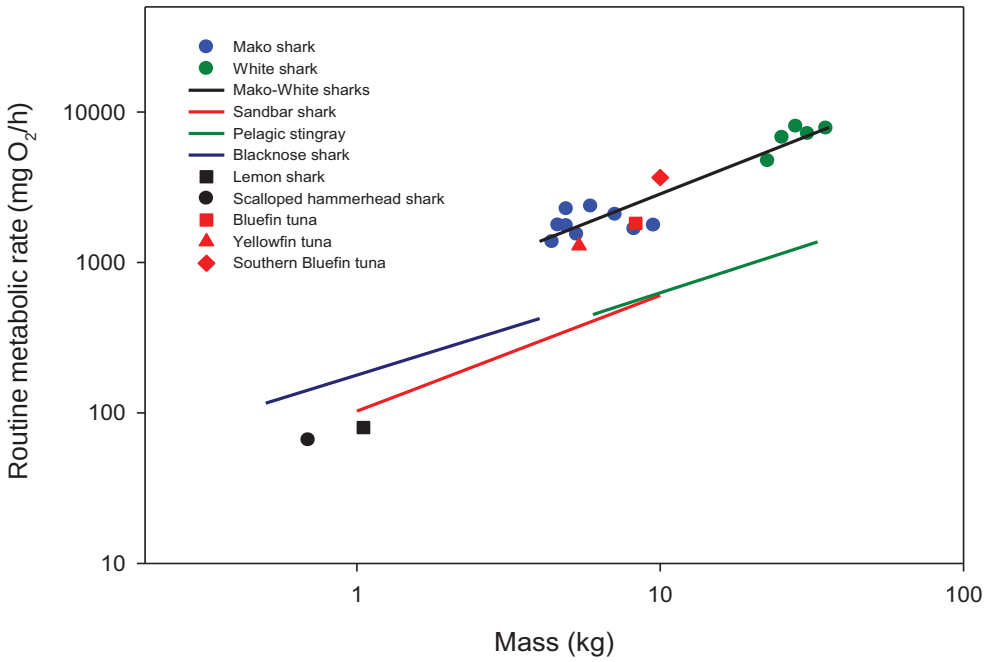

Figure 3. Routine metabolic rate (RMR, mg O_2/h) of the lamnid sharks, shortfin mako, *Isurus oxyrinchus* (Rafinesque, 1810) (Sepulveda et al., 2007), and white shark, *Carcharodon carcharias* (Linnaeus, 1758) (Ezcurra et al., 2012a), in relation to body mass (kg BM), compared to other active, pelagic sharks and tunas. Lines are for RMR calculated over a range of BM with the allometric equation $RMR = aM^b$. The line describing the RMR in relation to BM for lamnid sharks is $458.5M^{0.79}$, from log(a) = 2.66 ± 0.08 (SE) and slope (b) = 0.79 ± 0.08 (SE). Lemon shark, *Negaprion brevirostris* (Poey, 1868) (Bushnell et al., 1989), scalloped hammerhead shark, *Sphyrna lewini* (Griffiths & Smith, 1834) (Lowe, 2001), pelagic stingray, *Dasyatis violacea* (Bonaparte, 1832) (Ezcurra, 2001), blacknose shark, *Carcharhinus acronotus* (Poey, 1860) (Carlson et al., 1999) and sandbar shark, *Carcharhinus plumbeus* (Nardo, 1827) (Dowd et al., 2006), data were temperature adjusted to 17°C by using a Q_{10} of 2.3 (Carlson et al., 2004). Metabolic rates for yellowfin tuna, *Thunnus albacares* (Bonnaterre, 1788), Pacific bluefin tuna, *Thunnus orientalis* (Temminck & Schlegel, 1844) (Blank et al., 2007), and southern bluefin tuna, *Thunnus maccoyii* (Castelnau, 1872) (Fitzgibbon et al., 2006), were for fishes swimming at speeds of 0.65 - 1.0 body lengths/sec. Figure adapted from Ezcurra et al., 2012a.

The transport design yielded successful results with this highly active species. During most transports a declining trend was observed for mass-specific MO_2 values over time, presumably resulting from the shark's adjustment to the conditions of the transport tank. The mean mass-specific MO_2 for transported *C. carcharias* (246.5 ± 13.1 mg O_2/kg/h) was lower than values reported for the shortfin mako shark, *Isurus oxyrinchus* (Rafinesque, 1810), by Graham et al. (1990) and Sepulveda et al. (2007) at similar temperatures (369 ± 11 and 344 ± 22 mg O_2/kg/h, respectively). However, the lower mass-specific MO_2 in *C. carcharias* would be expected, as a result of the larger size of these sharks (four to five times greater in BM). To account for these differences in BM, metabolic rate data (mg O_2/h) for *I. oxyrinchus* (Sepulveda et al., 2007) and *C. carcharias* were pooled to estimate the scaling relationship for lamnid sharks described by the allometric equation $MR = 458.5 \times M^{0.79}$ (Figure 3). The resulting mass-scaling coefficient, b, for lamnids was very similar to the range of b values (mean = 0.8) reported for other elasmobranch and teleost species to date (Parsons, 1990; Ezcurra, 2001; Korsmeyer and Dewar, 2001; Dowd et al., 2006). An elevated metabolic rate for both of these endothermic lamnid sharks would be expected, based on their high activity level and capacity for high performance swimming (Bernal et al., 2001a; Donley et al., 2004). In addition, endothermy increases the efficiency of aerobic red muscles used in continuous swimming (Bernal et al., 2005) and has been theorized to provide a selective advantage for these species during 'bounce diving' forays into cool waters during oscillating vertical swimming patterns associated with prey search (Dewar et al., 2004; Sepulveda et al., 2004; Weng et al., 2007b).

Lamnids have metabolic rates much higher than those of ectothermic pelagic sharks, and are more similar to those of endothermic tunas (Figure 3). Although the transport temperature for *C. carcharias* in our study (17°C) was generally lower than that applied to other pelagic shark species, temperature adjustments of routine metabolic rate (RMR) reported for other sharks were performed using a Q_{10} value of 2.3 (Carlson et al., 2004). In addition, the BM of *C. carcharias* in our study (22.6 - 36.2 kg) was 2 - 30 times greater than that of sharks in other metabolic studies (Bushnell et al., 1989; Carlson et al., 1999; Lowe, 2001; Dowd et al., 2006). When differences in temperature and BM were taken into account, the RMR for lamnids was still approximately five times greater than that of ectothermic, obligate ram-ventilating sharks (Figure 3). Although they are more divergent, taxonomically, the metabolic rate for lamnids is closer to that of southern bluefin tuna, *Thunnus maccoyii* (Castelnau, 1872), Pacific bluefin tuna, *Thunnus orientalis* (Temminck & Schlegel, 1844) and yellowfin tuna, *Thunnus albacares* (Bonnaterre, 1788), at minimal swimming speeds (0.65 - 1.0 body lengths/sec) and similar experimental temperatures (Fitzgibbon et al., 2006; Blank et al., 2007; Figure 3). The convergent evolution of high performance swimming and endothermy in lamnid sharks and tunas has resulted in specialized morphology and physiology (i.e., streamlined body shape; internalized aerobic, red muscle capable of retaining metabolic heat; elevated enzyme activities associated with aerobic and anaerobic metabolism in white muscle; large gill surface area and low blood-water barrier thickness; and a circulatory system with a high oxygen delivery capacity to the tissues) in these distant groups (Bernal et al., 2001a; Bernal et al., 2001b; Korsmeyer and Dewar, 2001; Donley et al., 2004; Sepulveda et al., 2007).

Feeding While on Display

Upon arrival at the aquarium, YOY *C. carcharias* were weighed and measured (over the curve of the body and converted to straight length by linear regression), and placed on public display in the 3,800 m^3 Outer Bay exhibit. While on display, the sharks were offered food daily (Ezcurra et al., 2012b). Food items were individually weighed, tethered with cotton string to attach them to a feeding pole, and fed to the shark. This method reduced the potential for the shark to bite the feeding pole during burst swimming, or for other tank inhabitants taking food items offered to the *C. carcharias*. If a food item was shredded and dropped, an estimate of the weight of the food ingested was made. Mean daily ration for each week was calculated as a percent of BM per day (% BM/day) for each seven-day period that the sharks were on display (Figure 4). Food items fed to *C. carcharias* were sent for caloric analysis at NP Analytical Laboratories (St. Louis, Missouri, USA). Energy equivalent of total food consumption was determined by the feeding rate (% BM/day), energy content of the food type, and total duration in the aquarium. In addition, two dead YOY *C. carcharias* (whole fish)—by-catch of the commercial fishery—were sent to NP Analytical Laboratories for caloric analysis to determine the energy content of their tissue, allowing simplified energy budgets to be created for each shark.

After introduction into the Outer Bay exhibit, most YOY *C. carcharias* fed within seven days and continued feeding regularly. To initiate feeding for some sharks, live foods, such as *S. japonicus* or California skate, *Raja inornata* (Jordan & Gilbert, 1881), were offered near the surface. Feeding was occasionally difficult to initiate because of intraspecific variation in *C. carcharias* swimming behavior. For example, one shark spent the majority of its time near the bottom of the Outer Bay exhibit for the first month on display. Feeding the relatively small YOY *C. carcharias* in an exhibit with larger Galapagos sharks, *Carcharhinus galapagensis* (Snodgrass & Heller, 1905), scalloped hammerhead sharks, *Sphyrna lewini* (Griffiths & Smith, 1834), and pelagic teleosts, such as *T. orientalis*, *T. albacares*, and common dolphinfish, *Coryphaena hippurus* (Linnaeus, 1758), with BM in the range 40 - 140 kg, posed significant challenges due to aggressive competition for food. Therefore, food introduction to *C. carcharias* required surface pole feeding to reduce the potential for collisions with other fishes and to accurately record food intake. Within a month, YOY *C. carcharias* became the most aggressive animals in the Outer Bay exhibit and, at times, would charge at other fishes if they came close to the feeding station. Predatory behavior was observed in two of the five YOY *C. carcharias* that started feeding while in the Outer Bay exhibit. During its final five weeks on display, shark #04-01 fatally attacked two tope sharks, *Galeorhinus galeus* (Linnaeus, 1758), consuming the caudal fin and caudal peduncle of one of the sharks. It should be noted that this food intake was not included in the calculations below, as the estimated weight amounted to <1% of the diet on a wet weight (WW) basis. Shark #04-01 was also observed chasing *S. lewini* and *C. galapagensis*, prior to being released. *C. carcharias* #09-01 chased *S. lewini* and attacked a male *C. galapagensis*, which prompted aquarium staff to release the

aggressor. No other predatory behavior by *C. carcharias* was observed by aquarium staff or verified by security cameras mounted in the exhibit, which recorded continuously to a digital video recorder.

Four of the six YOY *C. carcharias* fed within a week of introduction into the Outer Bay exhibit, and fed consistently while on display (69 - 198 days). Four sharks showed a strong feeding preference for *O. tshawytscha*, which comprised 80.9 - 96.6% WW of their diet. Other individual feeding preferences were also noted, as one shark ate primarily *S. japonicus* (99.1% WW of diet). In addition to *O. tshawytscha* and *S. japonicus*, other bony fishes, such as sablefish, *Anoplopoma fimbria* (Pallas, 1814), striped bonito, *Sarda orientalis* (Temminck & Schlegel, 1844), and albacore, *Thunnus alalunga* (Bonnaterre, 1788) were regularly fed. Food items were supplemented with Vita-Zu Shark/Ray II tablets (Mazuri, Purina Mills LLC, Nutrition International LLC, Richmond, USA). As necessary, the antibiotic Enrofloxacin (Baytril, Bayer Corp., Whippany, New Jersey) was added to the food of sharks that had superficial wounds under the jaw, and at the tip of the rostrum, resulting from contact with the exhibit walls and windows.

Mean daily ration for each week showed an oscillating pattern with feeding peaks and troughs, and a significant decreasing trend over the period that the *C. carcharias* were on display (Figure 4). A period of approximately 3 - 8 weeks corresponded to a single cycle of feeding peak and trough, with greater oscillations tending towards longer cycles. Mean daily ration for each week for the four consistently feeding sharks ranged between a low of 0.2% BM/day to a high of 3.5% BM/day. The decreasing trend in mean daily ration as % BM/day was consistent, and the slope for linear regression was statistically significant ($p < 0.0013$). After 15 - 23 weeks on display a large decrease in the oscillations of feeding ration for sharks occurred, which stabilized near 0.9% BM/day. The grand mean daily ration for each week for all four sharks was 1.62 ± 0.15% BM/day.

The peak in mean daily ration recorded for *C. carcharias* in this study was similar to reported values for active pelagic sharks (Salini et al., 1999; Bush and Holland, 2002; Marin-Osorno et al., this volume). Daily ration reported by Salini et al. (1999) for juveniles of three species of carcharhinid sharks—2.9 - 3.4% BM/day for the whitecheek shark, *Carcharhinus dussumieri* (Müller & Henle, 1839), Australian blacktip shark, *Carcharhinus tilstoni* (Whitley, 1950), and sicklefin

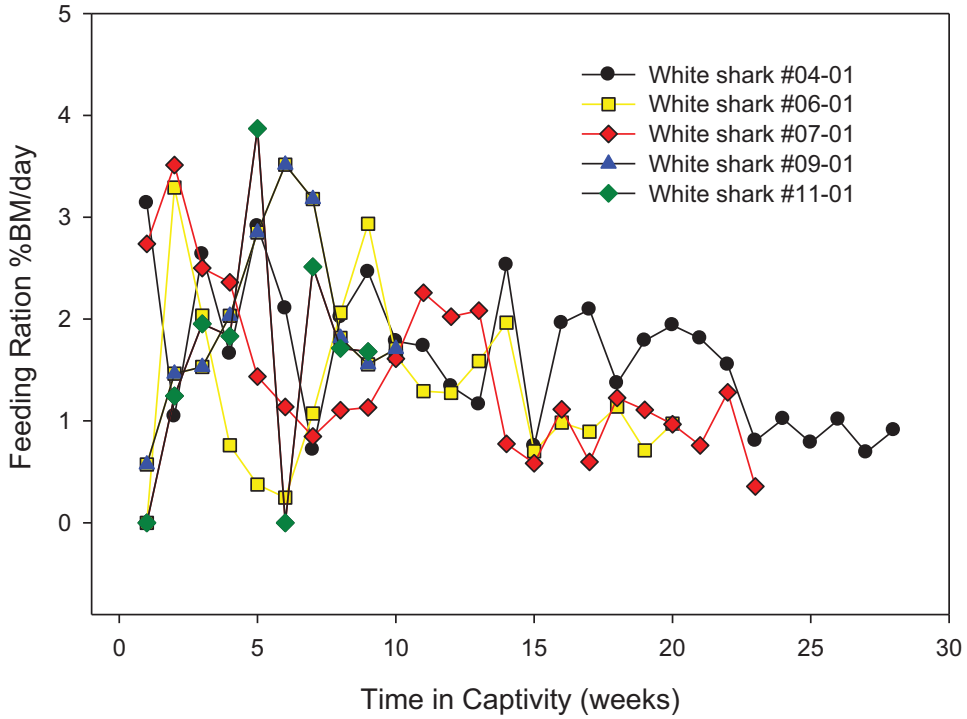

Figure 4. Feeding ration as percent body mass per day (% BM/day) for five aquarium YOY white shark, *Carcharodon carcharias* (Linnaeus, 1758), displayed at the Monterey Bay Aquarium from 14 September 2004 to 25 October 2011. Figure adapted from Ezcurra et al., 2012b.

lemon shark, *Negaprion acutidens* (Rüppell, 1837)—were similar to the initial peaks in mean daily ration of YOY *C. carcharias* in our study (3.1 - 3.5% BM/day). However, the feeding experiments done by Salini et al. (1999) were of very short duration (10 days) and, therefore, do not provide an indication of the range in daily ration over longer time periods in aquaria for these species. Peak feeding ration of tiger sharks, *Galeocerdo cuvier* (Péron & Lesueur, 1822), on display for up to seven years at the Acuario de Veracruz (Mexico) only peaked at 1.9% BM/day for sub adult sharks (1.9 - 3.1 m TL), even though these active, pelagic sharks were held at higher temperatures (Marin-Osorno et al., this volume). The highest daily ration calculated for an ectothermic, obligate ram-ventilating shark (3.54% BM/day) was for *S. lewini* (Bush and Holland, 2002), which was of similar value to that observed in our study. The peak in mean daily ration of *C. carcharias* in our study was lower than the daily ration calculated by Wood et al. (2009) for *I. oxyrinchus* (4.6% BM/day). However, daily ration for *I. oxyrinchus* was derived by calculating both the energetic needs of the shark and the amount of food required to satisfy those needs. The dominant food item in the diet of *I. oxyrinchus* in the northeast Atlantic ocean, as determined by gut content analysis, was bluefish, *Pomatomus saltatrix* (Linnaeus, 1766), which is 63% lower in energy content (4,800 KJ/kg) than the preferred food for *C. carcharias* in our study, *O. tshawytscha* (7,536 KJ/kg). If the main prey item of *I. oxyrinchus* were energetically denser, then the estimate of daily ration would have been lower. *C. carcharias* #09-01 fed on the least energetically dense food item (99% WW *S. japonicus*; 4,312 KJ/kg), and of all the sharks in our study, it fed at the highest mean daily ration for the entire period it was on display (2.03% BM/day).

The YOY *C. carcharias* (#08-01) displayed in 2008 fed only once during the eleven days it was on display. It was released after this period due to health concerns. This shark fed in the ocean pen, and although it adjusted to the Outer Bay exhibit very well, only consumed ~400 g of *O. tshawytscha* during a single feeding in the Aquarium. While on display, the shark lost 2.6 kg (10.3%) of its original 25.2 kg BM. Loss in BM due to starvation was used to estimate a maintenance ration of 0.94% BM/day for YOY *C. carcharias*, or 405 Kcal/day. This example corroborates the high metabolic demands of this active, endothermic species, and further, that feeding must occur frequently to maintain body condition and accommodate growth; a finding that is further supported by recent research on *C. carcharias* in the field, made possible by recent advances in tagging technology (Semmens et al., 2013). The inappetent *C. carcharias* (#08-01) was transported from the aquarium back to southern California, where it was tagged with a pop-off archival satellite tag (PSAT) and released. Data from the PSAT indicated that the shark survived for at least 30 days post-release (for more detail refer to Weng et al., 2012).

The *C. carcharias* acquired in 2011 (#11-01) did not adjust well to the Outer Bay exhibit and its duration on display was the shortest (55 days) for any of the five sharks that repeatedly fed while at the Monterey Bay Aquarium. Although this shark fed repeatedly, it did not feed consistently and stopped feeding for a period of 10 days (Figure 4). Observing the behavior of the shark suggested that repeated contact with the exhibit wall eroded its integument, facilitating the development of cellulitis and/or a systemic bacterial infection. An intramuscular antibiotic injection of Amikacin (5 mg/kg est. BM) was administered with a pole syringe. The antibiotic dose was supplemented with a vitamin B-complex, anecdotally reported to be an appetite stimulant. Prior to administration of the second dose, 48 hours later, the shark started to feed spontaneously. Rather than risk the development of tank margin avoidance behavior by the shark, due to repeated use of the pole syringe, the therapeutic regimen was changed to an oral administration of Enrofloxacin (5 mg/kg est. BM, po q 24 h), placed within the food.

After the shark resumed feeding, a change in its swimming behavior was observed. The shark appeared agitated and began to make contact with the exhibit walls more frequently. At 55 days post-introduction, the shark was released due to health concerns associated with ongoing damage from contact with the exhibit walls. The *C. carcharias* was transported to southern California for release, fitted with a PSAT, and an acoustic tag was surgically implanted into the coelomic cavity of the shark. Results from the PSAT showed that the shark died almost immediately after release. Since the shark could not be recovered, a post-mortem examination was not possible. Review of the available subjective and objective data suggested three possible causes of death: (1) non-consumptive post-release misadventure with a large predator; (2) fatal iatrogenic injury, such as hemorrhage, associated with the implantation of the acoustic tag; or (3) an irreversible cascade of metabolic aberrations associated with an acid-base imbalance caused by the cumulative effects of extraction from

exhibit, transport to southern California, pre-release handling/sampling/tagging, and transport to the actual release location. While any of these options were impossible to confirm, it was the considered opinion of the Aquarium team that the third explanation was most probable. This shark appeared to be an outlier from other YOY *C. carcharias* displayed at the Monterey Bay Aquarium, as evidenced by its feeding regime and growth rate, which was notably lower than any of the four consistently feeding *C. carcharias* on display (Table 1).

Growth While on Display

YOY *C. carcharias* on display in the Outer Bay exhibit grew in length (TL) at a rate twice that calculated for YOY *C. carcharias* in the wild, and growth rate correlated directly to time on display (Table 1). Shark #04-01 was on display for the longest period of time (198 days) and grew the fastest (~80.8 cm/yr); whereas shark #09-01, which was on display for only 70 days, grew at the slowest rate (~45.2 cm/yr). Mean growth for all four sharks was ~64.9 ± 8.5 cm/yr, whereas the predicted value calculated by a von Bertalanffy growth function for this species was 35 cm/yr (Cailliet et al., 1985). It should be noted that annual growth rates were estimates based on the time sharks were on display in the Aquarium, which was less than a year in every case. A YOY *C. carcharias* that had been tagged and released, as part of the Monterey Bay Aquarium's field research program in southern California, was recaptured 405 days later and, on measurement, yielded a growth rate of 33.4 cm/yr (Lowe, unpublished results). This observed growth rate was in close agreement with the published von Bertalanffy growth function (Cailliet et al., 1985), and corroborates the proposition that growth rates in the aquarium were approximately twice those in the wild, supporting the assertion by Van Dykhuizen and Mollet (1992) that growth of animals under human care may be higher than growth in the wild by a factor of two to three.

Growth in BM followed the expected general trend of highest growth for sharks that had the highest total food consumption (Kcal/day) while on display. *C. carcharias* #04-01 had the highest annual growth in BM (83.7 kg/yr) and had the highest total food consumption (261,979 Kcal in 198 days), followed by shark #06-01 (80.9 kg/yr and 201,410 Kcal in 138 days). Shark #07-01 grew at a slower rate (74.8 kg/yr and 180,232 Kcal in 161 days), and shark #09-01 grew the slowest and consumed the least amount of calories while on display (48.0 kg/yr and 64,882 Kcal in 70 days). The mean growth in BM for all four sharks was 71.6 ± 8.2 kg/yr, which is three times greater than the calculated rate (23 kg/yr) for first year of growth using the von Bertalanffy growth function from Cailliet et al. (1985), and allometric equations for TL and BM for *C. carcharias* from Mollet and Cailliet (1996). Growth of sharks in aquaria can be variable and depends on a variety of factors, with feeding ration thought to be a main determinant (Taylor and Wisner, 1989). Lamnid sharks have been reported to have high growth rates (e.g., 39 cm/yr for *I. oxyrinchus*) in the first year of life (Bishop et al., 2006), and a diet of energy rich salmonids has also been correlated with faster growth in lamnid sharks (Goldman and Musick, 2006). Many studies report faster growth in aquaria for species like the sandbar shark, *Carcharhinus plumbeus* (Nardo, 1827), bull shark, *Carcharhinus leucas* (Müller and Henle, 1839), lemon shark, *Negaprion brevirostris* (Poey, 1868), and *S. lewini* (reviewed in Mohan et al., 2004). However, studies on the broadnose sevengill shark, *Notorynchus cepedianus* (Péron, 1807) (Van Dykhuizen and Mollet, 1992) and the sand tiger shark, *Carcharias taurus* (Rafinesque, 1810) (Govender et al., 1991), have reported growth rates in aquaria that may be similar to growth rates in the wild. For this reason, caution should be exercised when interpreting growth rates of animals in human care.

Despite inherent variation, aquaria growth studies can add to our understanding of the biology of elasmobranchs that are difficult to acquire, or study long-term in the field (Mollet et al., 2002; Cailliet and Goldman, 2004; Mohan et al., 2004; Matsumoto et al., this volume). For example, a study using PSAT data from migrating *C. carcharias*, with validation from swimming kinematics of captive YOY *C. carcharias* at the aquarium, has shown that decreasing body condition factor, resulting in fewer lipid stores in the liver, decreases buoyancy in vertically migrating sharks, presumably resulting in an increased overall energy consumption (Del Raye et al., 2013).

Conversion Efficiency

Mean gross conversion efficiency (K_1) on a wet weight basis (K_1 WW) for YOY *C. carcharias* in the Aquarium was 27.1% ± 3.8, which is similar to reported values for wild sharks in the first year of life. K_1 WW was lowest for shark #09-01 (16.0%), and much higher for sharks #04-01, #06-01 and #07-01 (30.9%, 28.2% and 33.3%, respectively). Van Dykhuizen and Mollet (1992) reported K_1 values of 25 - 40% for *N. cepedianus*

in aquaria for the first year of life. K_1 values of 10-25% have been reported for other elasmobranchs and teleosts (Wetherbee and Cortes, 2004). Mean gross conversion efficiency (K_1) as energetic equivalents (EE) closely aligned with K_1 WW for three of the four aquarium *C. carcharias* (#04-01, #06-01 and #07-01). The largest difference between the two observed K_1 values (8.3%) was in shark #09-01. This shark had a higher K_1 EE (24.3%) resulting from the lower caloric value of its primary food item, *S. japonicus*. K_1 values have been reported to decrease with increasing age, and have been observed to be affected by the level of food consumption (Wetherbee and Cortes, 2004). Endothermy in lamnid sharks has been theorized to speed up digestive processes, shortening the time for gastric evacuation and enhancing food intake (Cortes and Gruber, 1990). Total evacuation time (36 h) was observed for *C. carcharias* while on display at the aquarium, which was faster than values reported for gastric evacuation in ectothermic sharks (Wetherbee and Cortes, 2004). In addition, peak feeding ration and K_1 values observed in this study were among the highest reported for any shark species to date.

Energy Budgets While on Display

Mean energy budget for an organism can be described by the following equation:

$$C = G + M + W$$

Whereby, C = total food consumption, G = somatic growth, M = metabolic cost, and W = energy loss to waste.

The energetic content of the two YOY *C. carcharias*, collected as bycatch and sent for caloric analysis, was determined by bomb calorimetry to be 1.715 Kcal/g or 7.18 KJ/g of tissue. This value was multiplied by the gain in BM for each shark, while they were on display, to determine the energy investment into somatic growth (G), which ranged from 24.3 - 29.7% (mean = 27.9% ± 1.6) of total food consumption (C). The largest and most variable component of an energy budget is the energy invested in metabolic costs (M) (Lowe, 2002). The amount of energy invested into M, as a proportion of C, ranged from 41.6 - 48.7% (mean: 45.1% ± 1.6). Energy loss to waste (W) was assumed to be constant (27% x C) (Wetherbee and Gruber, 1993; Wetherbee and Cortes, 2004). The mean energy budget for all four sharks while on display is thus described by the equation:

C = G + M + W: 100% = 27.9% + 45.1% + 27%

The mean energy budget for *C. carcharias* at the Aquarium reflected a high investment in G and was likely the result of higher growth rates for YOY sharks, compared to mature animals. For example, Schmid and Murru (1994) reported much lower growth (7% of C) for mature *C. leucas* in aquaria. Higher growth rates and potentially lower metabolic costs resulting from aquarium conditions are also presumably the cause for greater investment in G. In addition, the individual *C. carcharias* that did not feed (#08-01) incurred a BM loss that was 0.94% BM/day, which was equivalent to 405 Kcal/day, or 33% of the mean total consumption for all sharks (1215 Kcal/day). This estimate of M was comparable to our estimate from the energy budget, and was analogous to the value (44.9% of C) reported from an energy budget for pelagic stingrays, *Dasyatis violacea* (Bonaparte, 1832), in aquaria (Ezcurra, 2001). Of course, caution should be exercised when comparing M values determined from an energetics model derived for elasmobranchs in human care, to values of M reported from indirect calorimetry. This difference is highlighted by lower estimates of M for *C. leucas* from a bioenergetics study (Schmid and Murru, 1994), when compared to M values reported for carcharhinids derived from indirect calorimetry.

CONCLUSIONS

C. carcharias is a difficult species to display for a variety of reasons, not least of which includes the significant time and financial investment of capturing, holding in the field, and transporting these animals. Once a healthy *C. carcharias* is on display, individual differences in behavior further complicate husbandry efforts. However, our experience with this species has provided a better understanding of appropriate general husbandry practices, as well as the metabolism, feeding and growth rates, and energetic demands of this highly active, endothermic species. Quantifying these biological parameters in aquaria has also informed research methods, which, in conjunction with technological advances in the field of physiological telemetry, have further elucidated the high energetic demands of this species in the wild (Lowe and Goldman, 2001; Del Raye et al., 2013; Semmens et al., 2013). This research is a crucial mechanism to better understand the biology of, and needed conservation efforts for, this top-level predator (Weng et al., 2012; Lyons et al., 2013).

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Chapter 2

Notes on Husbandry of Whale Sharks, *Rhincodon typus*, in Aquaria

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Abstract: The whale shark, *Rhincodon typus* (Smith, 1828), is the largest fish in the world, yet individuals have been successfully kept in aquaria for over 18 years at the Okinawa Churaumi Aquarium. Using morphometric data from entangled and stranded *R. typus* (n = 33), an allometric equation was developed to estimate total length (TL) using the more readily obtainable pre-first dorsal length (PD1). Estimated TL using the allometric equation was in strong agreement with field measurements of living *R. typus.* This allometric method for TL estimation allows accurate tracking of growth rate in *R. typus*, and is useful for biological surveys and husbandry applications.

INTRODUCTION

Although it is well established that the whale shark, *Rhincodon typus* (Smith, 1828), is the world's largest living elasmobranch, their maximum size, and size at maturity, has not been definitively established. Very few of the largest specimens reported have been methodically measured (Castro, 2011). The largest individual measured (a male from Bombay, India) was 12.2 m in total length (TL) (Karbhari and Josekutty, 1986). Some reliable records of the body mass (BM) of large *R. typus* are available. BM was recorded in Okinawa for specimens of 3.94 m, 5.1 m, and 5.55 m TL, which weighed 468 kg, 1,400 kg and 1,973 kg, respectively (Uchida, 1983; Uchida, 1995). The heaviest BM on record for *R. typus* is 16,000 kg, from a 10.6 m TL individual landed in Taiwan (Joung et al., 1996).

Techniques for the care and scientific observation of *R. typus*, which have been maintained at the Okinawa Churaumi Aquarium since 1980, have been continuously refined and improved as our knowledge of animal biology and husbandry practices has grown. Accurate measurements of TL and BM are essential for *R. typus* husbandry in order to determine the appropriate food ration, medicine dosage rates, and size of equipment for transport, as well as for the assessment of maturity status.

Here, we present information on the capture, transport, and husbandry procedures used for *R. typus* at the Okinawa Churaumi Aquarium. We also propose simple, precise formulas to estimate the TL and BM of *R. typus* using an allometric equation that extrapolates from measurements of a key morphological feature on the shark.

ANIMAL HUSBANDRY

Since 1980, *R. typus* (n = 33) have been rescued from adverse situations and transported to the Okinawa Churaumi Aquarium. Of these animals, 31 individuals (94% of the total) were accidentally caught in set fishing nets along the coast of Okinawa (Table 1). The other two individuals were stranded on the coast or in the Port and were rescued by aquarium staff. Most incidences occurred in the summer, from May to August, and about 70% of animals were retrieved from water 24 - 28ºC. The condition of each individual varied; some were weak and exhibited hypophagia and poor physical condition, while others suffered from

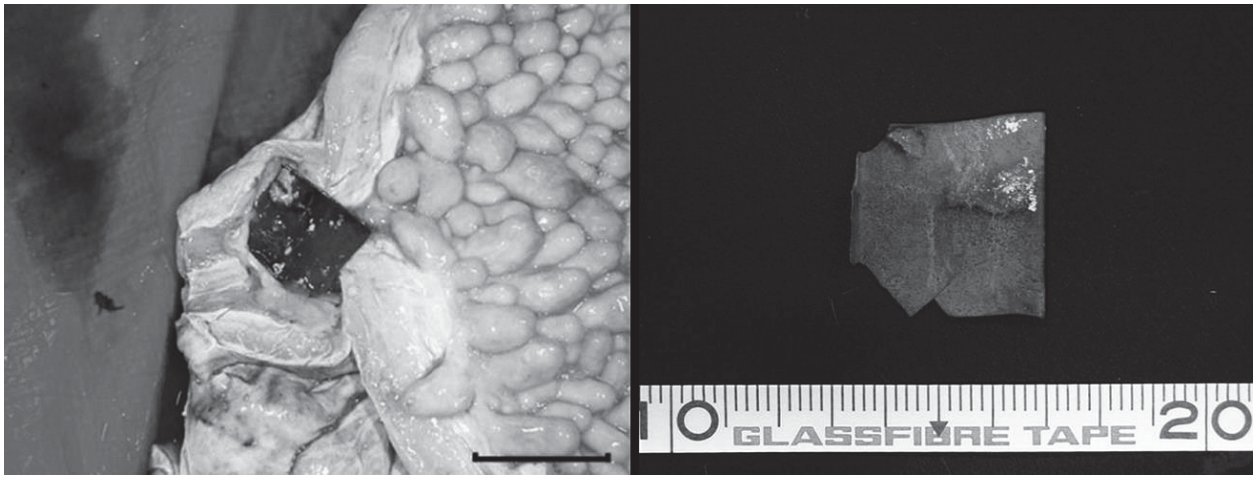

Figure 1. Aspiration of an alien substance from a whale shark, *Rhincodon typus* (Smith, 1828), which died after treatment in the sea pen, showing: (a) obstruction of the pylorus by a piece of plastic plate [scale bar = 5 cm]; and (b) an enlarged image of the causative agent.

Table 1. Whale sharks, *Rhincodon typus* (Smith, 1828), caught or stranded in Okinawa between 1980 and 2010, showing date caught, location and method of capture, sex and total length (TL).

No.	Sex	TL (cm)	Date of Capture	Method of Capture	Location of Capture
1	M	510	July 17, 1980	set net	Pacific Ocean
2	M	394	May 18, 1981	set net	East China Sea
3	F	443	July 5, 1982	set net	East China Sea
4	M	528	May 24, 1984	set net	Pacific Ocean
5	M	540	July 10, 1984	set net	East China Sea
6	M	425	July 10, 1985	set net	Pacific Ocean
7	M	425	June 8, 1986	set net	Pacific Ocean
8	M	365	June 29, 1986	set net	Pacific Ocean
9	M	633	June 6, 1992	set net	Pacific Ocean
10	F	435	September 11, 1992	set net	East China Sea
11	M	450	April 30, 1993	set net	East China Sea
12	M	450	May 8, 1993	set net	East China Sea
13	M	370	September 6, 1994	stranded	East China Sea
14	M	460	March 11, 1995	set net	East China Sea
15	M	310	May 6, 1997	set net	East China Sea
16	M	325	July 30, 1998	set net	East China Sea
17	F	620	May 25, 2000	set net	East China Sea
18	M	440	June 27, 2000	set net	East China Sea
19	M	430	June 29, 2000	set net	East China Sea
20	M	423	January 5, 2001	set net	East China Sea
21	F	490	May 7, 2001	set net	East China Sea
22	M	490	May 8, 2001	set net	East China Sea
23	M	535	May 26, 2001	set net	East China Sea
24	F	660	May 5, 2005	set net	East China Sea
25	M	910	April 18, 2006	stranded	East China Sea
26	F	590	June 27, 2006	set net	East China Sea
27	M	290	June 30, 2006	set net	East China Sea
28	F	305	July 4, 2006	set net	East China Sea
29	F	490	August 30, 2007	set net	East China Sea
30	M	370	July 11, 2007	set net	Pacific Ocean
31	M	440	November 8, 2007	set net	Pacific Ocean
32	F	500	April 2, 2008	set net	East China Sea
33	F	710	March 31, 2009	set net	East China Sea

the effects of low water temperature, infection, or accidental ingestion of foreign substances (Figure 1). Not all *R. typus* caught in set nets were transported to the aquarium; after taking detailed measurements and tissue biopsies, some sharks were tagged and released.

A shipping container designed for transporting *R. typus* was used to transfer animals from the set net to a sea pen. The containment vessel had holes at the front, or anterior end, and seawater flowed into the container when towed. The inside dimensions of the container were 2.3 × 7.4 × 1.7 m high, and it contained a double-hinged gate at the rear to provide easy access. Before taking *R. typus* aboard, a sedative (Midazolam; 4.5 mg/kg) was administered to make the shark easier to manage (Ueda et al., this volume). Once the shark was inside the container it was measured, injected with a microchip for ID, and a blood sample was taken. While the shipping container was towed toward the sea pen, staff members observed the animal's respiration rate and monitored dissolved oxygen concentration inside the container.

Offshore net enclosures, or sea pens, were used to acclimatize *R. typus* to aquarium conditions. The sea pens were 20 × 30 × 10 m deep. Water temperature at the sea pens ranged from an average of 21.4 ± 0.3ºC in the winter to 28.8 ± 0.6ºC in the summer.

Once acclimatized, *R. typus* were transported to the Okinawa Churaumi Aquarium and transferred to the Kuroshio tank (35 × 27 × 10 m deep; volume = 7,500 m^3). The Kuroshio tank contained seawater, supplied at a rate of 2,000 m^3/h. Seawater was abstracted from a location 300 m off the coast and at a depth of 20 m. Seawater was filtered and circulated at a rate of 12 turnovers/day. Water temperature in the tank ranged from an average of 21.4 ± 0.4ºC in the winter to 28.5 ± 0.7ºC in the summer.

MEASURING WHALE SHARKS

Precise TL and BM measurements were needed to assess growth rates, arrange transportation, and provide appropriate quantities of food and medicine to *R. typus*. However, the sheer size of R. *typus* makes measurement of live specimens difficult. In the field, researchers have estimated TL by comparing the animal to the length of a boat (Heyman et al., 2001; Hobbs et al., 2009), or snorkelers photographed with the shark (Graham and Roberts, 2007; Norman and Stevens, 2007; Bradshaw et al., 2008). Rohner et al. (2011) established a method of estimating TL in the field using laser photogrammetry and measuring the distance from the fifth gill opening to the first dorsal fin.

R. typus in aquariums offer the advantage of being easily observed at any time and allow for direct measurement of specific body parts (note: caudal fin flexion makes it difficult to measure posterior parts of the body). Further to the methods listed above, we propose formulas to estimate TL and BM from direct measurements of the pre-first dorsal length (PD1) (i.e., the length from snout tip to first dorsal fin origin). These formulae have been verified through back-analysis of morphometric data taken in the field and historical data extracted from animal records at the Okinawa Churaumi Aquarium.

Total length (TL) estimation

A total of 33 *R. typus* were caught near Okinawa from 1979 to 2010. Each shark was assessed using 66 morphometric characteristics, which included data from Uchida (1983). Terminology and abbreviations followed Compagno (2001). A geometrical (GM) regression provided certain functional relationships of size-on-size in white sharks, *Carcharodon carcharias* (Linneaus, 1758) (Mollet and Cailliet, 1996). In this study, a GM regression was applied to all morphometric data, and we chose to further assess characteristics that were highly correlated with TL, including a t-test to analyze sex-based differences. The most suitable characteristics for measurement of a living animal were chosen from the set that passed both tests. A predicted TL range was then calculated using these chosen characteristics.

There was a high coefficient of determination ($R^2 > 0.9$, $p < 0.01$) for eight morphometric characters: pre-first dorsal length (PD1), pre-second dorsal length (PD2), pre-caudal length (PRC; aka PCL in the literature), pre-pelvic length (PP2), pre-anal length (PAL), length of dorsal caudal margin (CDM), mouth width (MOW), and interorbital space (INO) (Table 2). A sex-based difference in MOW was revealed by t-test, so it was excluded from further analysis. CDM, PD2, and PRC were excluded, as caudal fin locomotion caused the body immediately posterior to the second dorsal fin to move, leading to discrepancy in meas-

Table 2. Morphometric characteristics of whale sharks, *Rhincodon typus* (Smith, 1828), highly correlated with total length (TL).

Morphometric characteristic	Number	Slope	95% upper limit	95% lower limit	Intercept	R^2	*t*-value	*p* value
Pre-first dorsal length (PD1)	33	0.964	1.060	0.868	0.443	0.925	19.488	<0.01
Pre-second dorsal length (PD2)	33	1.003	1.096	0.909	0.181	0.934	21.003	<0.01
Pre-caudal length (PRC)	33	1.005	1.096	0.914	0.098	0.938	21.626	<0.01
Pre-pelvic length (PP2)	32	1.029	1.139	0.919	0.235	0.916	18.055	<0.01
Pre-anal length (PAL)	32	1.045	1.165	0.926	0.065	0.904	16.767	<0.01
Length of dorsal caudal margin (CDM)	31	1.120	1.224	1.016	0.310	0.939	21.166	<0.01
Mouth width (MOW)	32	1.057	1.164	0.949	0.737	0.924	19.111	<0.01
Interorbital space (INO)	32	1.135	1.248	1.023	0.479	0.928	19.693	<0.01

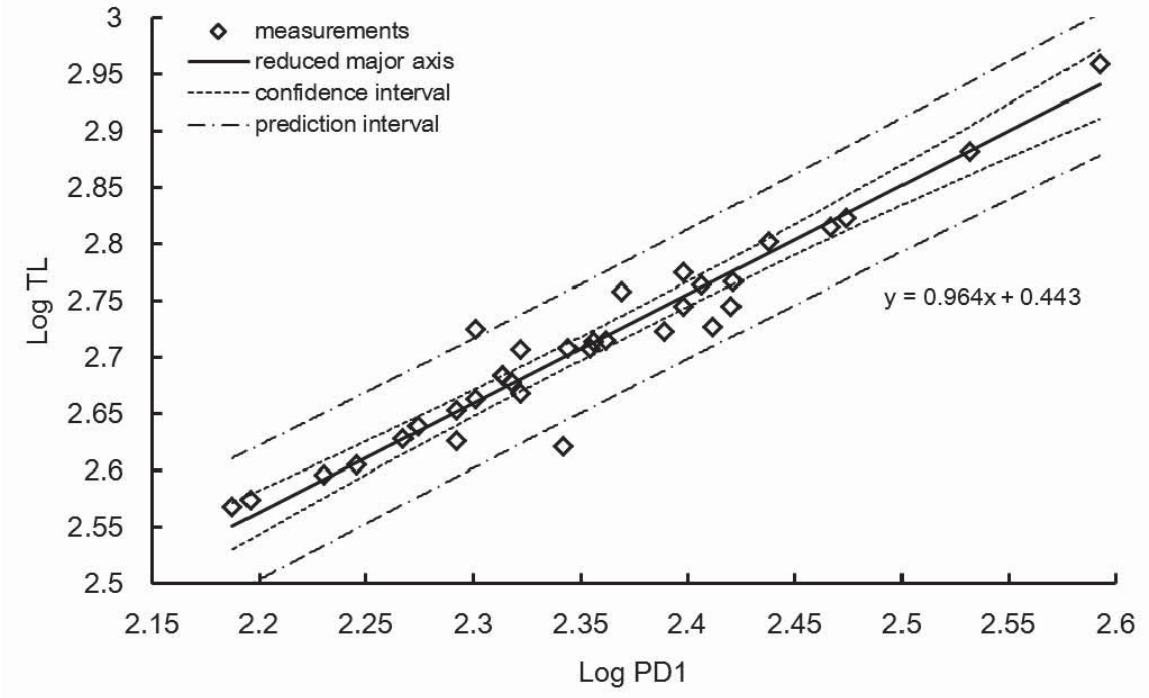

Figure 2. Correlation between logarithmic pre-first dorsal length (PD1) and total length (TL) in the whale shark, *Rhincodon typus* (Smith, 1828), by reduced major axis. Slope: y = 0.964x + 0.443.

urement. To measure PP2 and PAL divers had to swim to the ventrum of the shark, where it was difficult to obtain a precise measurement. INO was located on a stable portion of *R. typus*, but for live animals it was perpendicular to the body axis and difficult to measure. Pre-first dorsal length (PD1), or snout tip to first dorsal fin origin, is on the midline of the body, on the dorsal surface, and is not affected by the motion of *R. typus* through the water. For these reasons PD1 was deemed the most appropriate morphometric characteristic to use as an estimate of TL.

To estimate TL from underwater measurements of live *R. typus* we used the following equation:

log TL cm = 0.964 log PD1 + 0.443

The prediction interval of TL from this equation is approximately +14% and -12% of TL, according to the regression equation. The 95% confidence interval of the regression line and the 95% prediction interval of TL are shown in Figure 2.

Verification of TL estimation equation

The accuracy of the estimation equation was verified using five live *R. typus* at the aquarium. The sharks were captured and allowed to relax, after which TL and PD1 were measured. The estimated values of TL calculated by the formula were compared with the actual measurements of each shark.

There were minor differences between the measured and estimated values of TL (Table 3), while *R. typus* was free swimming (n = 5), with error ratios within the range of 1.4% to 3.3%. All five estimated TLs fell within the prediction interval (Figure 3), supporting the use of PD1 to estimate TL. In addition, error ratios of estimated TL to measured TL (Table 3), while *R. typus* was restrained (n = 3), were within the range of 0.3% - 2.4%. All three estimated TLs fell within the prediction interval (Figure 3). We therefore conclude that our equation provides a useful way to estimate TL from measurements of PD1 in restrained and swimming *R. typus*.

Body mass (BM) estimation

It is essential to know body mass (BM) for specimens in aquaria, in order to determine the correct amount of food ration and medication dosage rates. We analyzed morphometrics and weight data from eight *R. typus* that had been stranded or were fishery by-catch (7 males and 1 female), all of which died within a few weeks of rescue. Using this data we developed a BM estimation equation (Figure 4) as follows:

$$\mathbf{BM = 4.510\ TL^{3.280}}$$

Table 3. Comparison of field-measured total length (TL) of whale sharks, *Rhincodon typus* (Smith, 1828), at the Okinawa Churaumi Aquarium, to TL estimated by allometric equation.

No.	Estimated TL (cm)	Measured TL (cm)	Error[a] (cm)	Error / Measured TL[b]	Measuring Conditions
1	547	540	7	1.38%	free swimming
2	567	575	-8	-1.37%	free swimming
3	569	554	15	2.76%	free swimming
4	687	665	22	3.30%	free swimming
5	698	710	-12	-1.72%	free swimming
6	497	509	-12	-2.36%	fixed in container
7	702	710	-8	-1.14%	fixed in container
8	758	761	-3	-0.34%	fixed in container

[a] Error indicates gap between measured and estimated total length (TL)
[b] Error/Measured TL indicates ratio of error to actual measured total length (TL)

Although it was not tested for sex-based differences, the results of this equation were used as the basis for husbandry protocols.

Husbandry application based on total length (TL) and body mass (BM) estimates

Two species of frozen krill, *Euphausia superba* and *Euphausia pacifica*, were provided as food for *R. typus* at the Okinawa Churaumi Aquarium. Some additional food items were added or replaced, depending on the season (e.g., Japanese anchovy, *Engraulis japonicus* (Temminck & Schlegel, 1846), and sakura shrimp, *Sergia lucens*). *R. typus* were fed ~1% of their BM/day. An 8.5 m TL male *R. typus*, with an estimated BM (from the equation above) of 5,100 kg, consumed 100,384 ± 12,943 kJ per day; and a 7.1 m TL female, with an

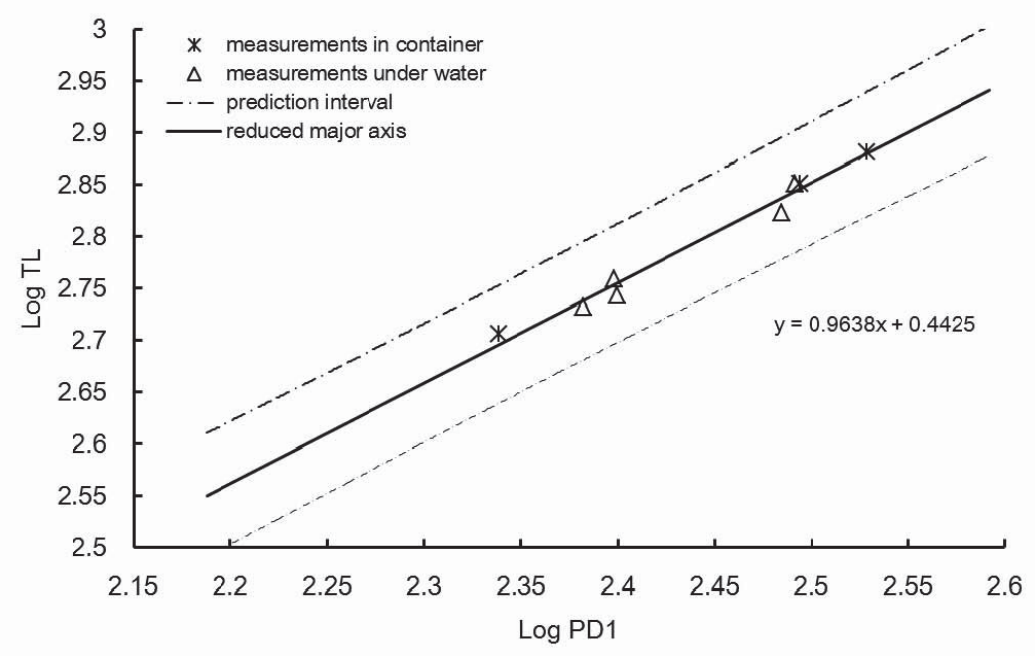

Figure 3. Comparison of field-measured total length (TL) of whale sharks, *Rhincodon typus* (Smith, 1828), to TL estimated by allometric equation. Slope: y = 0.964x + 0.443.

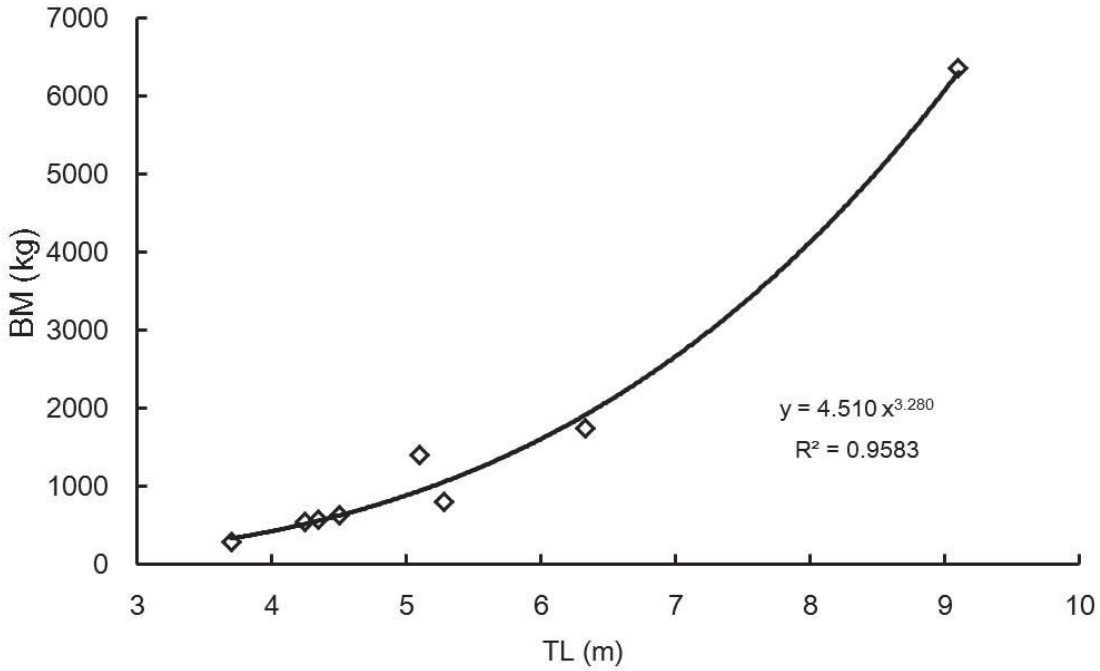

Figure 4. Relationship between field-measured total length (TL) and body mass (BM) in seven whale sharks, *Rhincodon typus* (Smith, 1828). Slope: $y = 4.510x^{3.280}$.

estimated BM (from the equation above) of 2,800 kg, consumed 60,511 ± 11,252 kJ per day. The ratios of caloric intake to BM of the two individuals were 19.7 kJ/kg and 21.6 kJ/kg, for the male and female *R. typus*, respectively. As a comparison, Motta et al. (2010) projected that the energy intakes of two wild *R. typus*, measuring an estimated 6.6 m and 4.4 m TL, were 28,121 kJ and 14,931 kJ, respectively. By applying our BM equation to the *R. typus* in Motta's study we estimated that the 6.6 m TL shark weighed 1,811 kg and the 4.4 m TL shark weighed 595 kg, and that the estimated daily energy intake for these animals was 12.7 kJ/kg and 25.1 kJ/kg, respectively.

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Chapter 3

Husbandry of the Tiger Shark, *Galeocerdo cuvier*, at the Acuario de Veracruz, México

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Abstract: Improved husbandry techniques at the Acuario de Veracruz have led to the successful long-term display of adult (up to 310 cm total length, TL) tiger sharks, *Galeocerdo cuvier* (Péron & Lesueur, 1822). A large exhibit volume (>900 m^3), rockwork on perimeter walls, a diverse diet, targeted pole feedings and a high feeding ration (up to ~2% body mass (BM)/day) are essential for managing the swimming behavior and energy requirements of this pelagic species. Following long-term exhibition within an aquarium, successful release of an adult male *G. cuvier* (308 cm TL) into the Gulf of Mexico was achieved and verified with a PSAT (pop-up satellite archival tag). Data from the PSAT indicated behavior consistent with a healthy wild *G. cuvier* during a period of 90 days post-release.

INTRODUCTION

During the past few decades, interest in maintaining the tiger shark, *Galeocerdo cuvier* (Péron & Lesueur, 1822), in aquaria for public display has increased dramatically. As a result, 20 institutions around the world, including one zoo and 19 aquariums, have exhibited this species. The display longevity of *G. cuvier* has increased from just 60 days in the 1960's to the present record of nearly eight years (2,885 days), recorded at the Acuario de Veracruz (Crow and Hewitt, 1988; Dehart, 2004; www1). Increased *G. cuvier* longevity in aquaria has been achieved through technological advances in exhibit design and a better understanding of the biology of elasmobranchs, both *in situ* and in human care.

However, the husbandry of *G. cuvier* continues to present challenges; predominantly related to their swimming behavior, difficulty feeding, and behavioral variability between individuals. *G. cuvier* in aquaria commonly swim against bare concrete exhibit walls (areas devoid of rockwork), which causes abrasions on the leading edge of the pectoral and caudal fins. These injuries may promote bacterial or fungal infections, which, if not treated, can lead to anorexia and even death in the spe-

cies (Dehart, 2004). Feeding *G. cuvier* in community exhibits is challenging and requires a heightened level of aquarist attention and effort. *G. cuvier* in aquaria may be out-competed for food by other dominant elasmobranch species, large teleosts, and even sea turtles, which are typically prey for this species in the wild (Marín-Osorno, 2002). Individual *G. cuvier* show a high degree of intraspecific variability in their adaptability to aquaria. Despite current best practice some *G. cuvier* do not adapt well to aquaria while others thrive, maintaining good health and living for long periods on display.

This chapter summarizes data from nine *G. cuvier* maintained at the Acuario de Veracruz from 1992 to 2013 (Table 1). Although further husbandry improvements are required to advance the successful exhibition of *G. cuvier*, we present methods that have allowed the Acuario de Veracruz to substantively improve display longevity for this species. It is hoped that this information may prove useful for other aquaria interested in displaying this challenging, but alluring, species.

ACQUISITION AND ACCLIMATIZATION

We have observed that *G. cuvier* are resilient to capture and transport stress in comparison with other carcharhinid sharks. The majority of *G. cuvier* obtained by the Acuario de Veracruz were collected opportunistically by artisanal fishers, many of whom had limited equipment or infrastructure to maintain the sharks. These fishers had limited knowledge of advanced transport methods used for large elasmobranchs. Sharks were captured on longline sets and transported in flooded compartments near the bow of small panga-type boats (< 7.6 m in length) with outboard motors. During transport, seawater was delivered to the shark via submersible pump or bucket. When fishers contacted the Acuario de Veracruz, staff were able to assist transport of the shark using a circular fiberglass tank.

During the 1990's *G. cuvier* acquired by the Acuario de Veracruz were placed in an ocean pen located on the Isla de Sacrificios, Veracruz. The two-meter deep pen was located over sand substrate and was of an irregular, oval shape with a volume of 2,000 m^3. Transport time to the pen ranged in duration from 40 - 120 minutes. Sharks were allowed to adapt to the semi-captive environment of the pen for two to eight months before being transported to the aquarium for display.

Capture protocols were revised in 2008 and newly acquired *G. cuvier* were introduced directly into the exhibition aquarium from the wild. Animals were transferred from the boat to the Aquarium via shark stretcher and hoist. Specimens were given a prophylactic buffered freshwater bath, to remove ectoparasites. Sharks were then temporarily moved into a holding tank, adjacent to the main exhibit, for hook removal and measurement of total length (TL). Occasionally sharks would rest on the bottom of the tank during this procedure. In these cases, divers would 'walk' the shark to assist in specimen recuperation. If the shark showed limited response to the 'walking' procedure, it was left to rest on the bottom of the tank until it began to swim on its own, at which point it was moved into the exhibit proper. The period of time that sharks rested on the bottom of the exhibit ranged from 2 - 12 h. Sharks were considered to have successfully acclimatized to the exhibit at the onset of feeding, which generally occurred 2 - 15 days post introduction.

EXHIBITION AND HUSBANDRY

Exhibit and system design

When the Acuario de Veracruz first displayed *G. cuvier* (1992 - 2002), they were maintained in the Reef Exhibit, a doughnut shaped aquarium of 1,250 m^3 volume. It was observed that *G. cuvier* did not flourish in this aquarium and a purpose-built exhibit to better accommodate the biology of the species was constructed. *G. cuvier* at the Acuario de Veracruz were thereafter maintained in a purpose-built kidney-shaped, multi-species, Shark Exhibit (26 m x 16 m x 4.5 m deep; 919 m^3 volume) with black, painted concrete walls. Artificial rockwork reefs had a low profile, providing adequate space for the sharks to swim above. Low-relief fiberglass rockwork panels were hung along the concrete walls to keep the *G. cuvier* from rubbing along the smooth perimeter of the exhibit. These panels were movable, allowing flexibility for adjustment to the observed swimming behavior of individual sharks. Exhibit lighting to simulate daytime consisted of nine 250 W LED lamps, while night lighting consisted of three 250 W LED lamps. The exhibit had two main viewing windows and an acrylic tunnel. An immersive educational 'shark program' was in operation, where visitors, enclosed within a protective acrylic box, were lowered into the center of the exhibit.

The exhibit was supplied with natural seawater and was maintained as a semi-open system, with a flow-through of 2.65 m^3/min. System water was

Table 1. A summary of nine tiger sharks, *Galeocerdo cuvier* (Péron & Lesueur, 1822), maintained at the Acuario de Veracruz from 1992 to 2013, showing gender, total length, hardiness, temperament and time on display. All specimens were caught by longline, transported by boat, and maintained in semi-open seawater systems (PSAT = pop-up satellite archival tag).

Specimen name	Gender	Total Length (cm)	Time in Holding Pen (Sacrificios Island)	Exhibit	Hardiness	Temperament	Display Longevity (years)
Delta	Female	308	2 months	Reef exhibit / Shark exhibit	Adapted readily	Extrovert; occasionally aggressive; dominant towards conspecifics during feeding	7.9
Viernes	Male	308	8 months	Shark exhibit	Adapted well	Non-aggressive	5.2
Beto	Male	310		Shark exhibit	Adapted well	Timid; opportunistic predation on juvenile conspecific and *M. atlanticus*	5+
Tito	Male	240		Shark exhibit	Delicate	Timid; aggressive in self-defense	3
Isabel	Female	240		Shark exhibit	Delicate	Timid; non-aggressive	1.9
Benito	Male	286		Shark exhibit	Adapted readily	Extrovert; occasionally aggressive; dominant towards conspecifics during feeding	0.5+
Claudia	Female	140		Shark exhibit	Delicate	Timid; non-aggressive	0.25
Diego	Male	150		Shark exhibit	Adapted readily	Extrovert; non-aggressive	0.22
Paty	Female	170	2 months	Reef exhibit	Delicate	Timid; non-aggressive	0.22

recirculated and processed by two protein skimmers, four mechanical filters, and two biological filters, before returning to the exhibit via gravity flow. A 10% water exchange occurred each week through regular life support system maintenance, and the exhibit was cleaned weekly by two divers (supervised by an attendant at the surface, as well as a paramedic).

Exhibit water quality parameters were consistent with those reported by Dehart (2004) as ideal for *G. cuvier* and other tropical elasmobranchs. Temperature was maintained in the range 22 - 25°C, and pH was maintained at ~8.0.

At the time of writing, there are two *G. cuvier* (286 cm and 310 cm TL) within the exhibit, along with 11 sandbar sharks, *Carcharhinus plumbeus* (Nardo, 1827) (180 - 200 cm TL), four nurse sharks, *Ginglymostoma cirratum* (Bonnaterre, 1788) (160 - 220 cm TL), and a community of 20 teleost species.

Feeding

G. cuvier should be fed on a daily basis (*ad libitum*), with a diverse, high-calorie diet. The food regime should be based on prey items *G. cuvier* would typically encounter in the wild, while accommodating the specific energy demands and feeding preferences of individual specimens. *G. cuvier* feeding protocols must be flexible and adaptable, otherwise there is an elevated risk of inter- and intra-specific aggression. Where possible, *G. cuvier* should be fed from dedicated feeding stations to reduce competition for food and to provide behavioral enrichment for the sharks.

G. cuvier at the Acuario de Veracruz were fed via a feeding pole (106 cm in length) from a different feeding station ("east end" of the exhibit) to other species ("west end" of the exhibit). Food items fed to *G. cuvier* ranged in size from 20 - 90 cm in length and 400 - 9,000 g in weight. If a shark did not consume the entire food item immediately, it was subject to competition from other shark species in the exhibit. Therefore food was offered to other species first, prior to feeding *G. cuvier*, in an effort to reduce competition for food items.

At the Acuario de Veracruz, *G. cuvier* feeding required substantial effort and care, especially when new animals were introduced. On occasion aquarists had to place food directly into the mouth of the shark via a feeding pole or by hand. Juvenile sharks typically began feeding sooner than sub adult or adult sharks. It was critical, during the period immediately following the introduction of a new specimen to the aquarium, to monitor sharks for signs of interest in feeding—e.g., rapid swimming across the area where food was offered and/or swimming in tight circles near the bottom of the exhibit in search of food items. Several specimens began feeding from the bottom of the exhibit and were encouraged to the surface, where food was more easily delivered using feeding poles. Other *G. cuvier* searched for food intensively throughout the exhibit, which facilitated an immediate introduction to surface feeding with a pole. Sharks were ultimately conditioned to use a dedicated feeding station, which further aided effective food delivery.

If a juvenile *G. cuvier* was regularly out-competed for food, it was removed from the exhibit and held in a separate pool until it was better able to integrate with the larger animal community. In the event of inappetence, force-feeding *G. cuvier* is not recommended. However, specimens should not be allowed to reach an advanced state of starvation, evidenced by a sunken ventrum, a loss of dorsal musculature, and the appearance of a disproportionately large head relative to the rest of the body. While oscillations in feeding rates are normal, it is estimated that a *G. cuvier* of ~190 cm TL should not go more than 25 days without food. A shark that has been anorectic for this period will have consumed the lipid reserves in its liver, negatively affecting buoyancy, compromising swimming performance, and further elevating their already high energy demands (Del Raye et al., 2013).

Diet

G. cuvier in the wild have a broad diet and are considered to be generalist feeders (Compagno, 1984). Unsurprisingly, at the Acuario de Veracruz, display specimens accepted a wide variety of foods. Despite this observation it was also noted that *G. cuvier* could tolerate a diet of low diversity, as long as it contained a preferred food item of high nutritional value.

Diet offered to *G. cuvier* should include a wide range of teleosts, smaller elasmobranchs and other marine organisms. Although *G. cuvier* eat marine mammals, turtles and marine birds in the wild, it is considered that these organisms may not be appropriate for use in aquaria from an ethical and legal standpoint. *G. cuvier* at the Acuario de Veracruz were therefore offered a variety of calorie-dense food items consisting of teleosts (i.e., little tunny, *Euthynnus alletteratus* (Rafinesque, 1810), and various jacks, Carangidae), elasmobranchs, and occasionally beef, chicken or horse, as an alternative to ma-

rine mammals and marine birds (Table 2). A supplemental multivitamin (Vita-Zu Shark/Ray tablets, Mazuri®, Purina Mills LLC, Nutrition International LLC, Richmond, USA) was also given. *G. cuvier* preferred food items with large amounts of red, vascularized muscle or lipids (e.g., tuna, shark liver, beef, and chicken). Food provisioning was dependent on the size and activity levels of individual sharks.

G. cuvier is an active species known to undertake long distance migrations (Holland et al., 1999; Kneebone et al., 2008; Meyer et al., 2009). This species therefore requires an elevated feeding ration to maintain healthy body condition. The feeding ration recorded for a single adult *G. cuvier* at the Acuario de Veracruz, from January 2012 to December 2012, was high for an ectothermic shark species (mean ± standard deviation = 0.97 ± 1.32% body mass (BM)/day, wet weight). Shark BM estimates were conducted using TL measurements in conjunction with growth and mass conversions from Kneebone et al. (2008). Feeding ration exhibited an oscillating pattern (as expected for a specimen offered food *ad libitum*) with mean weekly ration ranging from 0.23 - 1.90% BM/day. Observed feeding ration was much higher than that reported for temperate shark species and some tropical shark species. For example the adult ration for a broadnose sevengill shark, *Notorynchus cepedianus* (Péron, 1807), has been observed to be 0.2% BM/day (Van Dykhuizen and Mollet, 1992). Feeding ration for the sand tiger shark, *Carcharias taurus* (Rafinesque, 1810), and *G. cirratum* has been recorded as 0.3% BM/day, while feeding ration for the bull shark, *Carcharhinus leucas* (Müller & Henle, 1839), and *C. plumbeus* has been observed to be 0.5% BM/day (Schmid et al., 1990). However, feeding ration for *G. cuvier* was not as high as reported for active, endothermic sharks, such as the white shark, *Carcharodon carcharias* (Linnaeus, 1758) (1.6% BM/day; Ezcurra et al., 2012), and the shortfin mako shark, *Isurus oxyrinchus* (Rafinesque, 1810) (4.6% BM/day; Wood et al., 2009).

BEHAVIOR

Behavioral studies of animals in aquaria have largely revolved around marine mammals (Ramírez, 1999), with shark behavior in aquaria only a focus in more recent years (Moreno-

Table 2. Breakdown of food items ingested by a single adult tiger shark, *Galeocerdo cuvier* (Péron & Lesueur, 1822), displayed at the Acuario de Veracruz, over the period of a year (January 2012 to December 2012), expressed as a % of diet.

Common name	Scientific name	Percent of diet
Little tunny	*Euthynnus alletteratus*	59.00%
Jacks	*Caranx* sp.	15.00%
Nile tilapia	*Oreochromis niloticus*	5.50%
Beef steak	*Bos taurus*	4.10%
Blacktip shark	*Carcharhinus limbatus*	2.60%
Southern stingray	*Dasyatis americana*	2.30%
Atlantic sharpnose shark	*Rhizoprionodon terraenovae*	2.20%
Tiger shark	*Galeocerdo cuvier*	1.90%
King snake eel	*Ophichthus rex*	1.50%
Tarpon	*Megalops atlanticus*	1.40%
Red snapper	*Lutjanus campechanus*	0.90%
Cobia	*Rachycentron canadum*	0.90%
Chicken	*Gallus gallus domesticus*	0.70%
Silky shark	*Carcharhinus falciformis*	0.70%
Octopus	*Octopus* sp.	0.30%

Ballesteros, 2003; Sabalones et al., 2004; Henningsen et al., 2004); although some classic examples can be found (Clark, 1963; Myrberg and Gruber, 1974). Behavioral enrichment for aquarium species is also uncommon, especially for sharks. At the Acuario de Veracruz, we provided basic behavioral enrichment for the *G. cuvier* as follows:

1. Operant conditioning of *G. cuvier* through the use of visual or acoustic targets at feeding stations;
2. Variation in diet, including food items of high or low preference, food items of different size, and the manner of food item presentation—e.g., whole fish, filleted fish, and fish with or without viscera; and
3. Allowing food items to fall to the bottom of the exhibit in areas with decorative rockwork—encouraging an alteration to normal swimming behavior and the stimulation of multiple sensory systems while locating food and interacting with tank mates.

The predominant swimming behavior of *G. cuvier* at the Acuario de Veracruz was at the surface, along the exhibit perimeter. Management of this behavior, in combination with effective food introduction, is critical to successful *G. cuvier* care and is largely dependent on the dedicated efforts of the husbandry staff (Moreno-Ballesteros, 2003). To guide future husbandry efforts we have compiled an ethogram of *G. cuvier* behaviors displayed at the Acuario de Veracruz (Table 3), resulting from over 2,000 hours of observation.

In México, behavioral studies of sharks in aquaria, ethograms, and operant conditioning, are in their infancy (Moreno-Ballesteros, 2003; www2). Better documentation of *G. cuvier* activities in aquaria will allow for improved interpretation of specific behaviors and will lead to improved husbandry, animal welfare, and diver safety, as well as increased opportunities for research projects.

INTRASPECIFIC COMPATIBILITY

Multiple *G. cuvier*, including mixed gender and size, may be maintained together in an aquarium and, once established, will generally coexist without signs of stress. However, if distressed or new to an exhibit, juvenile *G. cuvier* (130 - 160 cm TL) may be susceptible to attack by larger conspecifics. Over a period of 15 years one such event was observed at the Acuario de Veracruz when a 310 cm TL adult male shark attacked a 150 cm TL juvenile male. It is recommended that juveniles not be introduced into displays with resident sub adults or adults. The establishment of a dedicated feeding station for each *G. cuvier*, or systematically feeding the dominant shark first, before feeding others, may be a useful strategy to help reduce aggressive intraspecific interactions.

The dominant male described above was also observed biting the upper lobe of the caudal fin of a slightly smaller male (286 cm TL), a few days after the latter was introduced into the exhibit. The resulting laceration healed in two months without medical intervention, and the shark swam and fed normally during its recovery. Both sharks were of similar size. It is speculated that this aggressive behavior may have been due to territoriality or some form of dominance hierarchy. No other aggression was observed between the two sharks.

No mating behavior has been observed between *G. cuvier* at the Acuario de Veracruz.

INTERSPECIFIC COMPATIBILITY

G. cuvier at the Acuario de Veracruz were generally compatible with a variety of other elasmobranch and teleost species. *G. cuvier* were typically timid and would commonly yield to other species of sharks swimming towards them, even when the *G. cuvier* was larger.

G. cuvier were frequently inhibited from foraging, or outcompeted for food, during feeding sessions. *G. cuvier* would avoid *G. cirratum* when they came to their feeding station and would not feed until the interloper had left the area. To mediate this behavior, aquarists would only feed *G. cirratum* when *G. cuvier* were not present, and would distract *G. cirratum* when *G. cuvier* were in the area.

In 2004, during a feeding session, a juvenile *G. cuvier* (146 cm TL) was bitten on the head by a *C. plumbeus*. This incident occurred when the juvenile *G. cuvier* swam away from its feeding station with a piece of fish in its mouth. The *G. cuvier* reacted to the *C. plumbeus* by biting it on the head. The *G. cuvier* sustained a substantial wound during this interaction, but fortunately the gills were not damaged and no significant blood loss was observed. The *G. cuvier* was fed again within

Table 3. Ethogram of behaviors displayed by nine tiger sharks, *Galeocerdo cuvier* (Péron & Lesueur, 1822) maintained at the Acuario de Veracruz, summarized from 2,000 hours of observation.

Behavior	Description of behavior
1. Slow swimming	Characterized by sharks slowly swimming along the bottom of the exhibit, or midway through the water column at a speed of 3 - 4 km/h. Slow swimming was generally exhibited by recently introduced *G. cuvier* and typically ceased days to a few weeks after arrival, at which point the shark would begin surface perimeter swimming.
2. Surface perimeter swimming	Occurred when a shark swam along the surface of the water, in close proximity to the perimeter of the exhibit. This behavior was frequently displayed by *G. cuvier* at the Acuario de Veracruz and is typical of healthy specimens. During surface perimeter swimming the head, pectoral fins and caudal fins may rub along the walls and form contact abrasions (Dehart, 2004). Surface perimeter swimming was occasionally punctuated by brief forays into the middle of the exhibit.
3. Food searching (surface)	When *G. cuvier* detected that food had been added to the exhibit, their slow swimming behavior changed to a more accelerated searching behavior. Sharks swam in large circles and often crossed the center of the exhibit. Upon location of a food item, sharks would frequently orient themselves at a 10 - 90° angle with respect to the surface of the water. At times, sharks would 'spy hop' and bite at food out of the water.
4. Food searching (substrate)	Characterized by a *G. cuvier* swimming in tight circles at the surface before swimming downward in a tight spiral pattern, approaching the substrate, and searching for a food item.
5. Wall biting	*G. cuvier* would occasionally bite randomly in the vicinity of a food item until it had been successfully grasped. This behavior often included making contact with exhibit walls.
6. Pole feeding	While ingesting food items presented via a feeding pole, sharks would swim at a 10 - 90° angle, in the horizontal plane, with respect to the exhibit walls.
7. Wall riding ('jogging') behavior	Wall riding, or 'jogging', was displayed when a shark reached a wall and, instead of changing direction in the horizontal plane, it would orient its ventrum to the vertical surface of the wall. In many cases the shark's ventrum would touch the wall. This behavior was considered undesirable and was typically observed in *G. cuvier* that had not adapted well to the aquarium. In some cases, the shark would continue its vertical momentum and 'bob' at the surface. In these cases, ventilation could be compromised and the shark could become fatigued. If not addressed, this behavior may result in a loss of body condition and possibly exhaustive collapse.

Table 3. Ethogram of behaviors displayed by nine tiger sharks, *Galeocerdo cuvier* (Péron & Lesueur, 1822) maintained at the Acuario de Veracruz, summarized from 2,000 hours of observation, cont.

Behavior	Description of behavior
8. Belly mouthing	This behavior was rare and only ever occurred between two *G. cuvier* at the Acuario de Veracruz. During feeding the dominant *G. cuvier* would swim parallel to the wall, under the subordinate shark. The dominant shark would open its mouth and push upwards against the ventrum of the submissive shark. This behavior never resulted in injury, but would cause the subordinate shark to actively avoid the dominant shark.
9. Food manipulation and rotation	When feeding, *G. cuvier* would typically rotate food fish items 180° and ingest them in the opposite direction to that initially caught. This behavior was adopted regardless of whether the food fish was presented head or tail first.
10. Food refusal	*G. cuvier* would occasionally accept a food item, drop it and swim away. It is speculated that this behavior was due to inappetence or a low preference for the food type presented.

two days and its food was supplemented with 3 g of vitamin C to boost its immune system. The head wound healed completely without further complication.

G. cuvier typically tolerated the presence of remora, *Echeneis naucrates* (Linnaeus, 1758), on their trunk, unlike *C. plumbeus* and *G. cirratum* that exhibited signs of stress (i.e., rapid swimming and rubbing) when *E. naucrates* were present. However, on one occasion, aquarists observed a juvenile *G. cuvier* attack and eat an ailing remora. A *G. cuvier* was also observed biting a large (170 cm TL) tarpon, *Megalops atlanticus* (Valenciennes, 1847), around the ventrum.

G. cuvier can be active nocturnal feeders in aquaria. It is recommended to employ a low-intensity night-light over an exhibit of *G. cuvier*, which ameliorates active nocturnal predation.

G. cuvier were not aggressive toward divers when they were performing routine maintenance or animal husbandry within the exhibit. Nevertheless, this species is potentially dangerous and appropriate precautions were always taken at the Acuario de Veracruz. It was speculated that any risk to divers would be increased by the presence of food in the water or when a *G. cuvier* seemed agitated. To mitigate this risk, divers only entered the exhibit after sharks had been fed and were calm.

CLINICAL ISSUES

G. cuvier in aquaria have been observed to readily heal from superficial wounds without the need for medical treatment or intervention. At the Acuario de Veracruz we observed this species to be very robust in comparison to other commonly maintained elasmobranch species. On the rare occasion that *G. cuvier* showed signs of infection, secondary to abrasions or lacerations, Enrofloxacin (Baytril®, Bayer Corp, USA) was administered intramuscularly at a dose of 10 mg/kg BM every other day (EOD).

TAGGING AND RELEASE

In accordance with the Acuario de Veracruz animal management plan, a 308 cm TL male *G. cuvier* ("Viernes") was outfitted with a pop-up satellite archival tag (PSAT, MK-10, Wildlife Computers) and released from the Aquarium into the Gulf of Mexico on 22 June 2007. The objective of the tagging study was to verify that a *G. cuvier* could successfully be reintroduced into the wild after maintenance in human care for over five years (1,899 days). The PSAT recorded data on light levels, water temperature and geographic location for a period of 90 days post release. The PSAT separated from the shark at the preprogrammed time (20 September 2007), surfaced northeast of the Mexican state of Tamaulipas, and began transmitting data.

After nearly a month of remaining in the waters off the state of Veracruz, the shark began a northward migration on 15 July 2007. During the 90-day tracking period, the shark traveled over 676 km (365 nautical miles). The *G. cuvier* remained at depths of less than 100 m for 87% of the time, although the shark made several deep dives (to a maximum depth of 744 m). Sea temperature data ranged from 22 - 28°C for 75% of the time, but dropped to a minimum of 6.6°C during a deeper dive. Deep dives are considered to be normal for *G. cuvier* and may be related to foraging behavior. PSAT data were consistent with the behavior of a healthy *G. cuvier* and it was hypothesized that the shark had been successfully returned to the wild.

CONCLUSIONS

Efforts to maintain *G. cuvier* in aquaria have been increasingly successful. One specimen was maintained at the Acuario de Veracruz for over seven years. This animal, along with other specimens, has provided valuable information about *G. cuvier* in aquaria, including specimen longevity, exhibit design for the species, captive feeding behavior, and growth rates.

Once acclimated to an exhibit, behaviors displayed by *G. cuvier* are highly predictable. With careful observation and knowledge of typical *G. cuvier* behavior, signs of poor adaptation, stress, or illness are easily distinguishable. Exhibit design is crucial and low relief rockwork around the perimeter is essential. The diet presented to *G. cuvier* should be broad and based on that of wild conspecifics. A flexible response to individual specimen food preferences, and feeding animals *ad libitum*, is necessary to meet the high energetic requirements of this species.

Maintaining *G. cuvier* in aquaria is not without controversy, as is the potential to release display animals back into wild after being held in human care (Weng et al., 2012). Data from our PSAT study indicate that it is possible to maintain a *G. cuvier* in an aquarium for an extended period and then successfully release the specimen back into its natural environment, providing evidence that applied husbandry protocols were responsible and effective. It should be noted, however, that the release of *G. cuvier* into the wild is subject to a number of ethical, legal (e.g., permitting), and ecological considerations, and should only ever be considered for healthy specimens able to integrate into their natural habitat and within their documented natural range.

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INTERNET RESOURCES

www1 http://elasmollet.org/Gc/Gc_captive.html
www2 http://www.youtube.com/terapiaanimal

The Elasmobranch Husbandry Manual II: Recent Advances in the Care of Sharks, Rays and their Relatives, pages 33-42.

Chapter 4

Collection, transport and handling of the Greenland shark, *Somniosus microcephalus*

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Abstract: During 2004 and 2005 the husbandry team at Ripley's Aquariums researched the possibility of collecting and displaying the fourth largest shark species, the Greenland Shark, *Somniosus microcephalus* (Bloch and Schneider, 1801). Extensive research, anecdotal reports, tagging studies and video documentation confirmed the presence of *S. microcephalus* in the St. Lawrence Seaway and its tributaries, in the province of Quebec. This relatively accessible location made the possibility of collecting and transporting *S. microcephalus* economically and logistically feasible. In the summer and fall of 2005, Ripley's Aquariums' staff was successful in locating, tagging and tracking live specimens of *S. microcephalus*, and collecting by hand (on SCUBA) a single female specimen of 3.72 m total length (TL) and 462.7 kg body mass (BM). The shark was transported by vessel across the St. Lawrence Seaway, and then by specialized tanker truck over land to the Aquatron Laboratory (Dalhousie University), where it was studied for several weeks. The shark did not adapt well to aquarium conditions and 18 days after collection was euthanized. Necropsy revealed the female *S. microcephalus* to be sexually immature, with an undeveloped uterus. The stomach was large, loose, relatively thin walled, and completely empty. The skull was thick and made of dense cartilage, and the cranial vault was large, full of fluid, and housed a small brain. This exploratory research effort shed new light on the behavior, age and anatomy of *S. microcephalus*, as well as the complex logistics involved in collecting and transporting large cold-water sharks.

INTRODUCTION

In April 2004, it was publicly announced that Ripley Entertainment would build its third aquarium in Niagara Falls, Ontario, Canada. At the launch event it was revealed that, for the first time, the Greenland shark, *Somniosus microcephalus* (Bloch and Schneider, 1801), might be displayed to the public in a large, cold-water exhibit. Never before had *S. microcephalus* been collected, transported, or maintained in an aquarium. Discussions with experienced *S. microcephalus* field researchers suggested that such an expedition was viable, and that much would be learned about the species (Benz, personal communication; Campana, personal communication; Hueter, personal communication). One of the goals of many public aquaria is to trial the exhibition of new and different species, so the research and development phase for collecting, transporting and maintaining a living *S. microcephalus* began.

GREENLAND SHARK BIOLOGY

S. microcephalus is the largest member of the family Somniosidae (Order Squaliformes). It is the fourth largest known shark, and second largest carnivorous shark, after the great white, *Carcharodon carcharias* (Linnaeus 1758). It is also the largest Arctic fish, known to reach a maximum of 6.4 m total length (TL). Most *S. microcephalus*, that have been reliably measured, range from 2.5 - 5.0 m TL, and weigh up to 1,100 kg body mass (BM). The body of *S. microcephalus* is cylindrical, with a distinct caudal keel and no anal fin. The head of the species is small, compared to the rest of its body, and two large spiracles are located dorso-caudal to the eyes. Detailed and contemporary morphological descriptions can be found in Castro (2011) and MacNeil et al. (2012).

The geographic range of *S. microcephalus* extends from the temperate northern Atlantic Ocean to the Arctic Ocean (MacNeil et al., 2012). The species may also extend further south, in deeper waters (Castro, 2011), as *S. microcephalus* has ostensibly been sighted and photographed in waters off Savannah, Georgia (Herdendorf and Berra, 1995) and in the Gulf of Mexico (Benz et al., 2007) Species identification from these reports remains in question. More recently, *S. microcephalus* was reported as caught on hook and line and landed during a research expedition in the Gulf of Mexico (www1), although positive identification of the species is unclear and it may have been a different species of Somniosid.

Because of its bathybenthic environment, typically inaccessible to SCUBA divers, *S. microcephalus* is very rarely observed directly and, due to their large size, deep-sea habitat, and lack of current commercial importance, their biology is poorly understood (Castro, 1983; Compagno, 1984; Ebert et al., 1987). Most of the historical information about this species derived from extensive liver oil fisheries throughout the

northeast Atlantic Ocean, especially Norway, operated during the 15th and 16th centuries (MacNeil et al., 2012). Sustained annual yields suggest the presence of abundant regional populations of *S. microcephalus* at the time (MacNeil et al., 2012). The harsh Arctic environment, and absence of directed commercial fisheries since the late 1960s, has led to a scarcity of modern studies on *S. microcephalus*.

The life span of *S. microcephalus* may be several hundred years. A tagging and recapture study by Hansen (1963) found that sharks grew 0.5 - 1.1 cm/y. During this long-term study, 411 *S. microcephalus* were tagged off the coast of Greenland and 28 specimens were recaptured. Of these sharks, the author only deemed three to have been accurately re-measured. One *S. microcephalus*, recaptured after 16 years at large, had grown only 8 cm TL (0.5 cm/y). Another specimen grew 1 cm TL after two years at sea (0.5 cm/y), and a third specimen, recaptured after 14 years, showed an increase of 15 cm TL (1.1 cm/y). These results suggest that *S. microcephalus* may be very long-lived and, depending on size at birth and the dynamics of growth rates throughout their life, may be one of the longest-lived vertebrates on the planet. A recent study, using eye lens radiocarbon dating, estimates a lifespan of least 272 years (Nielsen et al., 2016). Size-at-birth estimates for *S. microcephalus* range from 37 cm TL (Koefoed, 1957) to 100 cm TL (Kondyurin and Myagkov, 1983).

Diet and foraging

The diet of *S. microcephalus* appears representative of opportunism in a harsh environment. Prey identified from stomach contents include local benthic fishes and invertebrates such as Greenland cod, *Gadus ogac* (Richardson, 1836), Greenland halibut, *Reinhardtius hippoglossoides* (Walbaum, 1792), shorthorn sculpin, *Myoxocephalus scorpius* (Linnaeus, 1758), Atlantic wolffish, *Anarhichas lupus* (Linnaeus, 1758), as well as 'redfish', amphipods, sea urchins, gastropods, jellyfish, and skate eggs (Castro, 2011). Historical stomach content analyses of *S. microcephalus* included the remains of seals, whales and birds, as well as conspecifics (Jensen, 1914). A recent anecdotal report included the jawbone of a polar bear (www2), and a *S. microcephalus* rescued in Newfoundland was discovered attempting to ingest a moose (www3).

Teeth on the upper jaw of *S. microcephalus* are narrow, pointed and smooth. These teeth anchor the food item, as it is the lower jaw that does the cutting. The teeth on the lower jaw are larger and broader and curve sideways. By swinging its head in a circular motion, the shark can cut out a round plug from its prey item (Yano et al., 2007). The dentition of *S. microcephalus*, and the cruciate patterns of eroded pigment on its rostrum, suggests that this species is predominantly a scavenger. However, there are claims that, despite its lethargic appearance, *S. microcephalus* is a predator capable of short bursts of speed, and, under certain conditions, may hunt seals and even larger mammals, including beluga whale (Harvey-Clark et al., 2005). Watanabe et al. (2012) used data-logging tags to measure the swimming speed and tail-beat frequency of six free-swimming *S. microcephalus*. The sharks averaged a cruising speed of 0.3 m/s (0.76 mph), but were also capable of short bursts of speed. The researchers concluded that swimming performance was limited by water temperature, and that *S. microcephalus* would be unable to catch swimming seals. However, they conceded that Arctic seals sleep in water to avoid predation by polar bears, which may leave the seals more vulnerable to the cryptic, slow-swimming *S. microcephalus*.

Trimethlyamine *N*-oxide

S. microcephalus flesh contains one of the highest concentrations of trimethlyamine *N*-oxide (TMAO) on record (Seibel and Walsh, 2002). While the purpose of this osmolyte is not fully understood, it may assist in depressing the freezing point of bodily fluids, as it is also found in high concentrations in other deep-water and polar fishes (Treberg and Driedzic, 2002). The elevated TMAO concentrations, and its distinctive odor, have led to some interesting Inuit folklore. One origin story tells of an old woman who washed her hair in urine and dried it with a cloth, which blew into the sea and became the first Greenland shark (Caloyianis, 1998).

Historically, dogs played a key role in northern Greenland and the Canadian Arctic as draft animals for sleds. The *S. microcephalus* fishery was important, not only for liver oil, but also as a source of food for sled dogs. However, it became quickly apparent that, if fed to them fresh, shark flesh would intoxicate the dogs and render them useless (Jensen, 1914; Caloyianis, 1998). During digestion TMAO breaks down into trimethlyamine (TMA), which causes intestinal distress and neurological effects similar to extreme drunkenness. Eating too much TMAO can even lead to convulsions and death. Early settlers to

Iceland and Greenland developed a technique to avoid these ill effects: the shark flesh was buried in the ground for 6 - 12 weeks, exposed to many cycles of freezing and thawing, and then hung up to dry for several months. The end product was cut into bite-sized cubes and served as an hors-d'oeuvre called Hakárl (or kæstur hákarl), considered a delicacy in Iceland.

SOURCING A SHARK

The first underwater photographs of a living *S. microcephalus* were taken in the high Arctic in 1995 by natural history filmmakers, Nick Caloyianis and Clarita Berger (Caloyianis, 1998). These photographs prompted expeditions with scientist(s) George Benz in 1996, and Benz and Greg Skomal in 1999.

Skomal and Benz (2004) caught and tagged (with ultrasonic tags) six *S. microcephalus* off northern Baffin Island, Nunavut, Canada, and tracked the sharks for several hours each. The sharks were collected using baited hooks and lines dropped through ice holes. While successful for the researchers, this remote location was considered logistically challenging for live animal collection and subsequent transport to southern Ontario.

In early 2004, research revealed that a commercial fishing operation in Greenland, catering to high-end recreational fishers, had organized the "Greenland Shark Challenge" (www8). During this contest, which ran from February to April, *S. microcephalus* were fished through ice holes. The town that hosted the contest was located in northwestern Greenland, 590 km north of the Arctic Circle, and was home to the country's northern-most ferry terminal. Although more accessible for large scale transport equipment than Baffin Island, the distant continental location was also rejected as a source for live *S. microcephalus* for logistical reasons.

Undaunted, the team continued investigations and discovered reports that *S. microcephalus* were found at depths accessible by SCUBA, in the St. Lawrence Seaway, near the city of Baie Comeau in northern Quebec (www4; www5; www6; Harvey-Clark et al., 2005). This location was relatively accessible and represented a more logistically viable source for live *S. microcephalus*. During the summer of 2004, attempts were made to directly observe *S. microcephalus*, while diving on SCUBA, but no sharks were seen. Fortunately, a review of video footage taken in the region confirmed the presence of live *S. microcephalus*, and a decision was made to attempt collection and transportion of a shark the following year.

Permission to collect and transport two *S. microcephalus* (between September 15 and October 31, 2005) was requested from the Canadian Department of Fisheries and Oceans (DFO), Quebec Region, and was granted. Animal ethics approval for holding sharks in a facility accredited by the Canadian Council on Animal Care was also obtained.

In June 2005, a multidisciplinary team embarked on an exploratory reconnaissance expedition. This expedition was a crucial step toward successfully collecting a specimen later in the year. Staff were able to dive in the sheltered bay (Baie-Saint-Pancrace; 49.287314°N, 68.045956° W) where most of the sharks had been previously sighted, photographed and tagged (Stokesbury et al., 2005). This location represented the narrowest section of the bay (~1.5 km long x 0.25 km wide), which served to concentrate shark traffic and facilitate tag deployment by divers. Ripley's Aquariums' staff observed and photographed 40 unique *S. microcephalus*, over a period of six days, and assisted researchers from Dalhousie University tag eight sharks. Animals ranged from an estimated 2.5 - 5.0 m TL, and were located in water of 4.4°C and at depths of 23 - 28 m.

PREPARATORY LOGISTICS

A large seawater tank, located at the Aquatron Laboratory, Dalhousie University, in Halifax, Nova Scotia, was inspected and deemed to be an excellent staging facility for *S. microcephalus*. The tank was leased from the University for two years, starting in mid-2005. The concrete tank was 15.2 m in diameter and ranged in depth from 3.5 m at the perimeter, to 3.9 m at the center (volume = 684.05 m^3). The tank had 22 glass observation windows (each ~1 m^2), located around the perimeter, as well as an open top with a vertically retractable and rotatable cross-tank catwalk. Video recording equipment monitored the entire tank, making it an excellent observation platform. The tank was supplied with natural seawater from a nearby bay, and the physical plant was equipped with chillers and heat exchangers to provide tight temperature control when operated in either a

closed, or semi-open, mode. The Aquatron Laboratory was located approximately 12.5 hours by combined boat-truck transportation from the shark collection site near Baie Comeau.

Although located in North America, and reasonably close to larger cities, the town of Baie Comeau is relatively remote, making shark transport logistics challenging. The nearest bridge across the St. Lawrence Seaway is 415 km to the southwest, in Quebec City, adding 830 km to a trip that would otherwise only be 60 km by boat to the town of Matane, in the southeast. As a result of excessive road distances and inflexible public transit ferry options, it was determined that a chartered commercial fishing vessel (*F/V Le Maxime*) was the most reliable and convenient platform to collect and transport a shark. A dive boat was also chartered from Baie Comeau, as a tender, and a low-rise rigid hull inflatable boat (RHIB) was donated to the operation to help handle the shark at the surface once collected. When a shark had been secured, the *F/V Le Maxime* would ferry it to Matane, where it would be transferred to a tractor-trailer for the drive to the Aquatron Laboratory.

Based upon reported sizes of *S. microcephalus*, and of sharks observed in the Baie Comeau area, two large animal transport tanks were prepared; one for the *F/V Le Maxime*, to maintain the shark as it transited the St. Lawrence Seaway, and the other for highway transport once the collection team reached Matane.

The shipboard tank consisted of a large, fiberglass-reinforced plastic (FRP) trough-style tank (FRT-39, Red Ewald, Karnes City, Texas), which internally measured 4.88 m x 1.22 m x 0.89 m deep. The tank was insulated on the exterior with polystyrene spray foam. A pump aboard the *F/V Le Maxime* supplied flow-through seawater for continuous dilution, oxygenation and temperature control. An additional 12V submersible pump (Model 02, Rule ITT Industries, USA) was added to the system to provide water circulation (at 95 L/min) and atomized oxygen via compressed gas cylinders and diffusers.

The highway transport system consisted of a large, custom-made FRP tank (Waterdog Products, El Cajon, California), a life support system (LSS), and generator, mounted to a flatbed aluminum semi-trailer. The tank was designed to accommodate a *S. microcephalus* of 2.5 - 5.0 m TL, and internally measured 6.5 m x 2.2 m x 1.0 m deep. The tank was insulated to maintain a stable temperature and was baffled to reduce water surge using perforated plastic plates in each corner, and horizontally at a height of 0.93 m. The tank volume at 7 cm above the horizontal baffle plate (at the 1.0 m mark) was 14.58 m^3. The tank was outfitted with a large titanium chiller (5.6 kW (7.5 HP) AquaLogic MT-9, Aqua Logic Inc., San Diego, California) and heat exchanger (208 - 230 V three-phase Trane AWA090A3, Bridgeton, Missouri) with a PVC barrel and titanium tubes, to maintain the low temperatures (0 - 2°C) required to sustain the *S. microcephalus*. A large electrical generator (Model #DCA25USI MQ Power Whisperwatt Ultrasilent 25 KVA diesel, MQ Power Corp., Carson, California) was employed to accommodate the chiller and the balance of the LSS, which consisted of two, three-phase 1.5 kW (2 HP) pumps (Pentair Whisperflo, Pentair Aquatic Systems, Sanford, North Carolina), two 120 V submersible pumps (Model 18 Supreme Mag Drive, Danner Manufacturing Inc., Islandia, New York) delivering 5.69 m^3/min (1,800 GPM) and driving oxygen atomizers in the front corners of the tank, two 12 V submersible pumps (Model 10 Rule #2000, Xylem, Inc., Beverly, Massachusetts) delivering 7.57 m^3/h (2000 GPH) and driving oxygen atomizers in the rear corners of the tank, and four cartridge filters (150 sq. ft. Ultra-Mite Baker Hydro cartridge filter housing), loaded with 16-micron mechanical media (Baker Hydro / WaterCo, Augusta, Georgia). An additional self-priming pump (Model PR1C, Pacer Pumps, Lancaster, Pennsylvania) and hoses enabled the tank to be filled by an adjacent natural water source.

A large shark stretcher, sufficient for the length, girth and weight of *S. microcephalus* (4.3 m long x 1.0 m deep x 1.0 m wide, at the top, and 0.6 m wide at the bottom), was designed and fabricated (Ortega Sail and Canvas, Carlsbad, California). The stretcher included large aluminum stretcher poles for lifting, and was designed with removable panels at each end to prevent the animal from sliding out.

SHARK COLLECTION

S. microcephalus have traditionally been caught by native populations, commercial fisheries and for research, via hook and line (Jensen 1914; Hansen, 1963; Caloyianis, 1998; Skomal and Benz, 2004). It was planned, therefore, to fish for sharks with conventional bottom long line gear, outfitted with larger hooks (20/0) on the gangions.

However, observation of the slow swimming speed of *S. microcephalus* during the June 2005 tagging and reconnaissance expedition led the team to consider catching a specimen by hand using a "tailer". A tool used by recreational fishers to land large sharks, a "tailer" consists of a pole with a flexible tip and strong line, forming a noose, which is slipped around the tail and tightened to secure the animal (www7). Commercially available "tailers" (e.g., Aftco, Santa Ana, California) were not large enough for *S. microcephalus*, so a custom unit was built using an aluminum tube for the pole and a larger diameter stainless steel cable for the noose. A "tailer" is usually employed when an animal has already been caught on hook and line. Our plan was to have a SCUBA diver approach a slowly swimming shark and slip the "tailer" around its caudal fin. Although Borucinska et al. (1998) reported a high incidence of ocular infection with the copepod parasite *Ommatokoita elongata* in Arctic *S. microcephalus*, causing corneal opacity and likely loss of sight, the majority of shark's seen in the St. Lawrence Seaway had unaffected corneas and were highly likely to visually detect and avoid approaching divers (Harvey-Clark et al., 2005), presenting somewhat of a challenge.

Animal collection began in earnest on October 11, 2005, and several days were devoted to conducting practice sessions with equipment and personnel in Baie-Saint-Pancrace. Unfortunately, no sharks were observed during these practice sessions. However, shark-tagging studies in previous years suggested that sharks might be present, to the West, in the much larger Baie des Anglais. A reconnaissance dive confirmed the presence of *S. microcephalus* and plans were changed to focus collecting efforts in the new location.

On the morning of October 13, 2005, the first specimen collection dive was initiated. A large, untagged female shark was observed at nearly 30 m of depth (in 0.0°C water temperature), ascending from deeper water. The designated SCUBA "wrangler" placed the loop of the "tailer" around the caudal fin of the shark and tightened the padded cable around its caudal peduncle. A green float was released, indicating to the team on the surface that a shark had been successfully secured. The surface team then commenced the process of slowly pulling the shark back to the RHIB, via a long line attached to the "tailer". Once the green float had been deployed the dive team also slowly ascended, observing all required decompression safety stops. Following a controlled retrieval, the shark was brought to the surface at 49.267723° N latitude, -68.130539° W longitude.

The captured *S. microcephalus* was pulled alongside the RHIB with the large stretcher already in position. One pole of the stretcher was on the starboard gunwale and the other in the water, leaving the stretcher in a fully open position. The crew on the RHIB was unable to maneuver the large shark into the stretcher without assistance, so some of the surfaced dive team swam over to help. Once secured in the stretcher, atomized oxygenated seawater was directed over the gills of the shark using a 12 V submersible pump. The RHIB was then drawn alongside the anchored *F/V Le Maxime*, where a hydraulic crane hoisted the shark aboard and into the dedicated holding tank, also supplied with ambient seawater and atomized oxygen.

SHARK TRANSPORT

As soon at the shark, equipment, and personnel were aboard the *F/V Le Maxime*, the 64 km (3.5 h) shipboard transport across the St. Lawrence Seaway commenced.

A blood sample was drawn from the shark within an hour of capture using a 21 gauge butterfly needle set via the dorsal sinus below the second dorsal fin. Approximately 5 ml of blood was obtained and split between a lithium heparin tube and a plain tube with a serum separator. The blood was placed on ice and sent to a laboratory for analysis (Table 1).

Upon arrival in Matane the shark was measured in the on-board tank. Measurements were taken along the curve of the body and were recorded as follows: 3.8 m TL, 3.6 m fork length (FL), 3.2 m pre-caudal pit length (PCP), and 1.9 m girth. The animal was then transferred in its stretcher to the highway transport tank, using a land-based crane, and the 9 h highway transport to the Dalhousie University (730 km to the south) commenced. Dissolved oxygen and temperature was monitored (Model HQ10 dissolved oxygen meter, Hach Company, Loveland, Colorado) and remained stable throughout the transport. Water samples were taken from the tank at ~2 h intervals. Ammonia concentration climbed slowly, but steadily, from 0.011 mg/L up to 0.163 mg/L, while pH remained stable throughout the journey, at an average of 7.78.

Table 1. Blood chemistry results from two samples taken from a Greenland Shark, *Somniosus microcephalus* (Bloch and Schneider, 1801), at time of capture (day 0) and immediately before euthanasia (day 18), following a 12.5 h transport by sea and land, and 18 days in an aquarium.

Parameter	S.I. unit	At time of Capture (Day 0)	Pre-euthanasia (Day 18)
Sodium	mmol/L	252	276
Potassium	mmol/L	2.2	2.4
Chloride	mmol/L	248	277
Calcium	mmol/L	3.3	3.9
Phosphorous	mmol/L	1.98	
Magnesium	mmol/L	2.45	
Urea	mmol/L	231	232
Creatnine	mmol/L	0	
Glucose	mmol/L	4.2	
Cholesterol	mmol/L	2.51	
Total Bilirubin	mmol/L	4	
Alk Phosphatase	IU/L	10	
Creatnine Kinase	IU/L	110	
AST	IU/L	34	
ALT	IU/L	25	
GGT	IU/L	0	
Total Protein	IU/L	15	15
Albumin	IU/L	5	5
Globulin	IU/L	9.5	10
Lipase	IU/L	40	
Cortisol	nmol/L	<4.9	<4.9
Hematocrit	%	32	16
Hemoglobin	g/L	49	

SHARK HUSBANDRY

Upon arrival at Dalhousie University the *S. microcephalus* was briefly acclimatized, by adding Aquatron tank water to the highway transport tank, although water parameters were already very similar. The shark was then hoisted into the Aquatron Laboratory tank using a land-based crane and an internal crane rail. An attempt to weigh the shark was thwarted by equipment failure, so it was promptly released from the stretcher and it began swimming immediately without assistance.

Water quality in the Aquatron tank was considered to be ideal. The dissolved oxygen level of incoming raw water was >80% saturation, and was >100% saturation when supplemental oxygen was applied. Temperature ranged from 3.9 - 6.2°C, and pH ranged from 7.66 - 7.89. Ammonia (mean = 0.007 mg/L) and nitrite (mean = 0.008 mg/L) were well within acceptable limits.

The *S. microcephalus* was monitored closely throughout the 18 days it was in the Aquatron tank. Swimming behavior was documented continuously, using four fixed video cameras. Data recorded included: time to circumnavigate the tank, tail beat frequency, position in the water column, and swimming direction (Choromanski, in preparation). During this time the animal predominantly swam against the tank perimeter, eventually leading to areas of dermal erosion from contact with the walls. Many and regular attempts were made to discourage perimeter swimming and to steer the shark toward the center of the tank and/or to reverse swimming direction. Methods employed included: altering the photoperiod; eliminating illumination; lining the tank walls with PVC pipes hung vertically in the water; curtains of bubbles created by air stones in the swimming path; a movable physical barrier fabricated from a vinyl-covered PVC-pipe frame; and water currents created by incoming

or recirculated seawater. Although there were intermittent periods of improved swimming behavior, where the shark used a greater portion of the tank, it typically reverted to perimeter swimming.

Despite daily active and passive attempts by experienced husbandry personnel to induce the shark to feed, using a wide variety of local seafood items, the *S. microcephalus* never ate, nor showed any interest in food.

On October 31, 2005, after 18 days of continuous effort to acclimatize the shark to aquarium conditions, it was determined and accepted that these efforts would not be successful. A release-to-the-wild strategy was comprehensively considered. However, such an effort was deemed unsafe. A severe storm event earlier in the month had damaged Matane Harbor and rapidly dropping temperatures resulted in surface ice forming over the collection site. On consultation with local veterinarians, Ripley's Aquariums' personnel, Dalhousie University faculty, and the DFO, a decision was made to euthanize the shark.

EUTHANASIA AND NECROPSY

On November 1, 2005, the shark was transferred to a low-volume pool adjoining the Aquatron tank. Blood was taken from the ventral caudal vein and the sample was handled in the same manner as the post-capture sample (Table 1). The shark was then euthanized by a lethal dose (1,000 mg/L) of tricaine methanesulfonate (MS-222). Complete morphometric data was recorded, revealing the shark to be 3.72 m TL and 462.7 kg BM (Choromanski, unpublished results).

Gross examination revealed moderate to severe erosion of the dermis along all prominent anatomical features, including pectoral fins, dorsal fins, caudal keel, and caudal fin lobes; the result of contact with the perimeter wall of the Aquatron tank. Likewise, the snout, already scarred in the typical cruciate pattern observed in wild sharks, was eroded. A complete necropsy was performed on the shark and duplicate samples of organs were preserved in buffered formalin. The primary pathology observed during necropsy was the dermal abrasions noted above. Corneal edema was observed, as well as hemorrhage in the anterior chambers of both eyes.

Notable features observed during necropsy concerned the uterus, stomach and cranial vault. The uterus was immature and undeveloped. The stomach was very large, flaccid, relatively thin walled, and completely empty. It appeared as if the stomach could stretch readily and become engorged, presumably allowing the shark to quickly ingest a very large meal. The skull was remarkably thick and made of very dense cartilage. The cranial vault was large and full of fluid, housing a relatively small brain. The thick skull and high volume of cranial fluid may provide an advantage to a scavenger that uses excessive head-shaking to remove large chunks of flesh from prey items, as suggested by tooth morphology.

Blood chemistry

The clinical laboratory responsible for processing samples was inexperienced in the handling of elasmobranch blood, which limited the amount of meaningful data that could be derived. Nevertheless, some interesting results were obtained. Blood chemistry values (Table 1) were similar to those expected for elasmobranchs with high electrolyte and urea levels. Blood glucose was higher than expected, and packed cell volume (hematocrit) was notably robust for a slow-moving, cold water animal (George, personal communication). The shark showed marked anemia at the time of necropsy, which is common in chronically sick or stressed elasmobranchs, although onset in this case seems to have been rapid. There was little change in the other hematological and biochemical values obtained. Despite the shark not eating, its electrolytes and plasma proteins remained unchanged.

CONCLUSIONS

This exploratory research project was successful in developing techniques, equipment and logistics to collect a very large elasmobranch from a remotely located, cold water environment, and transport it long distances over water and land. This information can be used as a basis for future attempts with *S. microcephalus*, or modified for other large elasmobranchs. Although we were unable to maintain *S. microcephalus* long-term, the resultant necropsy provided valuable information on the biology of the species, about which little is known. The mere fact that an animal of this size (and estimated to be over 100 years old) was still sexually immature is particularly noteworthy. Teams attempting to collect, transport and display *S. microcephalus* in the future should consider the selection of smaller

specimens, as there was significant downward pressure on the body of the large shark when it was lifted out of the water. A modified apparatus, with capacity to lift the shark while surrounded by water, would help support the animal and avoid the impacts of any induced downward pressure.

Although this paper refers to Ripley's Aquarium of Canada in Niagara Falls, Ontario, the aquarium was ultimately never constructed. An aquarium of the same name was later built by Ripley's Aquariums in the city of Toronto, Ontario, which opened in October, 2013. Ripley's Aquarium of Canada does not display *S. microcephalus*.

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UNPUBLISHED RESULTS

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IN PREPARATION

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www2 http://www.independent.co.uk/environment/nature/clash-of-the-fiercest-predators-as-shark-eats-polar-bear-891512.html

www3 http://www.cbc.ca/news/canada/newfoundland-labrador/moose-eating-shark-rescued-in-newfoundland-harbour-1.2434102

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Chapter 5

Collection, transport and husbandry of the blue shark, *Prionace glauca*

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Abstract: The blue shark, *Prionace glauca* (Linnaeus, 1758), is a common, globally distributed shark species. Although beautiful and graceful it has rarely been maintained in public aquaria, and never for an extended period. The longest survival time for a *P. glauca* in an aquarium was 246 days at Tokyo Sea Life Park. Capture of *P. glauca* is relatively easy using longlines, game fishing gear (i.e., rod and reel) and set nets. These methods allow for the collection of animals in good condition. *P. glauca* can be successfully transported using a round tank with oxygen supplementation and a filtration system. Smaller animals (i.e., <100 cm TL) are optimal display candidates, as they better tolerate the biochemical challenges presented by capture and transport. Small *P. glauca* also seem to be more resistant to handling than larger specimens. Large-

volume exhibits (i.e., over 2,000 m^3), with ample space for swimming and gliding, few obstacles, and no potential predators—e.g., sand tiger sharks, *Carcharias taurus* (Rafinesque, 1810), and sandbar sharks, *Carcharhinus plumbeus* (Nardo, 1827)—are recommended, to provide an appropriate aquarium environment for *P. glauca*. It may be prudent to introduce *P. glauca* into an exhibit before other potential predatory species. *P. glauca* tend to rub their skin, fins and snouts on the smooth walls and windows of aquaria. Optimizing *P. glauca* swimming patterns to minimize contact abrasion is critical to their care in aquaria.

INTRODUCTION

The blue shark, *Prionace glauca* (Linnaeus, 1758), is the only species in the genus *Prionace*, and belongs to the family Carcharhinidae, commonly known as the requiem sharks (Compagno, 1984). This species of shark is found worldwide, often in offshore surface waters, in both temperate and tropical climates. Historically, *P. glauca* has been the most abundant species of large shark found throughout its range (McKenzie and Tibbo, 1964; Casey, 1982). *P. glauca* has an indigo-blue coloration on its upper body, bright blue sides, and a markedly white abdomen (Carwardine and Watterson, 2002). Tagging studies and catch records indicate that *P. glauca* exhibit extensive seasonal migrations (Stevens, 1976; Casey, 1982). *P. glauca* is the most heavily fished large shark species in the world, and bycatch in commercial fisheries, especially long line swordfish and tuna fisheries, accounts for the largest cause of adult shark mortality (Castro et al., 1999). The conservation assessment of *P. glauca* is “near threatened” (www1). Due to its unique shape, color, historical relevance, and conservation status, *P. glauca* represents an attractive species for public display. Yet, few institutions have displayed *P. glauca*, and never for an extended period of time.

This chapter presents an overview of capture and transport methods used for *P. glauca*, as well as basic husbandry methods specific to the species. The information provided is based on experiences drawn from maintaining a *P. glauca* at the Oceanário de Lisboa, Portugal, for seven months, along with information extracted from similar trials by other institutions. The experiences detailed in this article are intended to serve as a point of reference for future attempts at maintaining *P. glauca* in aquaria.

A number of institutions, including Sea World San Diego, Monterey Bay Aquarium, Aquarium of the Bay, Adventure Aquarium, and Tokyo Sea Life Park (Correia, 2004), have attempted to capture, transport and maintain *P. glauca*. The first known trials occurred in 1968 at Sea World San Diego, where a 15 m diameter tank was built to trial the maintenance of pelagic sharks, such as *P. glauca* and mako sharks, *Isurus oxyrinchus* (Rafinesque, 1810) (Powell et al., 2004). In order to understand how these and other scientists approached the capture, transport and maintenance of *P. glauca*, a questionnaire was sent to the nine institutions that have attempted to keep the species. Six questionnaires were returned providing information on more than 20 individual *P. glauca*, which has been summarized in Table 1. Five of the six responding institutions conducted trials with several sharks (3 - 7), some of which were released to the wild after spending variable lengths of time in human care.

SHARK COLLECTION

P. glauca are highly-pelagic, obligate ram ventilators, which prevents the use of any collecting method that does not allow the shark to swim freely. As such, trapping, gill netting, or pelagic trawling are not advisable collection methods, and would almost certainly result in *P. glauca* mortality. In light of these limitations, the use of long lines or standard game fishing gear provides a viable alternative for shark collection. Both of these capture methods allow the animal to swim continuously, even when being reeled in. However, collection in commercial set nets represents the ideal capture method, as the animal is allowed to swim freely, without hyperactivity, before being removed from the water.

For decades, game fishers have captured *P. glauca* using rod and reel, then tagged and released them. This methodology has been used in independent studies, as well as the National Marine Fisheries Service’s (NMFS) Cooperative Shark Tagging Program. Together, these programs list thousands of recaptures (Briggs, personal communication), providing evidence that

P. glauca survive a short struggle with anglers and subsequent tagging and release.

Many *P. glauca* detailed in the survey were caught using long lines, or game fishing rod and reel, although others were caught using set nets. The sharks caught in set nets displayed the highest survivability. Captured shark sizes ranged from 60 - 180 cm total length (TL). Most of the sharks were deemed to be in good condition after capture, with reported cases of post-capture complications predominantly involving larger animals (160 - 180 cm TL).

SHARK TRANSPORT

The majority of successful *P. glauca* transports used round tanks, with diameters ranging from 100 - 240 cm, and volumes up to 3.5 m^3 allowing the sharks to swim freely. Transport regimes typically employed oxygenation, via water replacement, or regulated diffusors fed by compressed gas cylinders, and/or some form of water treatment. Where reported, oxygen was maintained above 100% saturation. Water treatments typically consisted of a mechanical filter (e.g., a cartridge filter filled with pleated paper media), fed by a 12V submersible pump placed on the bottom of the transport tank. These regimens were adequate for transporting *P. glauca*, provided transport times were no longer than 4 h. Post-transport complications reported in the survey were predominantly related to excessively large sharks (160 - 180 cm TL). A shark transported in a rectangular tank did not fare well.

Our experiences transporting *P. glauca* at the Oceanário de Lisboa were consistent with the findings of other researchers. Smaller sharks (i.e., <100 cm TL) were relatively easy to transport using round polyethylene tanks (100 - 240 cm diameter). Mechanical filtration was provided by a canister (Model CFR 50, Jacuzzi, Chino, California) filled with activated carbon and a 50 µm pleated paper filter. Oxygen saturation was maintained as high as 200% using a cylinder of compressed medicinal grade oxygen. Further details of shark transport methods can be found in Correia (2001), Young et al. (2002), Smith et al. (2004), Correia et al. (2008), Correia et al. (2010) and Rodrigues et al. (2012). Multiple trials conducted directly by the team at the Oceanário de Lisboa suggested that 100 cm TL is the upper length threshold for transporting *P. glauca*, over which animals adapt poorly to the confines of a transport tank. For transports of longer duration, or for animals longer than 100 cm TL, a larger round tank with both oxygenation and filtration is strongly advised.

SHARK HUSBANDRY

Aquarium shape and size

As reported in the survey, aquaria used to maintain *P. glauca* varied considerably in shape, including circular, elliptical, and even rectangular. Aquarium volumes ranged from 20 - 157 m^3 for quarantine tanks and 250 - 7,000 m^3 for exhibit tanks. While some facilities maintained *P. glauca* in quarantine or holding tanks, before moving them into a display tank, other institutions maintained sharks in a quarantine tank or an exhibit tank exclusively. Although large-volume aquaria (i.e., >1,000 m^3) are recommended for *P. glauca*, it is suggested that smaller sharks (~70 cm TL) could survive, medium-term (i.e., several months), in smaller aquaria (e.g., 150 m^3). For example, an individual *P. glauca* was maintained at the Oceanário de Lisboa in a quarantine tank of 100 m^3 for a period of 161 days.

Food and Feeding

In general, as reported by survey respondents, initiating feeding in healthy *P. glauca* did not appear to be a challenge. Some *P. glauca* fed from the bottom of the aquarium, while others were induced to eat by target feeding. Two facilities reported a necessity to force-feed anorectic sharks. Food accepted by *P. glauca* included: squid, *Loligo* sp.; hake, *Merluccius* sp.; Atlantic herring, *Clupea harengus* (Linnaeus, 1758); European sprat, *Sprattus sprattus* (Linnaeus, 1758); tuna, *Thunnus* sp.; Atlantic salmon, *Salmo salar* (Linnaeus, 1758); and shrimp of the genera *Litopenaeus* sp. and *Penaeus* sp. Sharks were typically fed 3 - 5% of their body mass (BM) each day. In one case, a single *P. glauca*, which was eating well in a single-species aquarium, became anorectic when moved to a multispecies exhibit (Roche, personal communication). One researcher reported that *P. glauca* tended to swim along the perimeter walls of an aquarium, in some cases forming contact abrasions. Perimeter swimming typically ceased during feeding sessions, then returned once feeding was over (Ezcurra, personal communication).

Rudimentary growth data was reported in the survey for two *P. glauca*. One specimen grew from 60 cm TL to 100 cm TL in eight months (Arai, personal communication), while another shark

Table 1. A summary of attempts, by six institutions, to collect, transport, and maintain blue shark, *Prionace glauca* (Linnaeus, 1758) in aquaria (extended on the facing page).

Institution	Date	Shark Size (cm)	COLLECTION		TRANSPORT		
			Capture method	Animal condition	Transport method	Transport time (min.)	Animal condition
Tokyo Sea Life Park	22-06-99	60-70	Fixed fishing net; set-net; longline	Good	1 m^3 tank	120 (car)	Good to Average
	22-06-99						
	22-06-99						
	22-06-99						
	13-07-99						
	13-07-99						
Oceanário de Lisboa	01-10-02	90	Rod & reel; circle hooks	Good	100 cm circular tank; O_2 only	240 (boat)	Good
	01-10-02	90					Good
	24-02-03	120	Set-net	Good	160 cm circular tank; O_2 + filtration		Good
	15-07-03	180	Rod & reel; circle hooks	Poor			Bad
	30-09-04	160			240 cm circular tank; O_2 only		Average
	26-05-11	70	Set-net	Good	240 cm circular tank; O_2 + filtration	300	Good
	17-05-12	100		Good			
L´Oceanografic Valencia	04-08-05	120-150	Surface longline	Good	3.5 m^3 circular tank; O_2 + filtration	540	Good
	04-08-05						
	01-08-05					600	
	01-09-05				1 m^3 rectangular tank; O_2 only	540	Bad
Aquarium de San Sebastián	01-07-06	unknown	Surface longline	Good	Inmobilized with water pump directed into mouth	90 (boat)	Good
						30 (van)	Average
Tunipex, S.A.	01-06-11	100	Set-net	Good	160 cm circular tank	45 (boat)	Good
	14-05-12	100				240 (truck)	
	17-05-12	100				45 (boat)	
Monterey Bay Aquarium	multiple: 1995 - 1998		Handline with baited hook	Good	Vessel transport	60 - 180	Good

grew from 70 cm TL to 92 cm TL in six and a half months (mean ± standard error = 1.04 ± 0.20 cm/week; n = 2)

Longevity

The longest survival times reported in the survey, by Tokyo Sea Life Park, were 164, 224 and 246 days, for three separate *P. glauca* maintained in a large, toroid exhibit tank (dimensions = 28 m outer wall diameter x 20 m inner wall diameter x 7m deep). The *P. glauca* maintained at the Oceanário de Lisboa lived for a total of 194 days in human care. The remaining animals detailed in the survey survived for periods ranging from a few hours to 78 days (Table 1). Some of these animals were subsequently tagged and released (Ezcurra, personal communication; Graça, personal communication).

QUARANTINE TANK			EXHIBIT			FEEDING				Time in Aquarium
Shape	Dimensions (m)	Volume (m^3)	Shape	Dimensions (m)	Volume (m^3)	Standard feeding	Target feeding	From bottom	Force feeding	
N/A			Deformed toroid	28 (OD) 20 (ID) x7	2.2	Yes	Yes	Yes	No	246 days
										163 days
										unknown
										unknown
										224 days
										unknown
N/A			Cylindrical	35 x 7	5	No				1 hour
										2 days
										2 hours
										1 hour
Rectangular	9.6 x 7.0 x 1.5	100	Oval	1.8 x 2	250	Yes	Yes	No	Yes	194 days
			Cylindrical	35 x 7	5,000	Yes	Yes	No	No	4 days
Circular	5 x 1	27	Elliptical	80 x 30	6,000	Yes	No	No	No	45 days
										51 days
		20	Rectangular	40 x 30 x 6	7,000	Yes	No	No	Yes	60 days
N/A										0 days
Rectangular	4 x 5 x 1	2000	N/A			Yes	Yes	No	No	7 days
Circular	10 x 2	157	N/A			Yes	Yes	Yes	No	60 days
										30 days
										30 days
N/A			Oval bowl		4,500	Yes	Yes	No	No	5 to 78 days

Survival times reported in the survey depended on multiple factors. The three major causes of mortality were predation, physiological exhaustion and physical injury. All institutions reported challenges accommodating the swimming behavior of *P. glauca*, manifest as contact abrasions on the snout and fins from rubbing against the smooth walls or windows of the aquarium. This behavior has also been described for tiger sharks, *Galeocerdo cuvier* (Péron & Lesueur, 1822) (Dehart, 2004). One possible solution presented by Dehart (2004) was to build exhibits devoid of flat walls, as sharks tend to rub against smooth areas and not against decorative rockwork, an assertion corroborated by Marin-Osorno et al. (this volume). This counterintuitive suggestion contradicts prior assumptions that abundant rockwork along exhibit walls could

present a challenge to *P. glauca* and is worth serious consideration by researchers attempting to maintain this species in the future.

KEY FACTORS FOR SUCCESS

To better understand issues contributing to the successful maintenance of *P. glauca* in aquaria, survey respondents were asked to force-rank five key parameters based on their respective experiences. On a scale of "5" to "1", where "5" was of highest importance, the results were as follows (mean ± standard error; n = 5; ranked by highest to lowest): Specimen size = 5.0 ± 0.0; Aquarium size and shape = 3.8 ± 0.5; Presence of other species = 3.2 ± 0.7; Aquarium hydrodynamics = 3.0 ± 0.9; and Water quality = 2.0 ± 0.8.

The size of *P. glauca* was considered to be critical to transport success, and the success of their long-term maintenance in aquaria. While specimens should be small enough to ease transport challenges, it should also be noted that smaller specimens were more prone to become prey to larger species in a multi-taxa aquarium.

The next most highly ranked factor for success with *P. glauca* was tank size and shape. It is presumed that exhibits needed to be large enough to accommodate the swimming behavior of this highly pelagic species—i.e., an adequate horizontal dimension to allow the shark to swim and glide unobstructed. It should be noted, however, that a *P. glauca* maintained at the Oceanário de Lisboa for 161 days was maintained in a quarantine tank of only 100 m^3. In this case, manipulation of tank hydrodynamics (i.e., altering the direction and height of water currents) may have reduced the impact of smaller tank size, and the correspondingly modest horizontal dimension (a maximum of 9.6 m), on the swimming shark. Tank shape and internal topography was also identified as a determinant of success when maintaining *P. glauca* as the species is challenged by obstacles interrupting their swimming path (Roche, personal communication).

The third most important parameter for successfully maintaining *P. glauca* was the presence or absence of other predatory species. *P. glauca* were identified as susceptible to large predators, especially sandtiger sharks, *Carcharias taurus* (Rafinesque, 1810) and sandbar sharks, *Carcharhinus plumbeus* (Nardo, 1827). Every institution attempting to keep *P. glauca* with larger shark species reported at least one death due to predation. Tokyo Sea Life Park, reporting the longest survival times for *P. glauca*, did not maintain predatory species in the same aquarium.

Aquarium hydrodynamics and water quality were deemed less important for the successful maintenance of *P. glauca*, although elevated noise and elevated illumination were both reported as potential stressors (Murguia, personal communication).

CASE STUDY AT THE OCEANARIO DE LISBOA

This case study describes the capture, transport and husbandry of a *P. glauca* held at the Oceanário de Lisboa between June 22 and December 6, 2011.

Collection and Transport

A 70 cm TL *P. glauca* was captured in a tuna set net off the coast of Olhão (South of Portugal) on 26 May 2011. The shark was transported by boat to a commercial live fish facility (Tunipex S.A.), where it was held in a round staging tank (10 m wide x 1.8 m deep) for 27 days. The *P. glauca* began eating squid (*Loligo* sp.) from a pole two days after collection. Although food was offered several times per day, the shark fed intermittently during the first month. Starting on 1 June 2011 an antibiotic regimen of enrofloxacin (Baytril™ 5%, Bayer Portugal, S.A., Carnaxide, Portugal) was administered orally at a dosage of 15 mg/kg, every other day (EOD) for eight days, to treat abrasions on the snout (Graça, personal communication). Additionally, food was supplemented with six drops of Protovit™ (Bayer Portugal, S.A., Carnaxide, Portugal), a generic multivitamin complex traditionally used for pets, humans and wild animals. On 9 June 2011, as a result of continued intermittent anorexia, a new antibiotic regimen of ceftazidime (Cefortam™, Glaxo Wellcome, Portugal) was administered at a dosage of 30 mg/kg intramuscularly (IM) every three days. The antibiotic regimen was coupled with an injection of methylprednisolone sodium succinate (Solumedrol™ 40 mg/ml, Pfizer, Oeiras, Portugal), at a dosage of 0.5 - 1.0 mg/kg intramuscularly (IM). A mixture of two ointments containing codfish oil and zinc oxide (Mitosyl™, Sanofi Winthrop, Quétigny, France), and Centella asiatica (Madecassol™, Sofex Farmacêutica, Quelux, Portugal), was applied topically to stimulate healing of snout abrasions.

Less than a month after collection (22 June 2011), the *P. glauca* was transported 280 km by road to the Oceanário de Lisboa in a 2.4 m diameter tank with a volume of 3.2 m^3. The tank was equipped with a protein skimmer (Model EV240, Aquatic Ecosystems, Apopka, Florida) and a cartridge filter (Model CFR 50, Jacuzzi, Chino, California), containing activated carbon. Ammonia was maintained at 0.0 mg/L using 25 g of an ammonium quencher (Amquel™, Kordon, Wilbraham, Massachusetts), and pH was stabilized at approximately 8.0 with multiple additions of sodium bicarbonate (100 g) and sodium carbonate (100 g). The water was changed several times during the transport, with a net replacement of two transport vessel volumes (i.e., 6.4 m^3) during the 4 h trip.

Feeding and Body mass

On arrival at the Oceanário de Lisboa, the *P. glauca* was introduced into a rectangular quarantine tank (9.6 m long x 7.0 m wide), with a volume of 100 m^3. The shark exhibited normal swimming behavior and appeared to be in good condition. Although the shark began feeding immediately, the frequency at which it accepted food was inconsistent over the first two months. On four occasions, when the *P. glauca* had been anorectic, the shark was force-fed by manually restraining it in a vinyl stretcher and using a syringe, fitted with a long tube, to administer a mixed paste of fish, shrimp, mussels, tap water and elasmobranch vitamins (PSVO 10/3, Premix™, Viana do Castelo, Portugal). Within a day or two of force-feeding, the shark typically resumed eating normally. At the beginning of the third month the shark began to feed regularly, without further assistance. At this time, preferred food was small squid, “stuffed” with other food items, such as fish, shrimp or clams. Once the *P. glauca* was feeding consistently (10 August - 21 November), the shark was weighed regularly (n = 13) and an increase in body mass was observed from 1.08 to 2.96 kg, an increase of 174% BM during the period observed, or 11.8% BM/week.

Swimming behavior

The swimming behavior of the *P. glauca* was of primary concern to the husbandry team. Constant rubbing of the snout and fins along the wall or window of the tank resulted in numerous contact abrasions, in some cases requiring medical intervention. During its six months in quarantine the shark was readily caught (via plastic bag or stretcher), and regularly handled for the administration of intramuscular injections and/or topical treatments (see below), as well as for weighing and forced feeding, when required.

In an attempt to minimize contact abrasions, the water flow pattern in the quarantine tank was adjusted to disrupt repetitive swimming patterns adopted by the shark, in particular, swimming close to the perimeter walls of the tank. The first change directed incoming water across the surface of the tank, parallel to one of the longer walls, where it had formerly been directed downward at an angle of 45° toward the bottom. This change to water current resulted in an improvement to bilateral contact abrasions on the caudal fin and some other contact lesions elsewhere on the skin. The second hydrodynamic change, made some months later, resulted in water being introduced at the bottom of the exhibit, parallel to the floor and parallel to one of the longer walls of the tank; resulting in an improvement to contact abrasions on the lower lobe of the caudal fin and on the pectoral fins.

Medical management

During the first few weeks in quarantine, the *P. glauca* presented numerous medical challenges, including recurrent anorexia, as well as dermatological and ophthalmological pathologies. When the shark was anorectic, methylprednisolone sodium succinate (Solumedrol™ 40 mg/ml) was administered at a dosage of 0.5 - 1.0 mg/kg IM.

Some weeks after moving the *P. glauca* to the quarantine tank, the shark injured its left eye, presenting as severe traumatic keratitis (corneal inflammation) with a corneal ulcer penetrating to the iris. This condition was accompanied by increased anorexia and signs suggestive of general dehydration. The antibiotic ceftazidime (Ceftazidime 1g powder for injection) was administered at a dose of 30 mg/kg IM, once every two days. In addition, povidone iodine (Betadine™ dermal solution diluted 1:20 in water, Meda Manufacturing, Mérignac, France), in combination with a tobramycin-ophthalmic suspension (Tobrex™, Alcon Cusí, S.A., Barcelona, Spain) and carbomer-ophthalmic gel (Liposic™, Dr. Gerhard Mann Chem.-Pharm., Fabrik GmbH, Berlin, Germany), was applied topically to the eye while the shark was briefly restrained. Topical treatments were continued for more than two months and, despite the severity of the initial injury, the animal responded well to treatment with the cornea completely healing. There was also some regeneration of the iris and partial vision was restored.

While in the quarantine tank, the *P. glauca* regularly presented contact abrasions on the snout, caudal fin and elsewhere on the integument. In some cases, associated tissue inflammation necessitated medical intervention. Abrasions were treated with enrofloxacin (Baytril™ 5%) administered orally at a dosage of 10 mg/kg once every two days, for periods of 6 - 19 days. If little or no improvement was observed, ceftazidime was then administered at a dosage of 30 mg/kg IM, every three days, for periods of 4 - 30 days. Antibiotics were administered until the minimum prescribed treatment course had been met, and thereafter until significant improvement to lesions was observed. In two cases, scrapes of mucous from persistent lesions associated with the nares, gill slits, dorsal surface of the trunk, and the caudal fin, were positive for a protozoan (*Uronema* sp.) and flexibacter-like bacteria. These lesions were treated with antibiotics as detailed above. In addition, an immunostimulant (Ergosan™, Intervet/Schering-Plough Animal Health Aquaculture Centre, Essex, United Kingdom) was added to *P. glauca* food, once per day, and all lesions were treated topically with chlorhexidine gluconate (Desinclor™ 5% diluted 1:20 in water, Laboratorios Vaza Sl., Madrid, Spain) and a healing enhancement ointment consisting of codfish oil, zinc oxide (Halibut™, Farmalabor - Produtos Farmacêuticos, S.A., Condeixa-a-Nova, Portugal) and *Centella asiatica* (Madecassol™), which was applied whenever the animal was restrained for IM antibiotic administration.

Death and Necropsy

At the end of November, seven months after collection, the shark was deemed fit to move to the exhibit. At this time the *P. glauca* was feeding consistently, its eye lesion had healed, and contact abrasions had healed or were stable. The exhibit aquarium was a large, flume-like loop tank with a perimeter of 70 m, a width of 1.5 - 2.0 m and a depth of 2.0 m. The flume tank included a comprehensive water treatment system and incoming water created a strong, monodirectional current. When first introduced into the exhibit, the *P. glauca* swam normally, both against and with the water current. However, the shark would not feed. On day three, the swimming behavior of the *P. glauca* became strained and labored, and its swimming behavior and condition deteriorated quickly until its death six days later.

Necropsy revealed a gastric ulceration, as well as a rupture in the cornea of the right eye leaving the animal partially blind. The ultimate cause of death is unclear, but may have been related to chronic stress, the gastric ulceration, infection secondary to the chronic lesions, deterioration of general biochemistry, ante-mortem acidosis, or possibly a combination of some or all of these problems.

CONCLUSIONS

The longest period of time any institution has successfully maintained *P. glauca* in an aquarium is 246 days. Despite the many challenges associated with maintaining *P. glauca* in aquaria, collection and transport is relatively easy for small animals (up to 100 cm TL). Larger *P. glauca* (120 - 150 m TL) have also been transported successfully, but require large, sophisticated transport vessels.

Feeding initiation for *P. glauca* in aquaria does not appear to be a significant challenge, as most individuals commence feeding regularly within a few weeks, or even days, of collection. Nevertheless, during anorectic episodes, force-feeding may be required, and has proven to be a relatively straightforward operation.

One factor critical to the success of maintaining *P. glauca* in aquaria is the provision of ample horizontal swimming distance; allowing this pelagic species to adopt a swim-glide strategy to conserve energy reserves, and to minimize the formation of abrasions from repetitive contact with the perimeter walls of the exhibit. Periodically altering the hydrodynamics within an aquarium may be a helpful strategy to interrupt repetitive swimming behavior by *P. glauca* and reduce the incidence of contact abrasions. It may also be helpful to maintain this species in an aquarium without flat vertical surfaces by installing prominent rockwork along the perimeter walls, dissuading the shark from constantly contacting the otherwise smooth walls (refer also Dehart, 2004; Marin-Osorno, this volume).

P. glauca, especially smaller specimens, appear to be tolerant of handling for medical treatments. Some sharks responded favorably to treatment protocols and demonstrated an ability to recover from lesions and other medical challenges.

P. glauca should not be maintained with larger sharks as experience has shown them to be quite

vulnerable to predation. When considering the inclusion of smaller *P. glauca* (<100 cm) in a multi-taxa exhibit, introduction of the sharks, before any other species, may be a useful strategy to reduce loss through predation.

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INTERNET RESOURCES

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Chapter 6

Capture, Transport and Husbandry of Silvertip Sharks, *Carcharhinus albimarginatus*

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Abstract: There is a paucity of information about the husbandry of silvertip sharks (*Carcharhinus albimarginatus*) (Rûppell 1837), primarily because few facilities have acquired and displayed them. To date, only uShaka Sea World (South Africa), Resorts World Sentosa (Singapore), and Dubai Mall Aquarium (United Arab Emirates) have displayed *C. albimarginatus*. Additionally, *C. albimarginatus* have been successfully maintained in holding systems, for up to 12 months, at Cairns Marine in Queensland, Australia. Our collective experiences have demonstrated that *C. albimarginatus* are a very adaptable species and can be successfully collected, transported and displayed in multi-taxa aquaria. In general, institutions considering the display of *C. albimarginatus* should start with younger, smaller specimens, as they are more resilient and adaptable. It has been observed that *C. albimarginatus* is capable of rapid growth under human care.

INTRODUCTION

Silvertip sharks, *Carcharhinus albimarginatus* (Rûppell 1837), are an enigmatic, medium-sized requiem shark found in tropical Indo-Pacific waters. This species attains a confirmed total length (TL) of 275 cm (Last and Stevens, 2009); however, anecdotal reports suggest that this species measures up to 370 cm TL in remote locations off Australia (Squire, L. V., personal communication). *C. albimarginatus* are unmistakable, with relatively large eyes and distinctive white blazes on the tips of every fin, appearing to glow as they cruise through deeper water along the outer ledges of coral reefs and atolls. In the tropical Indo-Pacific, *C. albimarginatus* are revered by divers, as one of the most impressive apex predators to be encountered.

This chapter describes the collection, transport and basic husbandry of *C. albimarginatus*, based on experiences drawn from researchers at uShaka Sea World, Cairns Marine, Resorts World Sentosa, and Dubai Mall Aquarium.

NATURAL HABITS AND BIOLOGY

C. albimarginatus are typically at their highest densities, in strong currents, where reefs meets the steep drop-off of the continental shelf. Although they are primarily an offshore species, *C. albimarginatus* occasionally swim through reefs, where the topography allows for heavy currents (Last and Stevens, 2009). *C. albimarginatus* often demonstrate a degree of site fidelity, bordering on territoriality.

Aggression and territoriality has not been observed in *C. albimarginatus* in aquaria, although it is typical for smaller specimens only to be displayed. There are reports of deep-water divers encountering groups of up to six *C. albimarginatus*, which have been observed becoming more aggressive late in the day or near dusk (Squire, C., personal communication). However, there are no reported injurious encounters between *C. albimarginatus* and humans in the wild, even when commercial recreational dive operators attract specimens for "shark feeding" interactions.

The eyes of *C. albimarginatus*, by comparison to other carcharhinids, are particularly large, which is possibly an adaptation to living in deeper water. Supporting these large eyes is a brain that is also comparatively large (Jones, personal communication; Simmons, personal communication). *C. albimarginatus* have been noted, in aquarium environments, to have a highly sensitive sensory capacity for olfaction, sound and low-frequency vibrations (Squire, L. V., personal communication; Simmons, personal communication). Very little research has been conducted to quantify the sensory capacity of *C. albimarginatus*, and it is suggested that this should be an area of future focus to optimize aquarium conditions for the species.

Socially, *C. albimarginatus* can be found in intra- and interspecific groups, in particular with black-tailed reef sharks, *Carcharhinus amblyrhynchos* (Bleeker, 1856), generally comprised of similar-sized animals. *C. albimarginatus* have been observed to hunt cooperatively (Squire, L. C., personal communication).

COLLECTION AND TRANSPORT

In South Africa (uShaka Sea World)

Three collection trips for *C. albimarginatus* were undertaken on the east coast of South Africa, just south of the Mozambique border, in waters where rocky pinnacles reached ~25 m depth. *C. albimarginatus* were frequently seen swimming in close proximity to the ocean-going vessel, corroborating other observations that these sharks are attracted to boats (Compagno, 1984). *C. albimarginatus* were readily caught on baited hooks, predominantly in the evenings. After a short struggle, of no more than five minutes, sharks (60 - 80 cm TL; 4.0 - 7.0 kg) were transferred to an 8.0 m^3 (4.4 x 2.3 x 0.9 m) oval transport tank using a PVC stretcher. Fresh seawater and supplemental oxygenation were continuously supplied to the transport tank.

On each of the three collection trips, four sharks were transported, with other reef fishes, back to uShaka Sea World. The longest transport time was 44 hours. The sharks responded well to the transport tank, displaying normal swimming behavior.

In Australia (Cairns Marine)

C. albimarginatus were targeted with rod and reel, using baited circle hooks (O. Mustard & Son A.S., Gjøvik, Norway), along the steep outer edges and gaps of the Ribbon Reefs (northern Great Barrier Reef, Australia), adjacent to the continental shelf. Strong currents at this location allowed for limited fishing times, between tidal changes only. The best time to fish for small *C. albimarginatus* was at night. However, sharks were also occasionally collected during the day.

Small juvenile *C. albimarginatus* were targeted, with a preferred length of 75 - 130 cm TL and no greater than 150 cm TL. The use of "burley" (chum) was not effective when collecting *C. albimarginatus*, as its use would also attracted large, non-target shark species, including tiger sharks, *Galeocerdo cuvier* (Péron & Lesueur, 1822), great hammerhead sharks, *Sphyrna mokarran* (Rüppell, 1837), and *C. albimarginatus* in excess of 2.5 m TL. In practice, a baited hook alone was sufficient to attract and collect small *C. albimarginatus*.

C. albimarginatus in Australia, like those in South Africa, tended not to struggle for extended periods of time, allowing handlers to easily work with the animals. Once *C. albimarginatus* were brought alongside the collection boat, they were lifted aboard using solid, knitted-net scoop nets (built in-house by Cairns Marine). Sharks were placed in the transport tank, and, whilst still in the net, the hook was removed. Before release into the tank, *C. albimarginatus* were injected intra-

muscularly (IM) with a prophylactic antibiotic, enrofloxacin (Baytril®, Bayer Corp., USA), at a dose rate of 5 mg/kg. Shark weight was estimated and the injection quickly administered, in order to minimize restraint time.

The transport tank aboard the collection boat was 4.5 m long x 5.0 m wide, with a volume of approximately 22 m^3. The tank had rounded corners on all vertices, including the ceiling and floor. Two three-phase, 2.2 kW (3 hp) pumps provided both circulation and flow of raw seawater into the tank. When necessary the pumps could be reconfigured to recirculate water in a closed loop. The walls of the tank were padded with a smooth, high-density foam rubber to absorb possible impact by startled sharks when they were introduced into the tank. An added benefit of the padding was that it also served as a sound dampener. Pure oxygen (100%) was introduced into the holding tank, and dissolved oxygen saturation levels were maintained at ~150%.

C. albimarginatus settled quickly and small sharks (<110 cm TL) generally fed within a day of capture, while still in the transport tank. The transport tank described accommodated up to nine *C. albimarginatus* at any one time, varying from 75 - 140 cm TL. Larger *C. albimarginatus* could be aggressive and would occasionally bite smaller conspecifics in the confined quarters of the transport tank. Larger sharks also took longer to settle in the transport tank and additional *C. albimarginatus* were not added until resident sharks had relaxed and resumed normal swimming behavior.

Once collected, *C. albimarginatus* were transported to Cairns Marine, a trip of 25 - 26 hours duration (250 nautical miles). In some cases it took four days, from time of capture of the first shark, before sharks were released into the quarantine facility in Cairns. For this reason, it was important to feed smaller *C. albimarginatus* while they were board the collection vessel.

Transfer tanks used to move *C. albimarginatus* from the berth to the quarantine tank, were filled with water directly from the transport tank on the boat. The sharks were removed from the boat using the same hand nets employed during collection. The drive time from the wharf to the quarantine facility was ~20 minutes. Dissolved oxygen saturation of 150% was maintained throughout the transfer. Without the use of elevated dissolved oxygen levels, larger *C. albimarginatus* would not have survived the confines of the small containers.

Once the sharks reached the quarantine facility, they were given a second injection of enrofloxacin (IM) before being released into the quarantine tank, which measured 4.5 m wide x 11.5 m long. In general, acclimatization was not necessary, as water conditions between the transport tank and the quarantine tank were equivalent. Dissolved oxygen levels were allowed to gradually drop to the ambient level of 98 - 100%, once the animals were settled into the quarantine system. In general, larger *C. albimarginatus* took far longer to settle following a long-duration transport than smaller sharks.

AIR TRANSPORT

A number of *C. albimarginatus* have been transported by air. For example, two *C. albimarginatus* (110 cm TL and 120 cm TL) were successfully transported, each in a separate tapered cylindrical transport container (base diameter 2.2 m x tank-top diameter 2.4 m x 0.6 m deep). When packed, the containers weighed ~2,300 kg each. Transport time was ~40 h and the sharks arrived in excellent condition. The two sharks were acclimatized to ambient water conditions over several hours and both sharks ate within hours of transport. Another transport was conducted with two *C. albimarginatus* in a single container, in an attempt to minimize freight costs, but the sharks did not fare well. Overall, sharks of <135 cm TL did well in the transport tanks described. It is suggested that sharks of this size are the maximum recommended for shipments lasting up to 36 hours, under the conditions described above. For shipments over 40 hours duration, it is recommended to use larger, custom-built transport containers, providing a larger swimming area and a greater water volume.

QUARANTINE and ANIMAL HEALTH

None of the *C. albimarginatus* handled by Cairns Marine or uShaka Sea World showed signs of ectoparasites. Similarly, untreated, necropsied specimens were free of internal parasites (Jones, personal communication, Simmons, personal communication). Regardless, prior to moving *C. albimarginatus* into the exhibit at uShaka Sea World, all specimens were treated with a praziquantel bath (Sigma, USA) at 2.0 mg/L.

Sharks were transferred from the quarantine tank to the exhibit using an immersion anesthetic, 0.15 ml/L 2-Phenoxyethanol (Merck Laboratories, Johannes-burg) (Vaughan et al., 2008).

At uShaka Sea World, when required for medical procedures or specimen removal, larger *C. albimarginatus* were immobilized using a chemical anesthetic, administered by a pressurized dart (Paxarms, New Zealand). Darts were loaded with a combination of 0.1 mg/kg medetomidine (Kyron Laboratories, South Africa) and 1.0 mg/kg butorphanol (V-Tech Pty. Ltd., South Africa) (Penning, personal communication). Atipamizole (Antisedan®, Zoetis, USA), at a dosage of 0.5 mg/kg, was used to reverse the effects of butorphanol.

At Cairns Marine, *C. albimarginatus* were moved from quarantine tanks to transport containers without anesthetic, and without any apparent deleterious effect to the animals. The sharks remained calm, and did not struggle excessively, when netted or placed in vinyl bags for transfer. Once in transport containers the sharks were exposed to elevated levels of dissolved oxygen, which provided an additional calming effect (Gendron, 2004).

At uShaka Sea World, *C. albimarginatus* typically had a good appetite and ate regularly, so any shark that did not feed for more than two weeks was closely monitored. Two anorectic sharks that had not eaten for more than three weeks, visibly lost weight. A course of ceftazadime (Fortaz®, Glaxo-SmithKline Inc., USA) was administered by pressurized dart to each of the sharks, at a dosage of 25 mg/kg IM. Each shark received five doses, at 72-hour intervals. Both sharks resumed feeding within a week of the final dose.

ENVIRONMENT

Baselines for water quality and life supports systems for elasmobranchs can be found in Mohan and Aiken (2004). *C. albimarginatus* are a tropical species and require warmer water. Temperature ranges employed for maintaining *C. albimarginatus* have included 20 - 26°C at uShaka Sea World, 24 - 30°C at Cairns Marine, and 26 - 27°C at Resorts World Sentosa (Brett, personal communication).

Designers of exhibits for *C. albimarginatus* should refer to Powell et al. (2004). Our experience have shown that the slow, quiet swimming behavior of *C. albimarginatus* allow them to inhabit a variety of exhibit shapes and sizes. Sharks grew well at both uShaka Sea World and Resorts World Sentosa, with contrasting exhibit sizes of 1,400 m^3 and 3,400 m^3, respectively. Larger *C. albimarginatus* preferred larger, open areas, whereas smaller specimens navigated a reef-themed exhibit very well.

Minor abrasions to the rostrum of *C. albimarginatus* were occasionally observed.

FEEDING

C. albimarginatus typically started feeding within seven days of being placed on exhibit in the large ocean tank at Resorts World Sentosa. On some occasions, feeding commenced in as little as 24 hours (Barbosa, personal communication). Smaller *C. albimarginatus* began feeding almost immediately, while larger sharks took a little longer to commence eating. *C. albimarginatus* were readily trained to accept food from tongs at a dedicated feeding station. The sharks quickly learned to associate a variety of cues with feeding sessions (e.g., changes to water flows, removal of air stones from the exhibit, etc.) and would immediately exhibit foraging behavior.

At uShaka Sea World, *C. albimarginatus* were eating by the second day, following transfer of the sharks to the exhibition aquarium. The sharks ate a wide variety of fishes and squid species, and readily took food when scatter fed from the surface. When *C. albimarginatus* detected food was in the water, the sharks shifted from their typically slow, sinuous, quiet locomotion, to a more frenetic and accelerated swimming behavior, as they sought out prey.

C. albimarginatus feeding behavior and imprinting at Cairns Marine corroborated observations at Resorts World Sentosa. Sharks at Cairns Marine were offered fishes, including tuna steaks, and squid at a ration of 25 - 30% BM/week. Young, smaller sharks grew at an estimated rate of 2.90 cm/month TL, while larger, older sharks grew up to 1.25 cm/month TL.

INTERSPECIFIC COMPATIBILITY

At Cairns Marine, *C. albimarginatus* (100 - 110 cm TL) were successfully maintained with spotted eagle rays, *Aetobatus narinari* (Euphrasén, 1790),

for several months without conflict, injury or apparent stress to either species.

At uShaka Sea World, small *C. albimarginatus* were successfully maintained in a 770 m³ exhibit with other small shark species: blacktip reef sharks, *Carcharhinus melanopterus* (Quoy & Gaimard, 1824), and blackspot sharks, *Carcharhinus sealei* (Pietschmann, 1913), as well as some large bony fish species (e.g., grouper and snapper). After three years, the sharks were moved to a 1,450 m³ exhibit, which contained other carcharhinid sharks, sand tiger sharks, *Carcharias taurus* (Rafinesque, 1810), giant guitarfish, *Rhynchobatus djiddensis* (Forsskål, 1775), and giant grouper, *Epinephelus lanceolatus* (Bloch, 1790).

C. albimarginatus at Resorts World Sentosa shared their exhibit with a wide variety of shark species, without incident, including: grey reef shark, *C. amblyrhynchos*; blacktip sharks, *C. limbatus* (Müller & Henle, 1839); sandbar sharks, *C. plumbeus* (Nardo, 1827); Japanese wobbegong sharks, *Orectolobus japonicus* (Regan, 1906); ornate wobbegong sharks, *O. ornatus* (De Vis, 1883); whitetip reef sharks, *Triaenodon obesus* (Rüppell, 1837); scalloped hammerhead sharks, *Sphyrna lewini* (Griffith & Smith, 1834); and, bonnethead sharks, *S. tiburo* (Linnaeus, 1758).

At the Dubai Mall Aquarium, two *C. albimarginatus* were fatally bitten on the head by *C. taurus*. Tank theming in the exhibit included a cave-like structure, where *C. taurus* would congregate. As *C. albimarginatus* became more comfortable and exploratory they began to enter the cave area, with unfortunate consequences (Hamilton, personal communication; Tolliday, personal communication). After these events, feeding location and frequency, and food volume, were modified to minimize the chances of another negative interaction. Despite the smaller exhibit space at uShaka Sea World, there was no observed aggression between *C. taurus* and *C. albimarginatus*. It is speculated that less exhibit rockwork at uShaka Sea World allowed for more swimming space and therefore a reduced frequency of interactions between the two species, as well as greater opportunity for *C. albimarginatus* to evade *C. taurus*.

C. albimarginatus in aquaria have never displayed intra-specific competition, and so a large group can be exhibited together.

CONCLUSIONS

Our collective experiences have demonstrated that *C. albimarginatus* can be successfully collected, transported and displayed in a multi-taxa aquarium. The information in this chapter may not necessarily express ideal husbandry requirements for *C. albimarginatus*, but is intended to provide a solid basis for other institutions researching the display of this species. Ongoing research of *C. albimarginatus* in aquaria, and in the wild, will facilitate the development of more extensive and refined husbandry requirements for the species.

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Chapter 7

Notes on the husbandry of Manta Rays

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Abstract: Manta rays (*Manta* spp.) have been part of the living collection at the Georgia Aquarium since 2009. The large rays exhibited a great awareness of their surroundings, actively responding to visual cues and displaying a great capacity to negotiate their environs. These abilities, coupled with a healthy appetite, provided an opportunity to use operant conditioning as a husbandry management tool for regularly scheduled medical examinations, with no observable ill effects on the animals. The rays rapidly adjusted to surface feeding and were fed 6 - 8% of their body mass (BM)/week of superba krill, *Euphausia superba*, pacifica krill, *Euphausia pacifica*, Atlantic silverside, *Menidia menidia* (Linnaeus, 1766), gel food and vitamin supplements.

INTRODUCTION

Manta rays (hereafter *Manta*) are globally distributed across tropical to temperate waters (Compagno and Last, 1999) and are represented by two (possibly three: refer Marshall et al., 2009) species: *Manta alfredi* (Krefft, 1868) and *Manta birostris* (Walbaum, 1792). *Manta* are members of the family Mobulidae, a group of filter feeding rays that were originally known as “devil fish”, due to their large size and the presence of “horns” on their heads. Historical references to *Manta* as “sailor killers” and “vessel destroyers” can be found in Gill (1908). The reality, however, is that *Manta* are harmless planktivores, whose feeding strategy and morphology make them all the more fascinating. The gills of mobulids have evolved into rigid sieve plates, allowing them to collect tiny food particles from the water column (Cortes et al., 2008).

Mobulids are under increasing pressure from commercial harvest, and both species of *Manta* are described as “vulnerable” on the IUCN Red List (www1). Display of mobulids in aquaria provides an excellent opportunity to transmit an important conservation message to visitors. However, only a handful of institutions have the appropriate infrastructure, resources, and exhibit size and habitat, to meet the daily and long-term needs of *Manta*, so experience with this group of animals is limited. This chapter summarizes the experiences and techniques employed by the husbandry team at the Georgia Aquarium, while caring for *Manta*.

MANTA COLLECTION AND TRANSPORT

Collection efforts for *Manta* should focus on the nearest site of known aggregation, to minimize logistical challenges. As *Manta* are migratory,

seasonality should be considered during collection planning. *Manta* for the Georgia Aquarium were collected in South Africa and Florida, USA.

South Africa

In 2007, a young female *M. alfredi* was caught in a near-shore shark net, off the coast of Durban, South Africa. The ray was rescued and transported to uShaka Sea World, South Africa, where it recovered well. The *M. alfredi* flourished at uShaka Sea World but eventually outgrew its exhibit, reaching a disc width (DW) of 2.5 m. The size of the ray, coupled with concerns about risk of re-entanglement in shark nets should the animal be released, resulted in the animal being offered as a donation to the Georgia Aquarium.

To catch the *M. alfredi*, a net was used to guide it into a vinyl stretcher, which was then employed to lift the animal into a 6.1 m x 2.3 m x 2.1 m deep transport container (Johnson's Custom Fiberglass, Florida, USA). The ray was transported while recumbent, with oxygenated water passing over its gills. A team of 10 personnel from the Georgia Aquarium accompanied the *M. alfredi* throughout the transport. Dissolved oxygen concentration and pH were monitored using a Hach HQ40D Portable Meter (Hach Company, Colorado, USA). Ammonia was tested hourly using a Hach DR/850 Portable Colorimeter (Hach Company, Colorado, USA). Dissolved oxygen was maintained at 125 - 150% saturation and pH was adjusted and maintained to match water conditions in the Ocean Voyager exhibit. The *M. alfredi* arrived safely in Atlanta after a transport of 34 hours.

On arrival at the Aquarium, the *M. alfredi* was lifted from the transport box using a modified shark stretcher, designed to support the ray while out of water (Figure 1), and lowered into a bladder stretcher (Ortega's Canvas & Sail Repair, California, USA). The bladder stretcher was then used to transfer the ray to a 25 m x 12 m x 1.8 m deep holding tank. White PVC pipes were hung vertically, at 1.5 m intervals, along the dark gray perimeter of the tank, to act as a visual reference for the *M. alfredi* so it would avoid the walls (Figure 2). These white

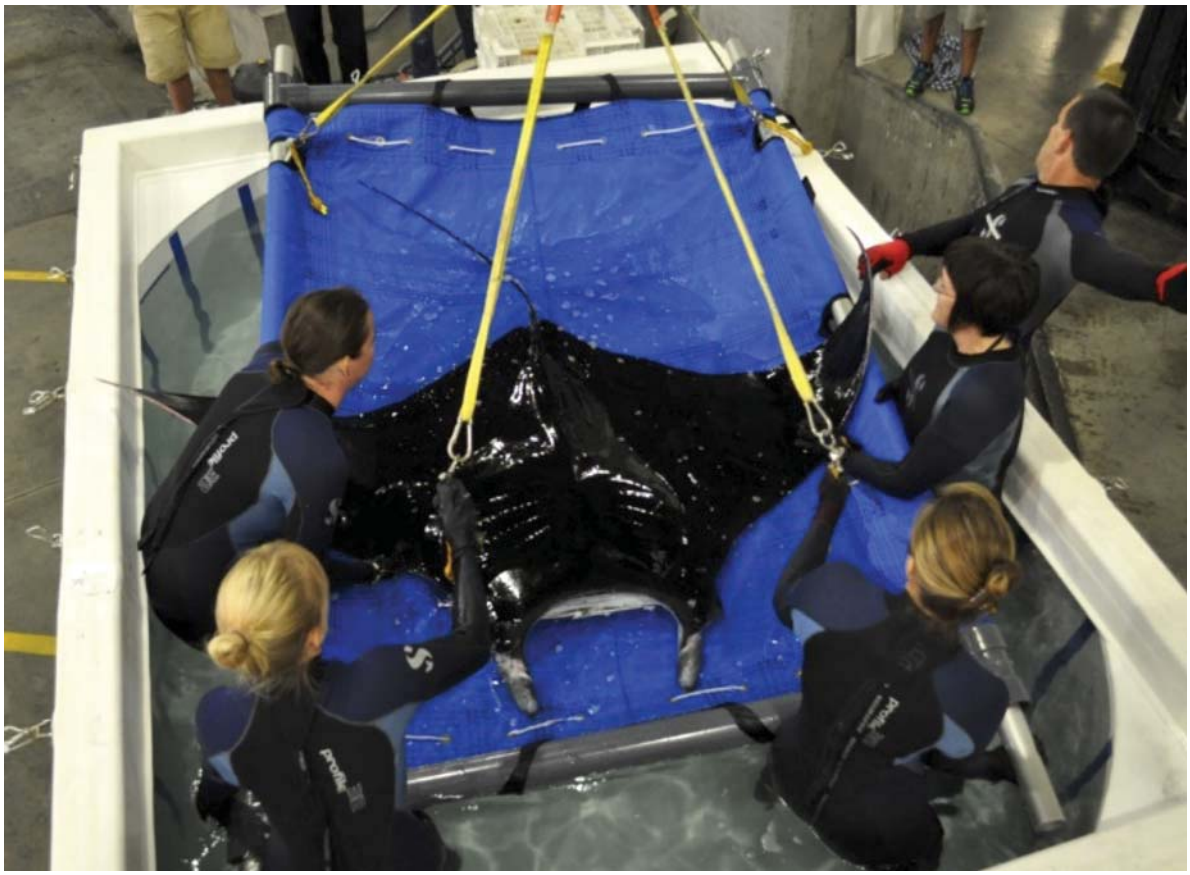

Figure 1. A *Manta alfredi* (Krefft, 1868) being transferred between tanks using a modified shark stretcher, designed to support the pectoral fins of the ray (Georgia Aquarium, Atlanta, USA).

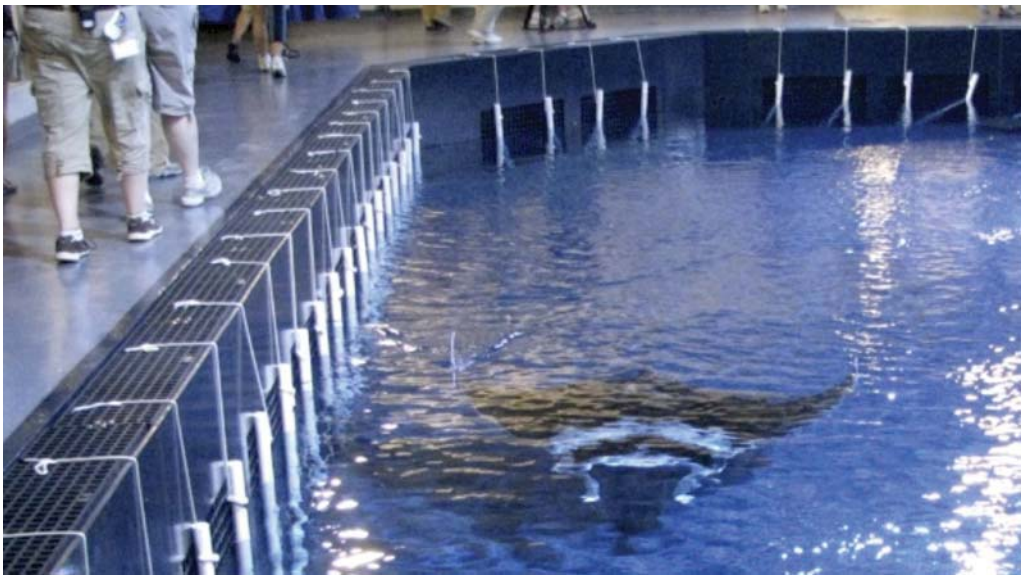

Figure 2. White PVC pipes suspended along the perimeter of a holding tank to provide a visual reference for *Manta* spp. (Georgia Aquarium, Atlanta, USA).

pipes were gradually removed as swimming patterns stabilized and the ray demonstrated that it could successfully negotiate the tank. The *M. alfredi* resumed feeding after 48 hours.

Florida

Three *M. birostris* were collected, via directed efforts, off the northeast coast of Florida. Once caught, the *M. birostris* were staged in a modular holding tank of dimensions 12 m x 12 m x 1.2 m deep. Each ray was offered food the day following collection. Feeding initiation occurred five days post-collection, for the first ray, and three days post-collection for the other two rays (held simultaneously). As soon as feeding commenced, operant conditioning was employed to train the *M. birostris*, using a ladle of food to guide the animals back and forth.

The *M. birostris* were fasted for two days prior to transportation to Georgia and transferred into a 4.6 m x 2.1 m x 0.8 m deep transport container (Johnson's Custom Fiberglass, Florida, USA) using stretchers designed to fully support the unique body form of the animals. The rays were then transported to the Georgia Aquarium via truck. Transport duration was 6.5 h and water quality parameters were monitored and maintained as described for the South African transport. All three *M. birostris* lay on the bottom of the container during transport, only occasionally moving their pectoral fins. All three *M. birostris* ate within three days of arrival at the Georgia Aquarium.

EXHIBIT DESIGN

Manta at the Georgia Aquarium were maintained in the Ocean Voyager exhibit with a volume of 15,300 m^3. An additional 8,700 m^3 of water was associated with the life support system for the exhibit, making a total volume of 24,000 m^3. The exhibit was designed to provide appropriate swimming space for numerous large pelagic elasmobranchs, as well as ample habitat for a wide variety of other species. The exhibit was 78 m long and ranged from 24.0 - 39.6 m wide (Figure 3). Water depth in the exhibit ranged from 6.1 - 9.1 m.

The exhibit was filled with synthetic seawater (Instant Ocean/Spectrum Brands, Blacksburg, Virginia, USA) and treated using a closed, recirculating life support system, including foam fractionation followed by high-rate sand filtration. Ozone disinfection and denitrification were applied on a side stream of 25% and 1% of system flow, respectively. All water then passed through a degassing tower filled with a 2.5 m deep layer of AccuPac® plastic media (Brentwood Industries, Reading, Pennsylvania), resulting in a total surface area of 107,000 m^2 (Dove, 2011). The degassing tower also served as the site for biological filtration (i.e., nitrification). Total water flow through the life support system was ~29,400 m^3/h, directed through two parallel process loops each processing ~14,700 m^3/h (Hall, personal communication). Filter backwash water was recovered, processed and re-used, to preserve both water and sea salt resources.

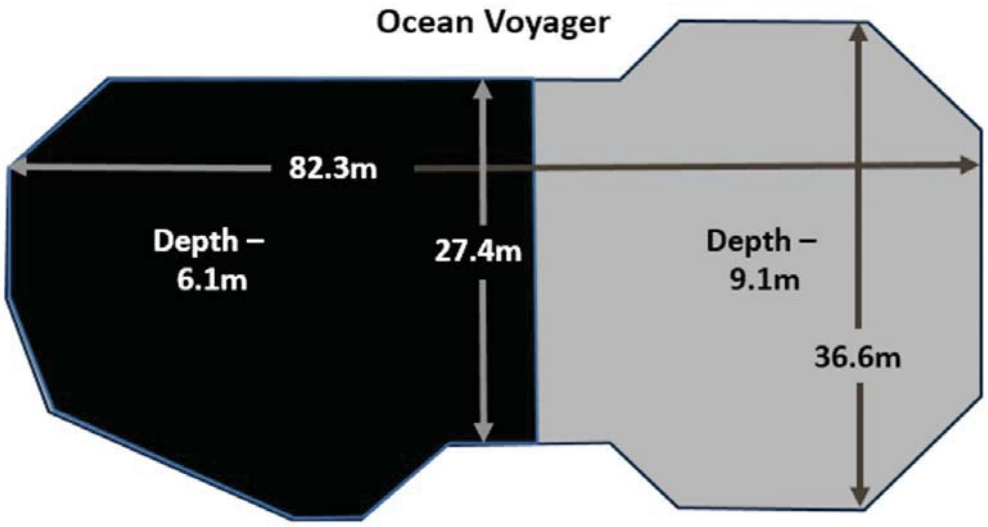

Figure 3. Plan view of the Ocean Voyager exhibit, Georgia Aquarium, USA.

Theming within the Ocean Voyager exhibit included concrete décor that depicted rocky outcroppings. Substrate comprised crushed coral/shell and silica sand in the 6 m and 9 m deep sections of the exhibit, respectively. Substrates were ~25 cm thick in both cases, but varied slightly due to water movement or animal activity (e.g., benthic rays disrupting the substrate). The most important portion of the habitat was the expansive water column, where *Manta*, sharks, and other large fishes (e.g., carangids) could glide for long distances and had ample space to change direction.

A large skylight provided exhibit lighting over the 9 m deep section of the exhibit. This illumination was augmented by an array of several dozen, paired 1,000 W metal halide lights (Lithonia Lighting, Conyers, Georgia, USA). These lighting fixtures provided illumination over the entire surface area of the exhibit. Lighting photoperiods were computer-controlled and could be adjusted, as determined by animal needs and other operational demands. A mechanized gantry, spanning the exhibit, facilitated access to the entire exhibit surface. This structure was an invaluable tool for maintaining overhead infrastructure (e.g., lighting fixtures) and for animal management.

A dedicated 22 m x 9 m x 1.5 m deep auxiliary pool, of ~300 m^3 volume, was connected to the main exhibit through a pre-cast opening in the tank perimeter. The auxiliary pool was absent of any décor and was separated from the main exhibit by a removable polypropylene mesh barrier. The auxiliary pool was used to acclimatize new animals, to initiate operant conditioning, to provide a controlled and protected area for animals to be rotated off display, and to carry out medical procedures.

HUSBANDRY

Water quality

Water quality requirements for *Manta* are consistent with other tropical, subtropical and temperate elasmobranchs. Water quality parameters measured in the natural range of the *Manta* should be used as the basis for establishing aquarium conditions. Water quality parameters maintained in the Ocean Voyager exhibit at the Georgia Aquarium are summarized in Table 1.

Table 1. Water quality parameters in the Ocean Voyager exhibit at the Georgia Aquarium, Atlanta, USA.

Parameter	S.I. unit	Range
Temperature	°C	21 - 25
Salinity	g/L	30 - 36
pH		7.8 - 8.3
Alkalinity	mg/L	200 - 250
Ammonia	mg/L	<0.01
Nitrite	mg/L	<0.01
Nitrate	mg/L	50 - 100

Spatial considerations
Manta at the Georgia Aquarium appeared to be very aware of their surroundings, actively responding to visual cues. Once a *Manta* was acclimatized to the exhibit, it would swim in close proximity to novel items; indeed the rays could be described as "inquisitive". As a consequence, great care was taken when hanging objects in the exhibit, as *Manta* are reputed to swim off at great speed, risking themselves and divers, when bumping objects in the water or becoming temporarily entangled. SCUBA divers working in the exhibit were constantly vigilant, as *Manta* would often glide close to areas being cleaned; possibly to investigate or even feed on detritus. When divers used mechanical scrubbers (Armada Systems, Inc. Florida, USA) powered from the surface, an additional diver was employed to prevent the *Manta* becoming entangled in the umbilical. Based on experiences at the Georgia Aquarium, it is recommended that infrastructure lines penetrating the water column (e.g., surface supply air lines, vacuum lines, etc.) be connected with quick-release or "tear-away" hardware, reducing the chances of a *Manta* becoming trapped.

Animal handling and Physical exams
At the Georgia Aquarium, *Manta* were periodically restrained for annual physical examinations or for medical interventions. To facilitate these procedures, *Manta* were behaviorally conditioned to swim into the restraint stretcher. When a *Manta* had not yet been conditioned, SCUBA divers carefully guided it into the stretcher. The stretcher was then raised to a depth where the *Manta* was just beneath the water surface, allowing unimpeded ventilation but preventing the ray from gaining purchase with its pectoral fins. Once the *Manta* settled (usually after 2 - 3 min), a gentle jet of water was directed into the mouth of the ray to facilitate oxygenation, and medical procedures could then commence. Morphometrics were recorded and blood samples were taken, either by caudal puncture, or from the vasculature in the posterior portion of the pectoral fins. Ultrasound was frequently employed to assess the condition of the coelom. Personnel inside the stretcher were careful to position themselves so they were not at risk of being struck by the pectoral fins of the *Manta*. Great care was also taken to avoid contact with the dorsal surface of the *Manta*, as experience demonstrated that any such contact left an obvious and persistent mark on the ray. Following medical procedures, the front panel of the stretcher was lowered and the *Manta* was allowed to swim out of the stretcher. As a precaution, SCUBA divers were stationed around the exhibit perimeter to guide the *Manta* away from the walls of the exhibit should it become necessary.

FOOD and FEEDING

***Manta* spp.**
Food consumed by wild *Manta* varies by region and is determined by their filter feeding behavior. In aquaria, *Manta* diet is frequently shaped by commercially available foods. *Manta* at the Georgia Aquarium were fed superba krill, *Euphausia superba*, pacifica krill, *Euphausia pacifica*, Atlantic silverside, *Menidia menidia* (Linnaeus, 1766), gel food (Mazuri Omnivore, Land O'Lakes, Inc., Minnesota), and vitamin supplements (Mazuri Shark/Ray Tabs II, Land O'Lakes, Inc., Minnesota).

Food items were typically introduced directly in front of the mouth of the *Manta* using a ladle attached to the end of a long pole. This technique was invaluable as a mechanism to ensure *Manta* received their daily ration, as well as a mechanism to behaviorally condition the rays.

Manta were fed twice daily, for a total of 6 - 8% BM (body mass)/week. *Manta* would occasionally supplement their diet by taking food intended for teleosts, when it was broadcast fed to the exhibit. When presented, gel would frequently lead the *Manta* to reject food and, occasionally, stop eating altogether for short periods. However, when broadcast fed, gel food was occasionally taken by *Manta* without an obvious change in behavior. Whole vitamin tablets were incorporated into the diet of *Manta*. These tablets were offered a few at a time, throughout feeding sessions, to minimize the risk of *Manta* rejecting the vitamins.

Manta rapidly became conditioned to external cues signaling the commencement of feeding sessions. These cues included a pump starting (used to propel food to the bottom of the exhibit), lowering the restraint stretcher into the exhibit and slapping the water surface.

Manta were occasionally observed skimming sand off the bottom of the exhibit, a behavior also noted in wild populations (Marshall, personal communication). Although this behavior was not cause for immediate concern, it was not encouraged as it was believed that excess ingestion of sand in aquarium

conditions could result in damage to the gastrointestinal system. *Manta* were frequently observed defecating sand.

General body condition was gauged by the dorso-ventral "fullness of body" between the pectoral fins of the *Manta*. Depression or concavity in the area of the coelomic cavity was used as an indicator of short-term malnutrition or anorexia, while distention of the coelomic cavity was an indication of excess material in the stomach.

***Mobula* spp.**

In 2008, six (three male and three female) Chilean devil ray, *Mobula tarrapacana* (Philippi, 1892), were caught by fishermen in Taiwan on behalf of the Georgia Aquarium and held in a 15 m x 30 m x 2 m deep outdoor pool. *M. tarrapacana* were induced to feed by suspending a permeable basket of food at the surface, creating an area of high food density. Once rays responded to the presence of food, the basket was moved toward the pool perimeter where additional food was offered using a ladle attached to a pole. The *M. tarrapacana* fed regularly for several weeks and even resumed feeding following a simulated transport. While similar in appearance to *Manta*, the subterminal mouth of *M. tarrapacana* made effective food delivery at the surface challenging. In addition, intraspecific variation in feeding behavior was high; some rays slowly swam at the surface or midwater, while others swam at high speed over the bottom. Ultimately, it was possible to transport only a single *M. tarrapacana* to Atlanta, which failed to resume eating post transport.

A single lesser devil ray, *Mobula hypostoma* (Bancroft, 1831), was acquired by the Georgia Aquarium in 2009. The animal was inappetent for 40 days and was sustained during this time by assisted feeding. A small-diameter nylon-reinforced tube was effective for feeding, as were larger Foley catheters. The feeding tube was inserted until it penetrated one-third of the distance into the coelomic cavity. The *M. hypostoma* was offered 3% BM of food per tube feeding session, consisting of *E. pacifica*, *E. superba*, and *Mysis relicta*.

As noted above, *Mobula* spp. are unable to process large amounts of food at the surface. Once the *M. hypostoma* started feeding voluntarily, it was transitioned from food introduction via a ladle at the surface, to food delivered from underneath by a SCUBA diver with a squeeze bottle. This procedure desensitized the ray to the presence of divers, which facilitated subsequent handling for clinical procedures.

The *M. hypostoma* was very responsive to visual cues. A large white placard, with contrasting black figures, was lowered into the exhibit to initiate feeding sessions and to demarcate a dedicated feeding station for the *M. hypostoma*.

BEHAVIORAL MANAGEMENT

Manta at the Georgia Aquarium proved to be remarkably agile. The rays demonstrated great capacity to negotiate the exhibit, turning quickly in a small space and readily swimming throughout the entire water column. This capacity, coupled with a healthy appetite and targeted feeding, provided an opportunity to use operant conditioning as a husbandry management tool. Using the feeding apparatus as a target, husbandry personnel were able to attract *Manta* from a distance >60 m and to actively guide the *Manta* into the restraint stretcher for physical exams. *Manta* were very responsive to guided directional changes and seemed remarkably adept at incorporating behavioral management strategies into their daily feeding routine. Behavioral conditioning was initiated as soon as *Manta* were collected from the wild, so quickly establishing a consistent feeding regime was paramount.

The steps to "stretcher train" a *Manta* were as follows: (1) a ray was fed from the surface and guided past a length of tarpaulin sheet, which was suspended against the wall of the exhibit; (2) a second tarpaulin, much shorter than the first, was suspended in the water some meters away from the first and the ray was guided between the two sheets; (3) the second tarpaulin was progressively increased in length until it matched the first; (4) the two tarpaulins were connected at the bottom, along the leading edge, by another short tarpaulin, using clamps that would break free if the guided ray turned prematurely; (5) the bottom tarpaulin was progressively lengthened to match the length of the "wall" tarpaulins; and (6) once the ray had routinely traversed the three tarpaulins, they were replaced with the vinyl restraint stretcher (Figure 4). Once a *Manta* had successfully traversed the tarpaulins (or the stretcher), they were re-engaged with the target, adjacent to the exhibit perimeter, and led away from the area. During training, the feeding ladle was occasionally lifted from the water and returned to the water to reinforce "targeting". When fully trained, it was possible to

Figure 4. Training a *Manta* spp. to swim into a restraint stretcher for physical exams and medical procedures (Georgia Aquarium, Atlanta, USA).

guide each ray through the stretcher during a single feeding session.

Manta were occasionally observed breaching or jumping clear of the water. As a precaution, an emergency response plan was formulated to address the unlikely scenario that a ray may leap clear of the water and onto the adjacent deck.

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Chapter 8

Husbandry of Bowmouth Guitarfish, *Rhina ancylostoma*

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Abstract: The distinctive bowmouth guitarfish, *Rhina ancylostoma* (Bloch & Schneider, 1801), has experienced an increase in popularity as an exhibit animal since its introduction to North American aquaria in 2005. Although the husbandry of this species is not particularly difficult, certain areas of care can present challenges. Successful transport of juvenile *R. ancylostoma* remains problematic. Quarantine protocols and exhibit designs for *R. ancylostoma* are consistent with those used for other free-swimming elasmobranchs. Medical challenges for *R. ancylostoma* in professional care are relatively few, and their diet consists of foods readily available to most public aquaria.

INTRODUCTION

The bowmouth guitarfish, *Rhina ancylostoma* (Bloch and Schneider, 1801), with its distinct prehistoric appearance, has recently become a popular candidate for display in public aquaria. From the 1960s onwards, the public display of *R. ancylostoma*, which are also referred to as 'shark rays' or 'mud skates' (McAuley and Compagno, 2003), was limited to aquariums in Australia and Japan (Yanagisawa et al., 1972; Tanase, 1989; Gordan, 1992). The species made its debut in

American institutions in 2005, at the Newport Aquarium, Kentucky, USA. At that time, an estimated six zoological institutions in the world were displaying *R. ancylostoma* (Dvornak, unpublished results). Since 2005, the number of institutions displaying *R. ancylostoma* has quadrupled. Given the increased popularity of *R. ancylostoma*, coupled with its listing as "vulnerable" by the International Union for the Conservation of Nature (McAuley and Compagno, 2003), a summary of current husbandry practices (i.e., transport regimes, quarantine protocols, exhibit designs, dietary needs, and medical challenges) was deemed an important inclusion in the *2nd Elasmobranch Husbandry Manual*.

NATURAL HISTORY

R. ancylostoma are the only representative species of the subfamily Rhininae (Family Rhinobatidae) (Eschmeyer, 2015). This robust marine species is most often found swimming around coral reefs or over open areas of mud or sand in the Indo-West Pacific, ranging from the Red Sea, Arabian Gulf and East Africa to Papua New Guinea, southwards to New South Wales, Australia and northwards to Japan (Compagno et al., 1989; Gordan, 1992). It is a coastal species, found in water depths of 3 - 90 m (Sommer et al., 1996).

Although the rounded snout and mouth lend themselves to the descriptive and most widely used common name "bowmouth guitarfish", it is the prehistoric look of the fish that is most intriguing and easily distinguishes it from other elasmobranchs. Most conspicuously, the dorsal surface over its cephalic region has nine distinct linear ridges of conical thorns that vary in length and prominence. The longest and most conspicuous nape-ridge runs medial and anterior to the first dorsal fin, while subsequent paired ridges run along the head and anterior portion of the dorsal surface. There has been speculation (supported by anecdotal observations in aquaria) that these ridges are used for defense (www1). Similar to elasmobranch teeth, individual thorns loosen, fall out and are slowly replaced by new thorns, which do not appear to be as sharp as their predecessor. *R. ancylostoma* have countershading consisting of a brownish gray dorsal surface and a white ventral surface. The dorsal surface may be mottled with white spots, the presence and intensity of which differ between individuals and temporally, with younger individuals having more intense patterns. Young *R. ancylostoma* have noticeable black bands between their eyes that fade with age (Last and Stevens, 2009). Adult *R. ancylostoma* reach a total length (TL) of 270 cm (Randall, 1995).

COLLECTION and TRANSPORTATION

The majority of *R. ancylostoma* displayed at zoological institutions have been the result of incidental collection or bycatch by local artisanal fishermen using a variety of techniques. In Taiwan and Indonesia, *R. ancylostoma* are caught in fixed set nets checked by fishermen several times a day, and three to four individuals may be caught annually (Fan, personal communication). *R. ancylostoma* have also been captured in '*hadra*' (tidal fish traps) in the Arabian Gulf (McEwan et al., 2000), in trawls, on long-lines (White et al., 2006), and on hook and line (Rees, personal communication). *R. ancylostoma* are typically harvested for their pectoral fins, which are dried for consumption (Frimodt, 1995) and can command a high price (McAuley and Compagno, 2003). Targeted collection for display in aquaria is challenging for a variety of reasons, including low density in the wild (Squire, personal communication; Fan, personal communication).

R. ancylostoma are not obligate ram ventilators; instead they use buccal pumping for gill ventilation. Transportation regimes appropriate for other benthic elasmobranchs can be readily adapted for *R. ancylostoma*. An emphasis on good water quality throughout the transport is recommended; in particular, adequate dissolved oxygen (DO), low ammonia concentration, and stable pH and temperature. Every effort should be made to ensure transportation routes are direct, to reduce the risk of unexpected delays in transit, and, in general, transport times should be kept to a minimum. Specifics regarding the transportation of elasmobranchs have been discussed previously in thorough detail (Smith et al., 2004).

Young-of-the-year *R. ancylostoma* (<70 cm TL) may be more susceptible to transport stress than juvenile and adult conspecifics. A weak correlation was observed between TL (at time of transport) and the duration of post-transport survivorship for 21 *R. ancylostoma* ($R^2 = 0.1498$, n = 21). Of nine *R. ancylostoma* transported at <60 cm TL, only three animals survived for longer than a year and five individuals were deceased within two months post transport. Conversely, of nine *R. ancylostoma* transported at >150 cm TL, eight animals survived more than a year thereafter.

Two separate attempts by the husbandry team at the Newport Aquarium to transport young-of-the-year *R. ancylostoma* resulted in shipment mortalities. In both cases, an aortic aneurism was noted during necropsy. On enquiry, other aquaria reported the death of small (<60 cm TL) *R. ancylostoma* within days or weeks of transportation. Histological analyses revealed severely low hepatic lipid levels in some of these smaller animals. Several reasons for deficient energy stores in young-of-the-year *R. ancylostoma* have been hypothesized: (1) animals were collected immediately post-partum, allowing insufficient time to forage and sequester hepatic lipid reserves; (2) hepatic lipid stores were depleted during hyperactivity associated with collection and transport; and (3) standard pre-transport fasting protocols further depleted already taxed hepatic lipid reserves. It is recommended that young-of-the-year *R. ancylostoma* not be transported if at all possible, and that pre-transport fasting protocols not be applied.

QUARANTINE and MEDICAL TREATMENTS

R. ancylostoma play host to a variety of parasites (Table 1) and suitable quarantine protocols are recommended for this species. In quarantine, *R. ancylostoma* should be observed for any abnormal behavior and a regular feeding routine must be quickly established.

Quarantine treatments applied to *R. ancylostoma* have generally included prophylactic *in situ* or bath administration of anthelmintics, such as praziquantel (National Fish Pharmaceuticals® Praziquantel Powder, Tucson, Arizona, USA) and fenbendazol (Panacur®, Merck Animal Health, Summit, New Jersey, USA). The use of the organophosphate trichlorfon (Dylox® 80, Bayer Corp., USA) to treat parasites may be contra-indicated in *R. ancylostoma*. In two separate cases, aquarium personnel reported the loss, or near loss, of *R. ancylostoma* after exposure to trichlorfon (Dvornak, unpublished results; Anon.[1], personal communication). However, Fan (personal communication) reports treating newly-collected specimens with 0.25 mg/L trichlorfon, dosed three times at eight day intervals, without any apparent problems.

R. ancylostoma spend a large amount of their time swimming (Gordon, 1992) and should be considered semi-pelagic (Powell et al., 2004). As with other active elasmobranchs, the size of a quarantine tank should be large enough to allow *R. ancylostoma* to swim freely and to accommodate a rest-glide/recovery phase while swimming (Klay, 1977). The use of

Table 1. Macroparasites associated with bowmouth guitarfish, *Rhina ancylostoma* (Bloch & Schneider, 1801).

Parasite species name	Parasite type	Reference
Nesippus vespa	copepod	Dippenaar et al., 2010
Pandarus cranchii	copepod	Izawa, 2010
Pandarus smithii	copepod	Izawa, 2010
Pontobdella macrothela	leech	de Silva, 1963
Branchotenthes robinoverstreeti	monogenean	Bullard and Dippenaar, 2003
Monocotyle ancylostomae	monogenean	Zhang et al., 2003
Carpobothrium rhinei	tapeworm	Sarada et al., 1995
Cephalobothrium neoaetobatidis	tapeworm	Sarada et al., 1992
Dollfusiella michiae	tapeworm	Campbell and Beveridge, 2009
Nybelinia southwelli	tapeworm	Palm and Walter, 1999
Stoibocephalum arafurense	tapeworm	Cielocha and Jensen, 2013
Tylocephalum carnpanulatum	tapeworm	Butler, 1987
Melogonimus rhodanometra	trematode	Bray et al., 1995

quarantine tanks with acute corners should be avoided. Specific considerations for the design of quarantine tanks suitable for large elasmobranchs have been thoroughly discussed by others (e.g., Choromanski, 2004). At the Newport Aquarium, *R. ancylostoma* were frequently observed swimming along the perimeter of the quarantine tank, regularly swimming against the side, or "wall-hugging", with their ventral surface rubbing the wall. In many cases, the head or pectoral fin of the animal would breach the surface. This behavior would occasionally result in pressure sores on the ventral surface of *R. ancylostoma*, most often on the pectoral fins, but sometimes on the pelvic girdle. "Wall-hugging" behavior was typically evident when *R. ancylostoma* were first introduced into a quarantine tank and normally ceased when they were transferred to the exhibit aquarium.

Relatively few other medical concerns specific to *R. ancylostoma* have been noted in aquarium specimens. At the Newport Aquarium, medical challenges were limited to acute dermal surface lesions affecting the rostrum and cornea, as well as chronic abrasions to the leading edge and apex of the caudal fin. Lesions typically arose from interactions with other animals and/or physical contact with exhibit décor. Repeated insult to chronic sites of injury would result in hypertrophy of the tissue immediately surrounding the wound. Other public aquaria reported similar challenges.

When pressure sores or lesions developed, oxytetracycline (Liquamycin® LA-200® injectable solution, Zoetis, Florham Park, New Jersey, USA) was administered intramuscularly (IM) to reduce the risk of secondary infection. Gordan (1992) reported success using oxytetracycline to treat pressure sores in *R. ancylostoma*. However, caution should be employed when using oxytetracycline, as the development of muscular necrosis and hemorrhaging has been noted following its use in this species (Anon.[2], personal communication). The prolonged use of quinolone antibiotics, such as ciprofloxacin (Ciprofloxacin, Dr. Reddy's Laboratories, Inc., Bridgewater, New Jersey, USA) and enrofloxacin (Baytril, Bayer Health Care LLC, Animal Health Division, Shawnee Mission, Kansas, USA), is discouraged for the treatment of injuries in *R. ancylostoma*, as they have been shown to cause chondrotoxicity (Pfister et al., 2007; Lim et al., 2008). The use of cefotaxime (Claforan®, Sanofi-Aventis, Bridgewater, New Jersey, USA) has shown promising results as an antibacterial option for *R. ancylostoma*.

As with many other elasmobranchs, the capacity of *R. ancylostoma* to heal from superficial abrasions and lacerations was impressive. Medical intervention should therefore be considered on a case-by-case basis, with a heavy emphasis on indirect measures—i.e., improvement to general overall health, nutrition and environmental conditions.

Sampling blood from *R. ancylostoma* was relatively easy. Tonic immobility, attained by placing *R. ancylostoma* in dorsal recumbence, was usually sufficient for the collection of blood samples. *R. ancylostoma* also tolerated restraint for short periods, while prone in the water column. Blood samples were easily drawn from the ventral coccygeal vein, which was also used successfully for the administration of intravenous therapeutics. Biochemistry reference values pooled from healthy, stress-free *R. ancylostoma* have been summarized in Table 2.

EXHIBITION

Water quality parameters for *R. ancylostoma* are relatively non-specific, matching those of other subtropical elasmobranch species (Mohan and Aiken, 2004). *R. ancylostoma* have been successfully kept in salinities from 28 - 36 g/L, however, they do not tolerate salinities below 25 g/L (Fan, personal communication). Water temperatures for *R. ancylostoma* should be kept at 24 - 28°C, as lower temperatures may result in sluggish behavior and decreased immunity (Gordan, 1992).

As noted above, *R. ancylostoma* are active, agile swimmers and should be displayed in exhibits designed for large, pelagic or semi-pelagic elasmobranchs (Powell et al., 2004). Although *R. ancylostoma* is frequently associated with reef structures (Gordan, 1992), and can navigate with ease, open areas within the exhibit water column should be provided to allow for all phases of the swim cycle (Klay, 1977). In addition, clear space must be provided on the floor of the exhibit to allow *R. ancylostoma* an opportunity to rest on the bottom. Substrate in these areas should consist of crushed coral gravel or sand, which helps prevent pressure sores as well as providing an opportunity for the animal to partially bury itself. Rocks, pipes, feeding targets and other foreign bodies in an exhibit may pose a potential heath risk to *R. ancylostoma*, as they have been observed biting hard, and ingesting small, objects. One researcher reported the death of *R.*

Table 2. Biochemistry reference values pooled from healthy, stress-free bowmouth guitarfish, *Rhina ancylostoma* (Bloch & Schneider, 1801), maintained at the Newport Aquarium. SD = standard deviation. Multiple samples were taken from a smaller number of animals, as indicated by the parentheses.

Parameter	SI unit	Mean ± SD	Range	Sample size (No. of animals)
Hematocrit	%	22.24 ± 3.24	16 - 30	45 (5)
Calcium	mg/dL	14.88 ± 0.75	13 - 16.6	51 (5)
Phosphorous	mg/dL	4.54 ± 1.16	2.2 - 7.7	56 (5)
Sodium	mMol/L	242.5 ± 9.66	231 - 264	22 (5)
Potassium	mMol/L	4.24 ± 1.13	1.5 - 7.3	56 (5)
Chloride	mMol/L	251.75 ± 19.05	218 - 280	12 (4)
TCO2 (Bicarbonate)	mMol/L	5.73 ± 2.53	0 - 9	13 (3)
Magnesium	mg/dL	3.07 ± 0.26	2.7 - 3.5	10 (4)
Blood Urea Nitrogen	mg/dL	978.2 ± 131.9	737 - 1162	13 (4)
Creatinine	mg/dL	0.23 ± 0.23	0 - 0.7	16 (3)
Total Bilirubin	mg/dL	0.09 ± 0.11	0 - 0.3	36 (4)
Glucose	mg/dL	44.4 ± 17.6	14 - 129	56 (5)
Cholesterol	mg/dL	101.36 ± 20.17	53 - 124	28 (5)
Creatine Phosphokinase	U/L	544.9 ± 680.2	16 - 3374	33 (5)
Lactate Dehydrogenase	U/L	102.2 ± 91.2	40 - 293	8 (4)
Alkaline Phosphate	U/L	71.57 ± 27.89	6 - 130	38 (4)
Alanine Aminotransferase	U/L	3.22 ± 4.22	0 - 14	31 (4)
Aspartate Aminotransferase	U/L	21.49 ± 17.07	3 - 70	31 (5)
Gamma Glutamyltransferase	U/L	14.61 ± 18.77	1 - 67	17 (4)
Amylase	U/L	4.43 ± 4.77	0 - 18	14 (4)
Lipase	U/L	58.11 ± 35.17	14 - 148	11 (3)
Total Protein	g/dL	3.16 ± 0.58	2.1 - 6.2	58 (5)
Globulin	g/dL	2.67 ± 0.28	2.2 - 3.1	23 (5)
Albumin	g/dL	0.48 ± 0.25	0 - 1.1	27 (5)
T3	ng/dL	71.75 ± 43.49	37 - 138	4 (2)
T4	ug/dL	1.267 ± 0.66	0.5 - 2.1	8 (2)

ancylostoma following the ingestion of some aeration tubing that had been buried in the exhibit (Anon.[3], personal communication). In another case, necropsy of a *R. ancylostoma* revealed a gut impacted with gravel (Dvornak, unpublished results).

COMPATIBILITY

Adult *R. ancylostoma* are generally not at risk from predation by other elasmobranchs typically exhibited in public aquaria, although tiger sharks, *Galeocerdo cuvier* (Péron & Lesueur, 1822), are known predators of this species (Simpfendorfer et al., 2001). However, it is recommended that juvenile *R. ancylostoma* (<100 cm TL) be kept separate from larger shark species to reduce the risk of predation. Non-fatal injuries resulting from interactions with other species have been recorded for *R. ancylostoma*. Loggerhead turtles, *Caretta caretta* (Linnaeus, 1758), and green turtles, *Chelonia midas* (Linnaeus, 1758), have been observed biting *R. ancylostoma* (Dvornak, unpublished results; Rees, personal communication), as have zebra sharks, *Stegostoma fasciatum* (Hermann, 1783), and lemon sharks, *Negaprion brevirostris* (Poey, 1868) (Rees, personal communication). Bannerfish, *Heniochus* spp., have been observed pecking around the eyes of *R. ancylostoma*, causing injuries to the dermis surrounding the orbit (Fan, personal communication).

Intestinal eversion occurs in *R. ancylostoma* (Dvornak, unpublished results), presenting an opportunity for injury to the valvular intestine when bitten by other fishes. An example of this

interaction was observed at The Scientific Center (Salmiya, Kuwait), when a golden trevally, *Gnathanodon speciosus* (Forsskål, 1775), was observed biting the valvular intestine of a *R. ancylostoma* (Dvornak, unpublished results). On this occasion the injury was minor and quickly healed, but during the recovery period the *R. ancylostoma* swam close to, or remained prone on, the substrate.

R. ancylostoma can prey upon other species within an exhibit, both benthic and pelagic (Borrell et al., 2011). Examples of species preyed upon by *R. ancylostoma* include: Sergeant baker, *Latropiscis purpurissatus* (Richardson, 1843); orangespot surgeonfish, *Acanthurus olivaceus* (Bloch & Schneider, 1801); juvenile spotted eagle rays, *Aetobatus narinari* (Euphrasén, 1790); juvenile giant guitarfish, *Rhynchobatus djiddensis* (Forsskål, 1775); bonnethead shark, *Sphyrna tiburo* (Linnaeus, 1758); and cownose stingray, *Rhinoptera bonasus* (Mitchill, 1815) (Gordan, 1992; Dvornak, unpublished results; Anon.[4], personal communication). Injuries from *R. ancylostoma* have also been reported for the common guitarfish, *Rhinobatos rhinobatos* (Linnaeus, 1758), and the giant shovelnose ray, *Glaucostegus typus* (Anonymous [Bennett], 1830) (Rees, personal communication).

R. ancylostoma appear to be tolerant of conspecifics. Several aquaria have exhibited more than one *R. ancylostoma* in the same exhibit, most displaying pairs but some maintaining as many as four to six individuals. It should be noted that feeding multiple *R. ancylostoma* can be a challenge, due to their aggressive nature when eating. Several staff members should be used during feeding sessions to manage individual *R. ancylostoma*, ensuring they get their food ration and do not sustain injury. Interactions between sexually mature *R. ancylostoma* should be closely monitored to ensure that breeding aggression does not result in serious injury (Dvornak et al., in preparation).

FOOD and FEEDING

R. ancylostoma have strong, multilobed jaws covered with small, rounded teeth, which are effective for crushing and chewing zoobenthic prey, such as crustaceans, mollusks (Compagno and Last, 1999), and fishes (Gordan, 1992). Although *R. ancylostoma* eagerly accept a wide variety of food types appropriate for medium to large elasmobranchs, a robust diet containing crustaceans, such as Caribbean spiny lobster, *Panulirus argus*, or American lobster, *Homarus americanus*, is strongly encouraged. At the Newport Aquarium, *R. ancylostoma* were regularly fed these and other high-quality food items. Supplemental elasmobranch multivitamins (i.e., Mazuri® Vita-Zu Shark/ray II tablets, PMI Nutrition International, Missouri, USA; and Elasmobranch Tablets®, International Zoo Veterinary Group, Keighley, UK) were regularly administered. Vitamin C (NutriBiotic 1000 mg capsules, NutriBiotic, California, USA) was frequently included in the diet at 6,000 mg per animal, five times per week, due to its role in cartilage synthesis (Janse et al., 2004) and its general restorative properties. The high-quality diet presented to *R. ancylostoma* is believed to be a key factor in the general well being of all specimens maintained at the Newport Aquarium. This level of dietary care represents a high financial commitment to *R. ancylostoma* and should be considered when planning to maintain the species in aquaria.

R. ancylostoma were fed at a rate of ~2 - 4% BM/day (i.e., body mass/day), although younger specimens require a higher ration. Some aquaria reported feeding *R. ancylostoma* to satiation in order to prevent aggression toward other exhibit animals (Rees, personal communication). Inducing inappetent, newly acquired juvenile *R. ancylostoma* to feed, has been achieved using live crustaceans, such as: Sally Lightfoot crabs, *Grapsus grapsus*; nimble spray crabs, *Percnon gibbesi*; small Atlantic blue crabs, *Callinectes sapidus*; whiteleg shrimp, *Litopenaeus vannamei*; northern pink shrimp, *Penaeus duorarum*; and white shrimp, *Litopenaeus setiferus*.

Broadcasting food can be an efficient way to feed *R. ancylostoma*, especially during quarantine. However, their aggressive and voracious feeding behavior can make this technique of food delivery in a mixed-species exhibit challenging. Target-training *R. ancylostoma* provides a valuable management tool for food delivery and also the application of clinical procedures. This species is highly motivated by food and easily target trained. At the Newport Aquarium, *R. ancylostoma* was readily trained to follow a schedule-80 PVC pole at the surface of the water. SCUBA divers could simultaneously feed two *R. ancylostoma* with the aid of hand-held targets, consisting of a food tube welded to the center of a flat plate of schedule-40 PVC. This technique was labor-intensive, but ensured that all specimens received their food ration.

CONCLUSIONS

R. ancylostoma have become more numerous in public aquaria in recent years, as their unique appearance makes them a popular exhibit species. There is an appeal to *R. ancylostoma* that, despite their prehistoric appearance, makes them endearing to guests and, therefore, an effective icon for aquatic conservation. As their numbers increase in public aquaria, so does the obligation to closely study these remarkable animals and further refine their husbandry. There remain challenges to the successful long-term display of *R. ancylostoma* in aquaria, including the transport of young specimens, captive reproduction of the species, and the effective rearing of newborn pups. As public aquaria work to illuminate solutions to these challenges, it is hoped that the knowledge gained about *R. ancylostoma* will help serve to protect wild populations of this remarkable species.

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Chapter 9

Husbandry of sawfishes

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Abstract: Sawfishes (Family: Pristidae) have been successfully kept in aquaria for decades and are unmistakable, their elongated saw-like rostrum comprising up to 25% of their total body length (TL). Pristids are hardy animals and thrive in a variety of aquarium conditions. Proper handling of pristids during capture and transport is just as important for the handler as it is for the fish. Even as juveniles, pristids are powerful and can cause serious injury to handlers if improperly secured or controlled. In some cases, it may be necessary to cover the rostrum of a pristid. Covers serve to protect the rostral teeth and help to protect handlers if struck by the saw. Long-duration transport of a pristid requires a tank that affords the animal enough space to rest on the bottom and to turn, but not enough space to encourage swimming. Whenever transporting pristids, dissolved oxygen should be maintained at 200 - 300% saturation. Pristids readily accept a varied diet and can take prey from the water column, using their saw to slash at their quarry, or the benthos, where they swim over and consume food items. Pristid reproduction in aquaria has only been recorded on one occasion for a single species, *P. pectinata*. The minimum requirements for pristid reproduction are unknown, although ample space, and natural fluctuations in water temperature, salinity, and photoperiod, are likely to be important.

INTRODUCTION

The family Pristidae is comprised of two genera, *Pristis* and *Anoxypristis,* and five species. The largetooth sawfish, *Pristis pristis* (Linnaeus, 1758), smalltooth sawfish, *Pristis pectinata* (Latham, 1794), and green sawfish, *Pristis zijsron* (Bleeker, 1851), are the main focus of this chapter. For the purpose of this publication, sawfishes are referred to as pristids and husbandry is generalized, unless otherwise specifically stated.

Pristids are found worldwide, from tropical, shallow marine coastal waters to estuarine and freshwater river systems (Last and Stevens, 2009). Pristids are large, charismatic, and currently represent some of the most critically endangered elasmobranchs (Simpfendorfer, 2000; Scharer et al., 2012) on the planet. Fisheries are believed to be the primary reason for decrease in pristid populations, as their large "dramatic" saw can easily become entangled in fishing nets, often resulting in injury or death (Simpfendorfer, 2000; Seitz and Poulakis, 2006; Stevens et al., 2008). Historically, the "saws" of pristids were harvested as a keepsake by fishers (Stevens et al., 2005). Pristids were also killed because they were considered dangerous and/or a nuisance (Squire, personal communication).

Seven species of pristids have been described. However, a recent study has led to the reclassification of three species into one. Faria et al. (2013) looked at the morphology, historical taxonomy, and mitochondrial DNA of all species of pristids, and found that, instead of seven distinct species, there are only five. *P. pristis* is now considered a circumtropical species that encompasses the former species of *Pristis microdon* (Latham, 1794)*, Pristis perotteti* (Müller and Henle, 1841) and *P. pristis*. Although *P. pristis* now encompasses three formerly separate species, they are ecologically different and the sub-group from the Indo-West Pacific, formerly *P. microdon*, will be referred to as such in this chapter. Pristids in public aquaria (at the time of writing) were obtained from specialist commercial aquarium fish collectors in Australia. The commercial collector was granted a permit to acquire the pristids in recognition of the educational value of these animals.

Relatively few pristids have been displayed in public aquaria. Pristids become large in size and require a correspondingly large exhibit to remain healthy as they grow. The studbook for North American aquaria lists the population of pristids as 5.7.0 *P. pectinata*, 10.6.0 *P. microdon*, and 7.6.0 *P. zijsron* (White, 2014). The European studbook lists aquarium pristid populations as 3.2.0 *P. microdon* and 3.3.0 *P. zijsron* (Duke, 2013). Australian aquaria currently maintain 6.7.0 *P. microdon*. Two specimens of *P. zijsron*, as well as 1.1.0 dwarf sawfish, *Pristis clavata* (Garman, 1906), are currently maintained in an aquarium in Japan.

GENERAL BIOLOGY

Pristids are unmistakable: their elongated saw-like rostrum comprising up to 25% of the total length (TL) of the animal (Thorson, 1982). Each species has a distinct number of "teeth" arranged along the length of the rostrum. The pattern, or spacing, between rostral teeth is taxonomically distinct. Furthermore, the number of teeth within each species can be regionally dependent. Wueringer et al. (2012) examined use of the saw by juvenile *P. microdon* and determined it to have exceptional electrosensory capacity, helping young animals detect and capture prey buried in the substrate.

P. microdon can be distinguished from other pristids by a distinct lower lobe on the caudal fin, a first dorsal fin anterior to the pelvic fins, and 17 - 24 pairs of rostral teeth (Last and Stevens, 2009; Thorburn et al., 2007; Whitty et al., 2009). Peverell (2008) found that *P. microdon* grew to a maximum of 6.0 - 7.0 m TL, and estimated their lifespan to be a maximum of 80 years. The largest specimen of *P. microdon* captured and physically examined was 5.82 m TL, had 30 ovacites and was determined to be 36 years old through laser ablation of vertebrae (Peverell, 2008). Size at maturity for *P. microdon* has been reported to be between 2.8 - 3.0 m TL for males and over 3.0 m TL for females (Thornburn et al., 2007; Peverell, 2008).

P. pectinata have 22 - 29 pairs of rostral teeth (Wiley et al., 2008). This species can be further identified by the first dorsal fin origin being anterior to the pelvic fin origins (Faria et al., 2013), as well as the lower lobe of the caudal fin, which is not well defined (Compagno and Last, 1999; Wueringer et al., 2009). There is some disagreement about maximum size and age-at-maturity for *P. pectinata*, but Carlson et al. (www1) states that age-at-maturity for males is around 7.5 years and 10 - 12 years for females.

P. zijsron are a large and robust species. *P. zijsron* are identified by 24 - 28 pairs of rostral teeth, no bottom lobe to the caudal fin and a first dorsal fin origin behind the pelvic fin (Stevens et al., 2005). Stevens et al. (2005) also noted that the pattern of rostral teeth could be used to identify the species. At the base of the rostrum the teeth are widely spaced, with spacing decreasing at regular intervals towards the tip of the rostrum. *P. zijsron* also exhibits a greenish-brown, or olive coloration on its dorsal surface, giving rise to its common name (Stevens et al., 2005; Duke, 2013). *P. zijsron* are believed to be the largest of the extant pristids, with reported lengths >7 m TL (although animals of this size are extremely rare) (www2). Age-at-maturity is estimated to be around 9 years (Stevens et al., 2005), with a theoretical maximum age of ~53 years (Peverell, 2008).

LEGISLATION

Regional, national and international legislation restricts, or severely constrains, pristid collection and trade, depending on purpose. The IUCN Red List denotes all pristids as "critically endangered" (www1; www2; www3) and they are all protected and listed under Appendix I of the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES).

In December 2014, all pristids were listed as "endangered" under the Endangered Species Act (ESA) of the United States. The U.S. distinct population segment of *P. pectinata* was initially listed under the ESA in April of 2003. This listing required that a recovery plan be developed and put into effect, and in January 2009 the Smalltooth Sawfish Recovery Plan was published. The ultimate goal of the recovery plan was to "...*rebuild and assure the long-term viability of the U.S. population of P. pectinata in the wild, allowing initially for reclassification from endangered to threatened status (downlisting) and ultimately recovery and subsequent removal from the List of Endangered and Threatened Wildlife (delisting)*..." (NMFS, 2009).

P. zijsron is listed as "vulnerable" under the Environment Protection and Biodiversity Conservation (EPBC) Act in Australia, and individual states in Australia have additional listings for the species. *P. zijsron* was listed as "vulnerable" in the Northern Territory under the Territory Parks and Wildlife Conservation Act of 2000. In New South Wales, *P. zijsron* was listed as "endangered" under the Fisheries Management Act of 1994 and in Western Australia it was listed as a "totally protected fish" by the Department of the Environment (www3). Queensland Fisheries have listed all pristids as a "no-take species", preventing commercial or recreational collection. However, under exceptional circumstances, a special fisheries permit can be obtained to allow for the collection of pristids for the sole purpose of public display.

If an aquarium is considering exhibiting a pristid, a deaccession plan to a suitable institution, consistent with the recommendations of the regional studbook, should be established before acquiring a specimen.

COLLECTION and QUARANTINE

Most pristids in public aquaria were obtained as juveniles, ranging between 1.0 - 1.8 m TL and estimated to be 1 - 3 years old. At this size, animals are easily handled and transports are relatively economical. In Australia, *P. zijsron* for public display were collected in saltwater foreshore localities, whilst most *P. microdon* were collected from either fresh or brackish water environments.

Pristids do well in round or rectangular quarantine tanks. Tanks should have a flat base and be free of objects and/or intrusive life support system equipment that could entangle or otherwise trap the pristid. The quarantine tank bottom should be bare or contain only a thin layer of sand.

Despite their shape and size *P. microdon* has an impressive ability to "climb" out of a tank using their pectoral fins for purchase, while propelling forward with their caudal fin. A 1.8 m TL animal was observed jumping clear of a holding tank, for a horizontal distance of 5 m. This remarkable capacity may stem from their adaptation to an annual migration up flooded river systems (500 km distance in northern Australia) during monsoon season, whereby the pristid must traverse small to medium-sized waterfalls and rapids. Holding tanks with high sides and secure, entanglement-resistant lids are therefore recommended for young *P. microdon,* to prevent them from propelling themselves out of the water.

Pre-transport quarantine

Pristids collected from the wild can carry a wide range of parasites, regardless of whether they have been collected from fresh or saltwater. Experiences at Cairns Marine (Stratford,

Queensland, Australia) have shown pristids to be susceptible to fungal infection (suspected, but not confirmed, *Saprolegnia* sp.) if maintained in fresh water immediately post capture. Fungal infections were apparent on the dorsal surface of the animal, presenting as opaque, whitish patches, and were likely the result of stressors experienced during the capture and transport process resulting in a compromised immune system. Fungal infection would result in death of the host within 24 - 48 h if not treated. For this reason, juvenile pristids collected from freshwater should be acclimated to a salinity of 12 g/L as quickly as possible.

Monogeneans (suspected, but unconfirmed, *Dermophthirius* sp.) have been observed on both *P. microdon* and *P. pectinata* (Squire, personal communication). If left untreated, monogeneans can lead to the death of the host. Mongeneans, as well as parasitic isopods and copepods, have been observed on animals collected in both fresh and saltwater, calling into question the efficacy of short-term fresh or saltwater baths as an anti-parasitic treatment. Praziquantel (Biltricide®, Bayer, Leverkusen, Germany), applied as a bath, or prolonged immersion treatment, has been an effective treatment for most species of monogeneans found on pristids, including *Benedenia* spp. Due to anecdotal reports of pristid mortality when exposed to trichlorfon (Dylox®, Bayer, Leverkusen, Germany) and other organophosphates, this chemical group is not recommended.

Pristids can be affected by sporozoans (parasitic, spore-forming protozoans). These parasites appear as small, white clusters or spots on the dorsal surface of the pristid and are difficult to eradicate. Affected host fishes may also have an increased ventilation rate, and become anorectic and dehydrated. Sporozoans observed on *P. microdon* were unaffected by fresh or saltwater baths, even when treatments were applied for extended periods of time. In addition, trials with quinine sulfate (Actavis, Devon, EX32 8NS, UK), chloroquin diphosphate (Sigma-Aldrich Corporation, Natick, Massachusetts, USA) and dimetridazole (Emtryl, BEC Feed Solutions, Carole Park, Queensland, Australia), appeared to have little impact as a treatment for sporozoa. A successful treatment for a sporozoan infection of a pristid in a Japanese aquarium employed a series of five copper baths, with a 48 h interval between each treatment (Ogawa, personal communication). Copper baths have been successfully applied to *P. microdon* at Cairns Marine at a dosage of 0.5 mg/L copper for 1.5 h, daily, for five days, followed by a two-day rest period, then a repeated five-day treatment. Dissolved oxygen was elevated to 150% saturation during copper treatments. Juvenile bull sharks, *Carcharhinus leucas* (Muller and Henle, 1839), which share a similar life history to that of *P. microdon*, have also been affected by sporozoans and successfully treated with a similar regime of copper at a dosage of 0.4 mg/L for 2 h, daily, for ten days.

Post-transport quarantine

Once a pristid reaches its destination aquarium, applied quarantine protocols should be as non-invasive as possible. Throughout quarantine, it is critical to continually monitor and assess the status of prisitids to ensure they remain healthy; body condition, feeding behavior, swimming behavior, body “attitude”, and general awareness of surroundings, should all be monitored. When resting on the bottom a strong, healthy *P. microdon* will elevate its rostrum at an angle of up to 30° to the plane of the substrate, whereas the rostrum of an unhealthy or “exhausted” *P. microdon* will rest flat on the bottom (Baggio and Squire, 2004). Conversely, *P. zijsron* will lie with their rostrum flat on the substrate, or parallel to the substrate if they are sitting high on their pectoral fins. A stressed or unhealthy *P. microdon* may exhibit an elevated ventilation rate. For young, healthy pristids a normal ventilation rate is 45 - 55 gill beats per minute (Baggio and Squire, 2004).

Many public aquaria have reported the application of praziquantel (Biltricide®, Bayer, Leverkusen, Germany) as a prophylactic treatment, prior to introducing pristids to a mixed-species exhibit aquarium. Due to the efficacy of praziquantel, no other chemotherapeutics have been reported as necessary.

Conversion to saltwater

Most public aquaria maintain adult *P. microdon* in seawater but they can be displayed long-term in either fresh or saltwater, regardless of specimen size. It has been observed that *P. microdon* acclimate to saltwater, from freshwater, more slowly than the reverse. The acclimation rate varies by individual and is dependent on the time elapsed since previous exposure to saltwater. As with any modification to the environment, acclimating pristids from fresh to saltwater requires careful observation, allowing the response of the animal to dictate the speed of change. If the change is too rapid the pristid may become wrinkled and emaciated due to

dehydration, and it may also become anorectic. Should this occur, salinity should not be adjusted further until the animal resumes eating regularly and body condition returns to normal. Thereafter, a period of two days should be allowed for recuperation before applying additional salinity changes. By using this method, the pristid is never deeply compromised and the conversion process can occur as quickly as possible, ranging from three days to several weeks. In most cases, pristids shipped (by Cairns Marine) from Australia to public aquaria were fully acclimated to saltwater—i.e., a salinity of 30 - 35 g/L.

When a pristid is first moved from quarantine to an exhibit aquarium, a regime of careful monitoring should be employed for several days to ensure that the animal is able to maneuver readily and is adapting well to its new environment.

HANDLING

Proper handling of pristids during capture and transport is just as important for the handler as it is for the fish. Even as juveniles, pristids are powerful and can cause serious injury to handlers when improperly secured or controlled. Nets should never be used to handle pristids, as their use inevitably leads to animal entanglement and broken rostral teeth. It is imperative that all persons involved in the capture and handling of pristids wear protective clothing, as their skin is highly abrasive.

Handling best practice varies depending on the size of the pristid, as well as the experience of those managing the animal. Pristids <1.2 m TL should not be handled in a stretcher. Restraint with a stretcher is clumsy, allows the animal to struggle, and, if not completely restrained, potentially break rostral teeth or sustain other damage. When handling a juvenile pristid, it is possible to simply grab the base of the rostrum over, or behind, the last set of rostral teeth, immobilizing the rostrum with the aid of either a glove or some kind of fabric for protection. The caudal fin is then immediately immobilized by a second person while the first person uses their free arm to hold the animal behind the first dorsal fin, effectively restraining the animal and supporting its abdomen. The pristid can twist and turn, but cannot gain purchase if handled in this manner. While this method may appear overly simple and overtly “hands on”, no injury to the pristid or handler will occur if conducted competently and quickly. For a pristid >1.5 m TL this method of restraint should be avoided, unless a highly experienced and suitably skilled team is available. Once the pristid had been secured a blindfold should be loosely applied over its eyes, which rapidly calms the animal and reduces attempts to “slash” at the handlers. Thereafter, rostral covers are easily applied if needed (see below).

Large pristids require a different method of capture and handling. A vinyl stretcher should be used, large enough to enclose and restrain the entire body and rostrum of the animal. Restrain in a stretcher allows for better control and minimizes unnecessary stress or tension on the caudal region of large pristids or their rostra. It is best practice to allow the pristid to slowly swim into the stretcher, but it is not always practical. As an alternative, an underwater catch and restraint bag, made from clear PVC, can be a very effective tool if tailored to the size and shape of the pristid. Once captured in the PVC bag, the pristid can then be placed into a stretcher for further restraint and handling.

Short, rigid PVC poles can be used to slowly guide a pristid into a stretcher or bag. By gently placing the pole beside the rostrum, or beside the pectoral fins, the animal can be guided in the desired direction. This process can be done from outside the tank or, very carefully, by a diver swimming above the pristid.

Very large pristids may require sedation to allow safe handling. AQUI-S® (AQUI-S New Zealand Ltd, Lower Hutt, New Zealand) has been used successfully to sedate pristids at a dosage of 20 - 25 mL/m^3, raised slowly over a period of 15 - 20 min. During sedation, pristids should be continually monitored for adverse reactions, or the potential for overdose, as a high variability of response to sedatives has been observed within the taxon.

If a pristid is restrained for an extended period, oxygenated water should be directed over the gills via the spiracles or mouth. Pristids are tolerant of high dissolved oxygen (DO) concentrations and a DO of 200 - 300% saturation is recommended during restraint. Oxygenation is best managed by placing the pristid into water—e.g., while still in the restraint stretcher—that has already been raised to the desired DO concentration. Oxygenated water directed over the gills using a submersible pump is also effective. Elevated

oxygen concentrations will help calm the animal and allow for easier manipulation during handling and transport.

Rostral covers
In some cases it may be necessary to cover the rostrum of a pristid. Covers serve to protect the rostral teeth and help to protect handlers if struck by the saw. Rostral covers can be made from a variety of materials, including outdoor carpeting and towels. The chosen material should be strong, able to withstand puncture, and be neutrally buoyant. Neoprene is not not advised for extended periods of time as it is positively buoyant, which may cause stress to the animals, and it is easily penetrated by rostral teeth. Outdoor carpet is recommended, which can be secured with pre-attached strips of heavy-duty Velcro®. Cable ties can be used to supplement the Velcro® if the rostral cover needs to be secured for an extended period of time.

Careful planning should be employed to ensure the rostral cover is sized to protect the entire length of the saw. To apply a rostral cover one handler should restrain the base of the rostrum, while a second handler controls the tip of the rostrum and carefully applies the device. The pristid may attempt to remove the cover in the same way they remove fish from their rostral teeth in the wild, by slashing their rostrum back and forth. Handlers must therefore exercise great personal care when handling the rostrum of a pristid.

A well-secured rostral cover may be left on a pristid during long-duration transports and will not cause undue stress to the animal. However, a rostral cover left on a pristid for more than three days may result in a bacterial infection, especially in elevated water temperatures.

Diving with pristids
Pristids are typically passive, docile animals and tolerate divers swimming nearby. Regardless, great care should be taken when diving with these powerful animals, the speed and force of a rostrum swipe can cause significant damage. Newly introduced animals should be given a wide berth until they become accustomed to divers. Abnormal swimming patterns or erratic behavior in the presence of a diver may indicate that a pristid is stressed, so remain vigilant for these tell-tale signs. If a pristid is in an exhibit used for a guest dive program, great care should be taken to ensure that divers do not attempt to touch or interact with the fish.

Training pristids
Pristids respond well to positively reinforced behavioral conditioning. Some aquaria have used training programs to improve the effectiveness of feeding protocols and minimize stress during husbandry procedures. Researchers at the Aquarium Mare Nostrum (Montepellier, France) target-trained two *P. pristis* while in quarantine, so that once the fish were moved onto exhibit they could be targeted to enter a prescribed shallow area where feeding behavior could be readily observed. Training took two months and six months, respectively, for the two pristids to routinely “station” on a target (Hirel, personal communication). The husbandry team at Sea World Orlando (Florida, USA) conditioned two *P. pectinata* (1.1.0) to swim into a medical pool at feeding times (Violetta, personal communication). Conditioning the pristids in this manner minimized handling during physical exams and medical procedures.

TRANSPORT

Long-duration transport of a pristid requires a tank that affords the animal enough space to rest on the bottom and to turn, but not enough space to encourage swimming. Pristids are capable of turning in a tank that is two-thirds their TL, even shallow tanks. Whenever transporting pristids, DO should be maintained at 200 - 300% saturation.

The husbandry team at Ripley’s Aquariums successfully transported three adult pristids by road for extended durations: a *P. pectinata* was transported from Vallejo (California, USA) to New Orleans (Louisiana, USA); a 3.4 m TL male *P. zijsron*, weighing 130.4 kg body mass (BM), was transported from Myrtle Beach (South Carolina, USA) to Toronto (Ontario, Canada) via Gatlinburg (Tennessee, USA); and a 4.2 m TL, 207.7 kg BM, female *P. zijsron* was transported from Gatlinburg (Tennessee, USA) to Toronto (Ontario, Canada). In all three cases a dedicated transport trailer was used, complete with temperature control, oxygenation, mechanical filtration and a portable power supply. Neoprene rostrum covers were used while handling the pristids, but were removed for transport. Chemical sedation was not applied.

The team at Cairns Marine successfully transported a 3.5 m TL, 150 kg BM, *P. microdon* in a commercial passenger aircraft from Australia to Singapore. The specimen was transported in a completely sealed container, without a supporting

compressed oxygen supply or external power supply. A rostral cover was used throughout the process and sedation was applied when the animal was placed into, and removed from, the transport tank.

EXHIBITS

Space

An exhibit for pristids must be adequately sized to accommodate the rapid growth of juveniles and the large size of adults. Table 1 summarizes exhibit dimensions for a variety of aquaria that currently display pristids. Exhibits should include ample areas of clear substrate for pristids to rest, as they spend a significant portion of their time on the bottom. In some institutions, pristids have been observed using the top of acrylic tunnels to rest. In addition, pristids require "runway" space for "take-off" and "landing", so open areas of sand are essential to accomodate their natural swimming behavior.

Although their body shape would suggest otherwise, pristids are capable of swimming backwards and they are very agile. Pristids can maneuver in tight spaces and seldom become entangled in theming or rockwork, unless disorientated during the period immediately post transport or from some other significant stressor. In the wild, pristids easily negotiate regions of intertwined mangrove roots and fallen trees, and are adept at avoiding entanglement. If a pristid becomes entangled in an aquarium it should not be attributed to the act of a "clumsy" animal, rather it should be addressed as a symptom of poor exhibit design.

Lighting

Aquarium lighting technology has advanced rapidly in recent years, especially with the advent of LED lighting. Facilities displaying pristids have reported using LED lighting, as well as metal halide lights with a color temperature range of 10,000 - 14,000°K. The photoperiod for pristid exhibits vary. Some aquaria adjust exhibit photoperiods to mimic natural seasonal changes. In other cases, exhibit lighting is dictated by hours of operation. Some aquaria use natural light to illuminate their exhibits, although depending on the latitude natural light may not adequately simulate natural conditions for pristids. Although there is little information about an appropriate lighting spectrum or photoperiod for pristids, mimicking natural conditions is recommended. It is not known if photoperiod plays a role in pristid reproduction.

Compatibility

In general, juvenile pristids are at risk from larger predators. Juvenile pristids should thereore be maintained in an appropriate smaller aquarium until they reach a size suitable for a larger, multi-taxa exhibit.

Adult and sub adult pristids have frequently been displayed in multi-taxa exhibits without problems. Exhibit cohabitants have included large grouper, *Epinephelus* spp., various shark species, such as the sand tiger shark, *Carcharias taurus* (Rafinesque, 1810), and the sandbar shark, *Carcharhinus plumbeus* (Nardo, 1827), as well as a wide variety of smaller teleost species.

Some aquarists have observed pristids preying upon smaller teleosts. Some "nuisance" teleost

Table 1. A summary of sawfishes (*Pristis* spp.) held at aquariums worldwide, showing dimensions of pristid exhibits and animal sex ratios. Number of animals is represented by x.x.x = male.female.unknown

Aquarium	Exhibit dimensions (m)	Volume (m^3)	Pristids displayed
Georgia Aquarium (Atlanta, GA USA)	79.5 x 30.5 x 7.5	24,224	3.1.0
John G. Shedd Aquarium (Chicago, IL USA)	10 x 27 x 5.5	1,514	0.1.0
L'Oceanogràfic (Valencia, Spain)	30 x 82 x 5.5	6,900	1.1.0
Sea World (Orlando, FL USA)	15 x 38 x 4.5	2,839	1.1.0
Aquarium Mare Nostrum (Montpellier, France)	18 x 18 x 9	1,800	2.0.0
Atlantis Paradise Island (New Providence, Bahamas)	45 x 27 x 4 / 30 x 15 x 1.5	5,206	3.5.0
Ripley's Aquarium (Myrtle Beach, SC USA)	32 x 25 x 4.5	2,082	2.1.0
Ripley's Aquarium of the Smokies (Gatlinburg, TN USA)	34 x 25 x 4.5	2,082	1.1.0
Ripley's Aquarium of Canada (Toronto, Canada)	37 x 25 x 4.5	2,801	1.1.0
The Deep (Hull East Yorkshire, UK)	17 x 28.2 x 10	2,400	1.1.0

species (e.g., *Balistes* spp. and *Heniochus* spp.) have been observed biting the upper lobe of the caudal fin of pristids, causing lesions and inflammation and risking secondary bacterial infection.

Water quality

A detailed description of life support systems and water quality requirements for elasmobranchs can be found in Mohan and Aiken (2004). In general, reported life support systems for pristids include: high-rate mechanical sand filtration, foam fractionation, ozone application (for micro-flocculation and oxidation), biological filtration and heat exchange. Water quality parameter ranges reported by aquaria maintaining pristids have been summarized in Table 2.

P. microdon have a preferred temperature range of 24 - 32°C. Juvenile *P. microdon* at Cairns Marine decreased their feeding rate by half at 23°C, then half again at 22°C. The *P. microdon* would only feed periodically at 21°C, and became moribund at 19°C (Squire, personal communication). Permanent long-term exposure to temperatures of 22 - 23°C may inhibit breeding, growth and overall health of *P. microdon*. *P. zijsron* are able to tolerate high temperatures, similar to *P. microdon*, but are also able to tolerate cooler temperatures; as would be expected from their natural range.

Most aquaria holding pristids maintained a constant water temperature throughout the year. For the few facilities that did adjust temperature the largest shift was 4°C, with an average annual temperature change of 2°C. It should be noted that, in general, juvenile pristids are less tolerant of variations to water temperature than adults.

Table 2. Pooled and summarized water quality parameter ranges reported by aquaria (n = 10) maintaining sawfishes (*Pristis* spp.) worldwide.

Parameter	S.I. unit	Range
Exhibit volume	m^3	794 - 24,224
Temperature	°C	20 - 26
Salinity	g/L	28 - 35
pH	pH	7.7 - 8.3
Dissolved Oxygen	% saturation	90 - 100
Ammonia	mg/L	0.0 - 0.09
Nitrite	mg/L	0.0 - 0.10
Nitrate	mg/L	0.0 - 400

Pristids typically breed in the summer, where tropical water temperatures reach ~30°C.

Open or flow-through seawater systems allow for natural water quality ranges and fluctuations. The pristid exhibit at Atlantis (Paradise Island, Bahamas) has an open system, drawing in natural seawater, and is open to the exterior, allowing in natural light. This system allows the animals to experience annual fluctuations in both water parameters (e.g., temperature and salinity) and photoperiod (Liu, personal communication). It may be no coincidence that the *P. pectinata* at Atlantis are the only pristids to have successfully reproduced in an aquarium to date. Those aquaria that maintain pristids in closed or semi-open systems are encouraged to simulate annual fluctuations to water parameters, consistent with changes observed in the natural range of the species in question. Water temperature and variations to water temperature may be crucial for pristid reproduction in aquaria.

FOOD and FEEDING

Diet

Pristids readily accept a wide variety of foods. Table 3 summarizes food types successfully fed to pristids in public aquaria. In general, diet for pristids in aquaria should be varied and should be prepared in a suitable manner to prevent nutritional or mineral deficiencies. A vitamin supplement such as Mazuri Shark/Ray II tablets (Mazuri®, Purina Mills LLC, Nutrition International LLC, Richmond, USA) should be provided at the prescribed dose. All food items should be sized appropriately for easy ingestion. Larger food fishes may need to have their head removed to prevent complications presented by bigger bones. Squid has been reported by many institutions to be a dietary staple for pristids. It should be noted, however, that the “quill” of the squid should be removed prior to feeding, as they can remain undigested in the gastrointestinal tract for extended periods, possibly causing complications.

Aquaria reported two different feeding regimes for pristids: feeding to satiation or feeding a set % BM/week in the range of 1 - 4%. The frequency at which food was offered to pristids varied between aquaria, from daily to twice per week. In the wild, *P. zijsron* and *P. microdon* can grow up to 30 cm/year TL for the first five years of their life (up to ~2.4 m TL). When fed to satiation in aquaria, these two species have been observed to grow

Table 3. A summary of food items fed to sawfishes (*Pristis* spp.) as reported by aquariums worldwide.

Scientific name	Common name
Caranx crysos	Blue runner
Clupea spp.	Herring
Doryteuthis opalescens	Opalescent squid
Mallotus villosus	Capelin
Melanogrammus aeglefinus	Haddock
Merlangius merlangus	Whiting
Merluccius spp.	Hake
Family: Mugilidae	Mullet
Oncorhynchus spp.	Salmon
Suborder: Pleocyemata	Shrimp
Pseudocaranx dentex	White trevally
Salmo spp.	Salmon
Sarda spp.	Bonito
Family: Scombridae	Mackerel
Urophycis spp.	Hake

up to 60 cm/year TL during the first year (Squire, personal communication).

When formulating a feeding regime, the age of the pristid should be taken into consideration. Neonate or juvenile pristids grow rapidly and require a higher % BM of food than adults. Pregnant pristids may be fed to satiation, to ensure that mother and pups receive the necessary nutriment. Increases to food ration should be made carefully, however, as it has been observed that some species (e.g., *P. zijsron*) are susceptible to obesity. To date, this propensity has not been observed in *P. microdon* or *P. pectinata*.

Feeding

Pristids are easily conditioned to different feeding methods and locations. Wild pristids can take prey from the water column, using their saw to slash at their quarry, or the benthos, where they swim over and consume food items (Wueringer et al., 2012).

Some aquaria reported feeding pristids at the water surface, by carefully throwing (or releasing with tongs) food items near the rostrum of the fish. Swimming pristids would swing their saw toward the "prey" item, catch it, then push the food to the bottom of the exhibit where it could be restrained and consumed. This feeding technique required a large open area in the exhibit, allowing space for the pristid to swing its rostrum and maneuver to the bottom.

As an alternative, many institutions have reported introducing food to pristids near the bottom of an exhibit using a feeding pole. Pole feeding allowed the aquarist at the surface (or SCUBA diver) to "guide" a pristid to a designated feeding location—e.g., an open area of substrate or the top of an acrylic tunnel. Some aquaria have used SCUBA divers to pole feed swimming pristids from underneath.

Another technique to feed pristids is to simply broadcast food and allow it to settle to the floor of the aquarium. A variation on this technique is to introduce food by allowing it to fall down an open PVC pipe directed toward the bottom of the aquarium, a technique employed at L'Oceanogràfic (Valencia, Spain) (Taura, personal communication).

At Ripley's Aquarium, Myrtle Beach (South Carolina, USA), *P. microdon* preferred to feed from the surface, *P. pectinata* remained on the bottom and fed off a pole, and *P. zijsron* ate regularly and equally from both the surface and the bottom.

Pristids are readily trained and are good candidates for operant conditioning. Regardless of how accustomed a pristid is to a particular foraging technique, they can easily adapt to feeding in a different manner. This plasticity is useful should an animal become too large to feed in a specific location (e.g., a feeding platform or narrow stretch of sand). In addition, conditioning individual pristids to a dedicated feeding station is a useful technique to minimize intra- and interspecific competition for food, and allows for a more accurate record of food intake. The husbandry team at Sea World Orlando (Florida, USA) trained *P. pectinata* to swim from the exhibit into an adjacent holding pool where they were then fed from the surface, or where feed was broadcast into the pool (Violetta, personal communication).

Feeding records should always be maintained for pristids. Unusual patterns in feeding behavior or food intake, or a decrease in appetite, may be an early indication of a health challenge.

CLINICAL CONSIDERATIONS

Pristids are hardy and can be maintained long-term in aquaria. An individual *P. pectinata* caught in 1968 lived for 44 years in an aquarium (White, 2014). At the time of writing, the oldest living pristid in an EAZA aquarium is 21 years old (Duke, 2013).

Most aquaria reported a "minimum intervention" approach to pristid husbandry, only examining animals when a problem was detected. Annual physical examinations were not routinely carried out on pristids, as capture and restraint was considered stressful and risky for the animals and aquarists. However, some aquaria have trained pristids to enter holding pools or designated areas, facilitating restraint with a stretcher for physical examinations. If routine physicals are possible, husbandry teams are encouraged to measure and weigh pristids, as well as take blood samples for analysis. Table 4 summarizes pooled data from blood taken during routine physical examinations of 12 healthy *P. pectinata* in U.S. aquaria.

The most common, although infrequent, medical challenge reported for pristids in aquaria was a bite wound inflicted by other species. On one occasion a pristid had its rostrum fractured in two places by a *C. taurus*. The rostrum of the pristid was "splinted" and after five months of recovery had completely healed (Blair, personal communication).

Table 4. Pooled blood data (mean and standard deviation) collected from twelve healthy *P. pectinata* in U.S. aquaria (n = 22 samples extracted from 12 animals)

Parameter	S.I. unit	Mean ± SD
Hemoglobin	g/dL	6.64 ± 1.78
PCV		23.44 ± 4.57
TP		6.27 ± 1.92
WBC	mm^3	11,645.00 ± 8,391.12
Bands	%	0 ± 0
Neutrophils	%	53.80 ± 28.22
Lymphocytes	%	42.06 ± 27.37
Monocytes	%	1.11 ± 1.37
Eosinophils	%	3.22 ± 11.24
Basophils	%	0.12 ± 0.49
Platelets H-N-L		N
Glucose	mg/dL	30.40 ± 9.51
BUN	mg/dL	913.71 ± 417.11
Uric Acid	mg/dL	0.86 ± 0.37
Cholesterol	mg/dL	98.39 ± 48.35
Triglycerides	mg/dL	165.68 ± 72.08
T. protein	g/dL	3.40 ± 0.84
Albumin/Globulin	g/dL	0.43 ± 0.12
Globulin	g/dL	3.69 ± 2.74
AST	U/l	20.44 ± 16.15
LD	U/l	148.69 ± 176.34
Calcium	mg/dL	9.77 ± 4.89
Phosphorus	mg/dL	86.83 ± 119.12
Sodium	mEq/L	146.99 ± 121.68
Potassium	mEq/L	76.00 ± 113.21
Chloride	mEq/L	157.72 ± 106.05
CO_2	mEq/L	7.61 ± 5.65

CONCLUSIONS

P. microdon, P. pectinata and P. zijsron have been successfully maintained in aquaria for several decades, with few challenges. However, reproduction has only occurred at one aquarium (Atlantis, Paradise Island, Bahamas), for a single species, *P. pectinata*, resulting in two male and two female pups. The underlying minimum requirements for pristid reproduction are unknown, although natural fluctuations in water temperature, salinity, and photoperiod are likely to be important. Aquaria are encouraged to replicate natural environmental conditions in their pristid exhibits wherever possible. Large expanses of open sand on the bottom of exhibits are likely to further facilitate reproduction.

The meta-population of pristids in aquaria needs to be carefully managed. Coordination between regional studbook keepers and aquaria maintaining pristids is paramount to ensure optimal numbers and sex ratios are maintained. The option for national and international transport of pristids provides a valuable population and studbook management tool for this highly threatened and charismatic taxon. By carefully recording successful husbandry practices, opening lines of communication between facilities that maintain pristids, and working together, we can improve all aspects of pristid care and increase the chances of further reproduction in aquaria.

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Chapter 10

Husbandry of Whale Sharks

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Abstract: Whale sharks, *Rhincodon typus* (Smith, 1828), have been on continuous public display at the Georgia Aquarium (Atlanta, USA) since 2005. The Aquarium successfully transported six *R. typus* from Taiwan over a three-year period from 2005 to 2007. A behavioral conditioning plan was developed to manage the sharks both in the field and in their exhibit at the Aquarium. Feeding techniques were modified over time to meet the changing requirements of the species. Dietary ration for *R. typus* was in the range of 3 - 5% body mass (BM)/week. *R. typus* presented the following challenges: habitat use, extended periods of inappetence, gastric stenosis, contact lesions, anemia and behavioral stereotypy, requiring the development of some novel treatment strategies.

INTRODUCTION

Whale sharks, *Rhincodon typus* (Smith, 1828), are large epipelagic fish with a tropical and semi-tropical circumglobal distribution, including coastal and offshore waters (Compagno, 2001; Stevens, 2007). Although relatively common in shallow waters associated with the Seychelles (Rowat and Gore, 2007), the Sea of Cortez (Nelson, 2004) and Western Australia (Taylor 1996), *R. typus* are capable of migrating long distances and may be found in other locations. Large seasonal aggregations of *R. typus* have been observed, the most well-known occurring in the coastal waters off the Yucatan Peninsula, Mexico (de la Parra-Venegas et al., 2011).

R. typus are filter feeders, eating a wide array of plankton at different locations throughout the year. Many, but not all, *R. typus* aggregations appear associated with an abundance of food, be it newly spawned fish eggs, fish fry, copepod blooms, krill blooms or other concentrations of plankton. Near-shore aggregations of *R. typus* have not only drawn the attention of field biologists, but also of eco-tourism operators, in particular along the Yucatan Peninsula (Dove, personal communication). With a declining global population, *R. typus* are classified as “endangered” by the International Union for the Conservation of Nature (IUCN) (Pierce and Norman, 2016) and are listed in Appendix II of CITES. Recent examples of commercial harvest in China (www1) and the Philippines (www2) underscore the important role of public education in the conservation of this impressive and benign species.

While field research continues to add to our knowledge of *R. typus*, the public aquarium community has made great strides in its understanding of the

species. Advances in basic husbandry, diet formulation and the development of behavioral management techniques has enabled institutions with the appropriate resources to present *R. typus* to the public in the form of permanent educational displays. Led by the pioneering efforts of aquarium professionals in Japan, especially at the Okinawa Expo Churaumi Aquarium and Osaka Kaiyukan Aquarium, *R. typus* has been on display at seven institutions worldwide since 2015 (www3; Kawahara, personal communication).

ACQUISITION AND TRANSPORTATION

Due to their large size and, typically, their availability in remote locations, rigorous planning must be employed when developing logistics for the acquisition, staging and transportation of *R. typus*. Strong partnerships must be established with various stakeholders (e.g., local fishers, foreign and domestic regulatory agencies and commercial transport companies) to ensure the success of any *R. typus* acquisition effort.

Acquisition

Georgia Aquarium acquired six *R. typus* off the East Coast of Taiwan using an existing shore-based trap-net fishery. The fishery harvested *R. typus* to supply a quota-based seafood market up until 2010 (Hsu, 2012). Two *R. typus* were collected on behalf of the Georgia Aquarium each year, for three years, from 2005 to 2007.

Specimens for acquisition were selected based on specific criteria: (1) excellent physical condition, with no observable injuries or net damage; (2) no evidence of an historical injury resulting in permanent maiming or other significant body form alteration; and (3) a juvenile specimen <4.5 m total length (TL), a response to the physical constraints of available transport equipment. Both genders were considered candidates for acquisition. If a specimen met each of these criteria, and sea conditions permitted, contracted trap-net fishermen were directed to move the specimen into a dedicated hexagonal staging sea pen (15 m per side x 15 m deep), located 150 m offshore. Selected specimens were moved to the fixed sea pen using a small hexagonal towed sea pen (5 m per side x 3 m deep).

Transport

Large-animal transports require a sizeable team and a great amount of planning and coordination. The team at the Georgia Aquarium used a large group of its own staff and partnered with dozens of other individuals and entities for each of the *R. typus* transports. Two sharks were transported each year from 2005 to 2007.

Extraction of *R. typus* from the sea pen was one of the most delicate and risky phases of the animal transport. Personnel safety was paramount throughout each phase of the operation. Sea conditions influenced the ability to move heavy gear, positioning and stability of the support vessel, and

Figure 1. A whale shark, *Rhincodon typus* (Smith, 1828), restrained in a stretcher in preparation to be lifted out of a sea pen and onto a boat for transport to shore.

the operation of the lifting crane, all of which determined safety throughout the operation. If sea conditions permitted, the operation would begin. A large, multilingual team would surround the perimeter of the sea pen and use a net to direct one of the *R. typus* toward a large stretcher. The stretcher was designed to provide as much support as possible for the shark, yet drain rapidly as it was lifted clear of the water (Figure 1). The *R. typus* was lifted into a 6.1 m x 2.3 m x 2.1 m deep fiberglass transport box strapped to a fishing vessel, known locally as a '*twan'*. This "dry" lift, a constraint imposed by the limited capacity of the crane on the '*twan*', represents the only time throughout the transport that a shark was moved without the support of surrounding seawater. The transport box aboard the '*twan'* was supplied with oxygen-enriched seawater at a flow rate of ~4.0 L/min. Once the first *R. typus* was loaded, the process was repeated for the second shark, which was loaded into a transport box on a second '*twan*'.

Once secured, the two sharks were transported to a nearby harbor. The *R. typus* were then transferred to custom-built transport containers on a freight vehicle. Paired cranes lifted each shark, surrounded by seawater, in a heavy-duty bladder stretcher (Ortega's Canvas & Sail Repair, California, USA) (Figure 2). The transport containers (Johnson's Custom Fiberglass, Florida, USA) measured 6.1 m x 2.3 m x 2.1 m deep, had windows to allow for visual monitoring and permitted access to the shark in transit. On arrival at the airport, the transport containers were loaded onto a cargo plane for a short domestic flight and then transferred to another cargo plane for the trans-Pacific flight to the United States. The transport containers were positioned so the *R. typus* were pointing toward the rear of the plane, ensuring that their heads remained underwater during take-off and landing. Once the cargo plane landed in the USA, the transport containers were off-loaded in Atlanta and driven to the Georgia Aquarium. Transport time from the sea pen in Taiwan to the Georgia Aquarium was 36, 32 and 28 hours in 2005, 2006 and 2007, respectively.

Throughout each transport, mechanical filtration was achieved using cartridge filters. A battery-powered pump and spray bars provided gas exchange. Dissolved oxygen and pH were monitored continuously using a Hach HQ40D Portable Meter (Hach Company, Colorado, USA). The target ranges for oxygen and pH were 125 - 150% saturation and 8.1 - 8.3, respectively. Ammonia was tested hourly using a Hach DR/850 Portable Colorimeter (Hach Company, Colorado, USA). Adjustments to system water were made throughout the transport using AmQuel® Plus™ (Kordon LLC, California, USA), sodium bicarbonate and sodium carbonate, if water quality fell outside target parameter ranges.

Once the sharks arrived at the Georgia Aquarium, they were acclimatized to water conditions within the Ocean Voyager exhibit and then lifted into the habitat using the bladder stretcher and a 20-ton

Figure 2. A whale shark, *Rhincodon typus* (Smith, 1828), in a water-filled bladder stretcher, is lowered into a transport box in preparation for shipping by air.

hoist (Gajjar Engineering Systems Inc., Georgia, USA). As a precaution, colored ropes were suspended along the walls and acrylic windows to visually alert the sharks to the configuration of the habitat and perimeter boundaries. SCUBA divers monitored and, when required, "guided" the sharks until they consistently navigated the exhibit without difficulty, a process that typically took 1 - 4 hours.

EXHIBIT DESIGN

R. typus at the Georgia Aquarium were maintained in the Ocean Voyager exhibit habitat (volume = 15,300 m^3), one of the largest indoor artificial habitats wholly dedicated to the display of fishes. An additional 8,700 m^3 of water was associated with the life support system of the exhibit, resulting in a total volume of 24,000 m^3. The exhibit was designed to provide appropriate swimming space for numerous large pelagic elasmobranchs, as well as ample habitat for a wide variety of other species. The exhibit was 78 m long and ranged from 24.0 - 39.6 m wide. Water depth in the exhibit ranged from 6.1 - 9.1m.

The exhibit was filled with synthetic seawater (Instant Ocean/Spectrum Brands, Blacksburg, Virginia, USA) and treated using a closed recirculating life support system, which included foam fractionation followed by high-rate sand filtration. Ozone disinfection and denitrification were applied on a side stream of 25% and 1% of system flow, respectively. All water then passed through a degas tower filled with a 2.5 m deep layer of AccuPac® plastic media (Brentwood Industries, Reading, Pennsylvania), resulting in a total surface area of 107,000 m^2 (Dove, 2011). The degassing tower also served as the site for biological filtration (i.e., nitrification). Total water flow through the life support system was ~29,400 m^3/h directed through two parallel process loops, each treating ~14,700 m^3/h (Hall, personal communication). Filter backwash water was recovered, processed and re-used to preserve both water and sea salt resources.

Theming within the Ocean Voyager exhibit included concrete décor depicting rocky outcroppings, which provided overhangs and a variety of refugia for smaller species. Substrate was comprised of crushed coral/shell and silica sand in the 6 m and 9 m deep sections of the exhibit, respectively. Substrates were ~25 cm thick in both cases, but varied slightly due to water movement or animal activity (e.g., benthic rays disrupting the substrate). The most important portion of the habitat was the expansive water column, where *R. typus* and other large fishes (e.g., mobulids) could glide for long distances and had ample space to change direction.

Water quality requirements for *R. typus* are consistent with other tropical, subtropical and temperate elasmobranchs. Water quality parameters measured in the natural range of the *R. typus* should be used as the basis for establishing aquarium conditions. Water quality parameters maintained in the Ocean Voyager exhibit at the Georgia Aquarium are summarized in Table 1.

A large skylight provided exhibit lighting over the 9 m deep section of the exhibit. This illumination was augmented by an array of several dozen paired 1,000 W metal halide lights (Lithonia Lighting, Conyers, Georgia, USA). These lighting fixtures provided illumination over the entire surface area of the exhibit. Lighting photoperiods were computer-controlled and could be adjusted, as determined by animal needs and other operational demands. A mechanized gantry, spanning the exhibit, facilitated access to the entire exhibit surface. This structure was an invaluable tool for maintaining overhead infrastructure (e.g., lighting fixtures) and for animal management, through the deployment of nets or other tools.

A dedicated 22 m x 9 m x 1.5 m deep auxiliary pool, of ~300 m^3 volume, was connected to the main exhibit through a pre-cast opening in the Ocean Voyager tank perimeter. The auxiliary pool was absent of any décor and was separated from the main exhibit by a removable polypropylene mesh barrier. The auxiliary pool was used to acclimatize new animals, to initiate operant condi-

Table 1. Water quality parameters in the Ocean Voyager exhibit at the Georgia Aquarium, Atlanta, USA.

Parameter	S.I. unit	Range
Temperature	°C	21 - 25
Salinity	g/L	30 - 36
pH		7.8 - 8.3
Alkalinity	mg/L	200 - 250
Ammonia	mg/L	<0.01
Nitrite	mg/L	<0.01
Nitrate	mg/L	50 - 100

tioning, to provide a controlled and protected area for animals to be rotated off display, and to carry out medical procedures.

Aquarium guests could view exhibition animals via nine acrylic windows of various shapes and sizes, including a tunnel that bisected the exhibit. The final view into the Ocean Voyager exhibit was through a 23 m wide x 9 m tall acrylic window. Guests could also view the exhibit from above, when participating in behind-the-scenes tours, and from within the exhibit, as part of a fee-based guest dive program.

FOOD AND FEEDING

R. typus under managed care should be offered food items consistent with the anatomical features of the shark and their associated filter feeding behavior (described by Motta et al., 2010). Feeding should be initiated as soon as practicable after an animal has been acquired and food should be offered on multiple occasions throughout the day, with a short overnight fasting period only, to meet the high energetic demands of the species. Food should be offered at a consistent time each day, in a consistent manner, and feeding sessions should be initiated with an unambiguous audio or visual cue. The filter feeding behavior of *R. typus* means that food can be introduced by being poured directly into the mouth of the shark, while it is oriented vertically in the water column, or by presenting the food in a line in front of the open mouth of the shark, as it swims just below, and skims, the water surface.

Feeding initiation

Once *R. typus*, acquired on behalf of the Georgia Aquarium, had been introduced into the sea pen in Taiwan, efforts were directed toward initiating feeding and desensitizing the shark to humans. At the outset, a small raft was tied in the middle of the sea pen with a mesh bag suspended underneath. Frozen blocks of Atlantic krill, *Euphausia superba* (Dana, 1850), were placed into the mesh bag to defrost, creating a concentrated cloud of food beneath the raft. This technique worked most effectively when the floor of the sea pen was raised to a depth of 5 m, bringing the shark into closer proximity to the food cloud.

Behavioral conditioning was considered an invaluable tool for both feeding and general husbandry of *R. typus* and was therefore initiated shortly after the sharks were acquired. Once a shark was eating consistently, food was offered by hand, from the raft, to desensitize the animal to the presence of humans (Figure 3). The sharks rapidly acclimatized to feeding while hanging vertical in the water column beside the raft. This process was occasionally compromised when the shark physically contacted the raft, so feeding was modified so that food could be offered a short distance away using a plastic ladle attached to a bamboo pole. As the husbandry team optimized food presentation, the time for new specimens to start feeding became shorter. The first shark collected

Figure 3. A whale shark, *Rhincodon typus* (Smith, 1828), hangs vertically beside a raft, as it is hand fed from a ladle.

Figure 4. Two whale sharks, *Rhincodon typus* (Smith, 1828), being fed simultaneously from a raft using ladles attached to bamboo poles. This technique allowed for both sharks to be guided under stimulus control.

did not eat until 30 days had elapsed, while the other five specimens ate within two weeks of being acquired.

Once a shark was feeding regularly from the ladle, it was incrementally conditioned to follow the 'target' (i.e., the ladle) around the raft and feed while swimming. By target feeding the shark it was possible to lead the animal and vary its swimming pattern (speed, direction, etc.) as desired.

The process of conditioning *R. typus* to feed while swimming became more difficult once a second shark was introduced into the sea pen. Feeding two *R. typus* required two people in the raft to feed both animals simultaneously, each shark being fed by a dedicated person. The more experienced shark was fed as it was led around the raft, while the second animal was fed alongside the raft, remaining in a vertical position with its mouth pointed toward the surface. Once the second shark was conditioned to follow the food ladle, both sharks could be fed while circling the raft (Figure 4). Communication was paramount during feeding sessions to ensure the two sharks did not collide or interfere with each other. In each of the three years *R. typus* were managed in the sea pen, it was observed that the second specimen acquired began feeding in a shorter period of time than the first specimen acquired.

Feeding on exhibit

Once *R. typus* were on exhibit at the Georgia Aquarium, feeding was quickly initiated using the same methods employed in Taiwan. Sharks were fed from a raft and targeted with a food ladle on a pole. Food was offered to the sharks the day following arrival. The sharks resumed feeding in seven, five and one day(s) in 2005, 2006 and 2007, respectively.

Over time the sharks were conditioned to feed from two dedicated platforms located on a movable gantry, positioned above the exhibit (Figure 5). As additional sharks were introduced into the exhibit, two additional feeding stations were

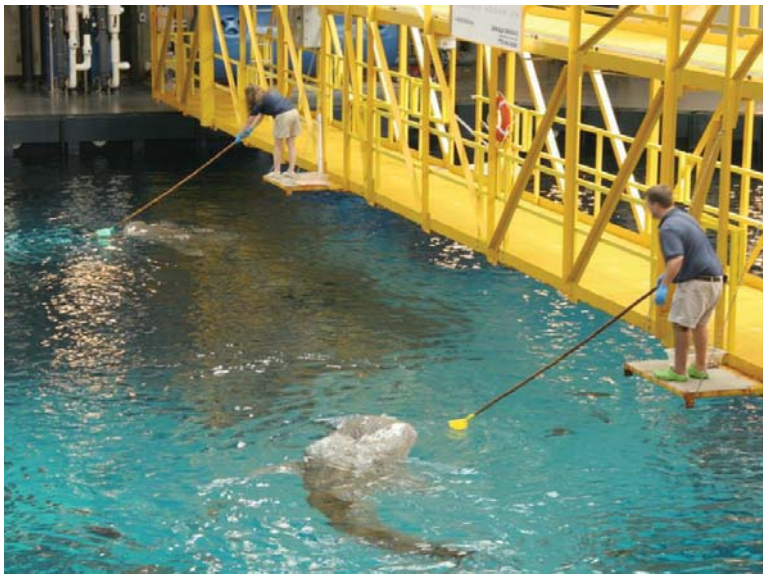

Figure 5. Two whale sharks, *Rhincodon typus* (Smith, 1828), being fed simultaneously from platforms located on a gantry above the Ocean Voyager exhibit, Georgia Aquarium, Atlanta, USA.

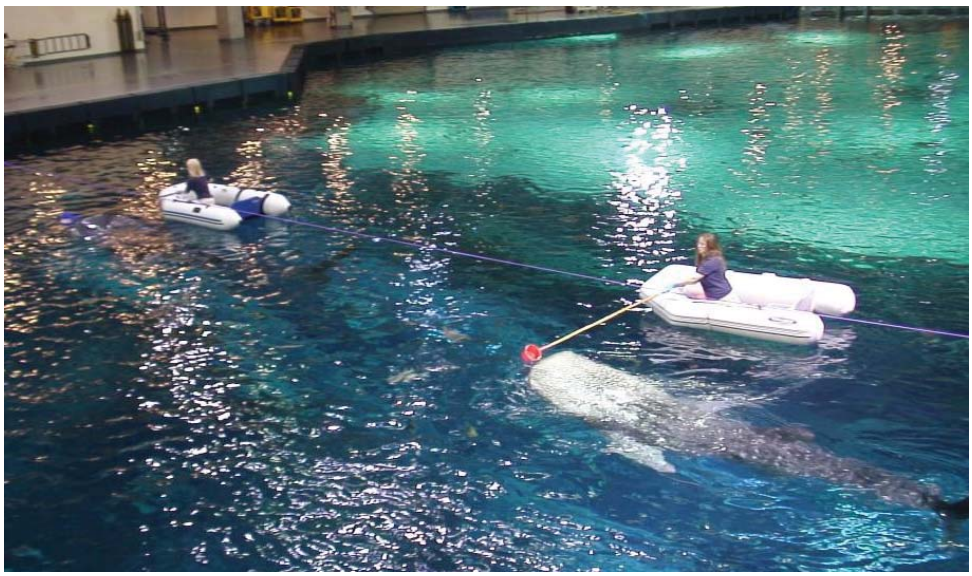

Figure 6. Two whale sharks, *Rhincodon typus* (Smith, 1828), being fed simultaneously from rafts floating above the Ocean Voyager exhibit at the Georgia Aquarium, Atlanta, USA.

added in the form of two rafts (Figure 6), enabling four animals to be fed simultaneously. *R. typus* were only reinforced with food when they approached their designated feeding station. A pictorial overview of feeding techniques used for *R. typus* at the Georgia Aquarium can be downloaded from the online resources for this manual (www4).

Over time the sharks grew too large to be fed from the overhead platforms and it became necessary to feed all the sharks from dedicated rafts, which could slide back and forth along two ropes running the length of the exhibit. This approach allowed for longer, straighter feeding runs. Having dedicated feeding stations for each shark allowed for better control of the animal collection and a clear mechanism for tracking the amount of food consumed by each shark. However, this process was labor intensive, as it required a dedicated staff member for each shark at every feeding session.

As feeding times approached, *R. typus* typically started to swim more quickly, and as rafts were launched and attached to their guide ropes, the sharks would consistently approach their dedicated feeding station. Once feeding sessions concluded, the sharks would return to their usual, more sedate, swimming behavior.

Diet and ration

R. typus at the Georgia Aquarium were offered a diet of commercially available foods, including, but not limited to: *E. superba*, North Pacific krill, *Euphausia pacifica* (Hansen, 1911), Atlantic silverside, *Menidia menidia* (Linnaeus, 1766), squid (*Loligo* sp.), gel food (Mazuri® Omnivore, Land

O'Lakes, Inc., Minnesota, USA) and vitamin supplements (Mazuri® Vita-Zu® Shark/Ray Tabs II, Land O'Lakes, Inc., Minnesota, USA). *R. typus* were fed four times per day, twice in the morning and twice in the afternoon. Dietary rations varied between individuals, but were in the range of 3 - 5% body mass (BM)/week. Composition of the daily diet was regularly adjusted to ensure nutritional balance, accommodate specimen food preferences and to meet behavioral conditioning objectives. An example of the daily food ration for a female *R. typus*, collected during 2015, is provided in Figure 7. The same individual was also administered 22 multivitamins with its food ration every day, except during 2009 when it was provided 11.5 multivitamins. The shark was also provided 12 and 8 vitamin C tablets daily in 2006 and 2007, respectively.

Food preferences

R. typus were observed actively rejecting some food types. For example, when gel food was first introduced to the sharks, it was ingested and then expelled during some of the earlier feeding sessions. A process of desensitization was therefore used to incorporate gel food into the diet. Gel food was introduced in very small amounts with other foods and the proportion slowly increased over several days, using the feeding response of the sharks as a guide for the rate of increase. Through this desensitization process, it was possible to raise the proportion of gel food in the diet up to a maximum of 18%. Vitamin supplements were also observed to be distasteful to *R. typus*, so they also were added to the diet through a process of slow desensitization.

Gut transit time

Gut transit time is an indicator of digestive function. It is therefore useful for husbandry personnel to quantify gut transit time as it can inform animal health management. The husbandry team at Georgia Aquarium added 50 inert colored plastic beads (Airstrike, Inc., Arkansas, USA), of 2 - 4 mm diameter, to the diet of each *R. typus*. Each shark was fed different colored beads. Following each feeding session, SCUBA divers verified that the beads had been ingested and were not re-

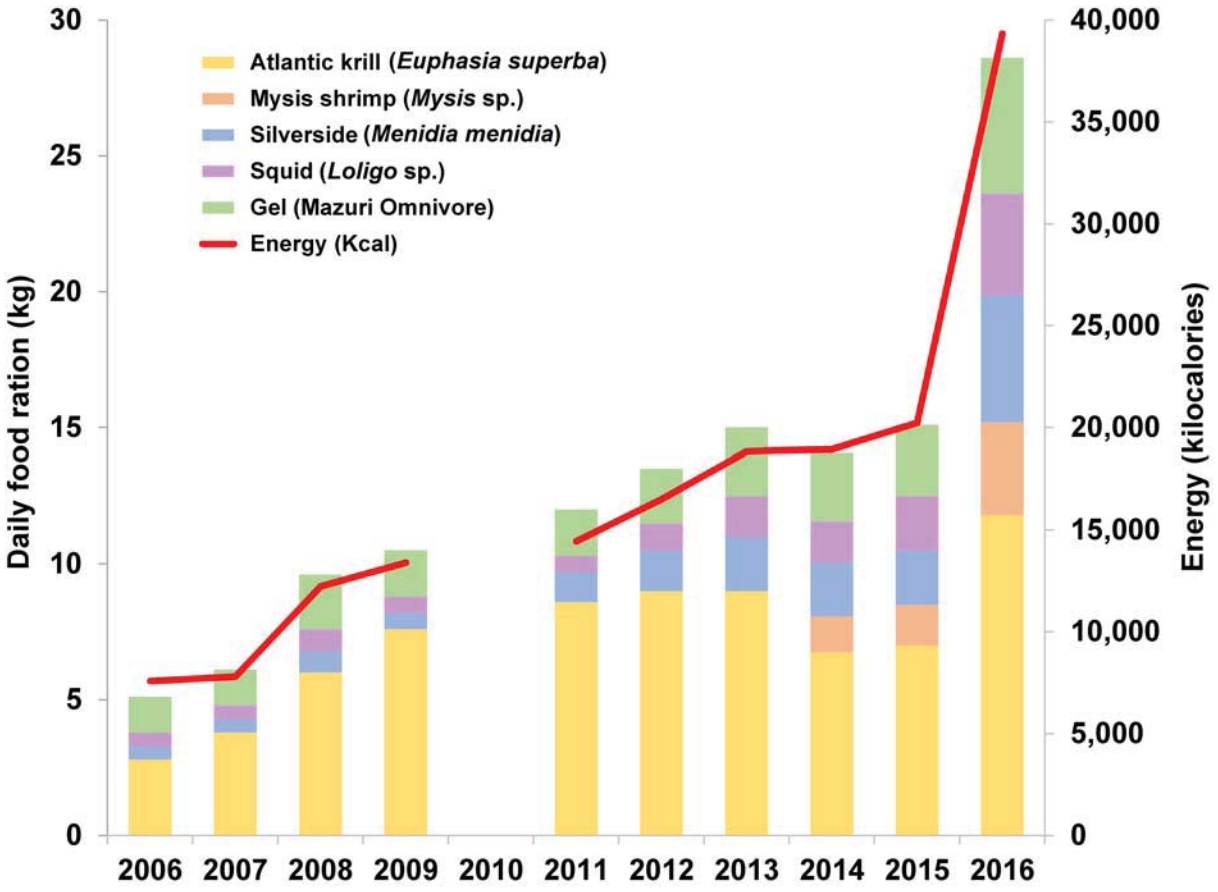

Figure 7. Daily food ration for a female whale shark, *Rhincodon typus* (Smith, 1828), collected in 2015. Energy (kilocalories) consumption was extrapolated from a nutritional analysis conducted on a sample of food in 2016. Data for 2010 was not available.

jected by the shark and left on the bottom of the exhibit. Any beads not ingested were removed. The exhibit was then surveyed each day to see if any of the ingested beads reappeared. Based on the recovery of ingested beads, the husbandry team was able to postulate a 3 - 4 day gut transit time for healthy *R. typus*. If *R. typus* digestive dysfunction was suspected—as indicated by body appearance, feeding response or swimming behavior—gut transit time was verified by feeding beads to the shark and waiting for their reappearance. If the beads appeared prematurely, or only after a protracted time, further investigation or therapeutic measures were undertaken.

ANIMAL MANAGEMENT

Animal assessment

R. typus should be examined from every angle, on a daily basis, to carefully evaluate and track their overall condition. Observations should be recorded using a standardized reporting mechanism. Any changes to feeding behavior, swimming behavior (e.g., swimming speed or position), anatomy (e.g., fin attitude), or body coloration should be recorded and closely tracked. It was observed at the Georgia Aquarium that water temperature changes could lead to behavioral changes and even a cessation of feeding activity in *R. typus*. All possible variables should be carefully evaluated when changes to *R. typus* behavior or appearance are observed.

It was not uncommon for *R. typus* at the Georgia Aquarium to contact the perimeter walls or décor, which occasionally resulted in minor abrasions. These abrasions were typically superficial, but were monitored carefully to ensure they healed quickly. A variety of mechanisms were used to minimize the frequency of *R. typus* contacting the exhibit perimeter, including PVC pipes hung vertically along walls, vinyl curtains, floating plastic barrels and/or buoys, and bubble 'curtains' to interrupt repetitive swimming patterns. When working with *R. typus* it is important to consider the sheer size and weight of the animal. A brief contact with the surrounding infrastructure can cause damage to both the animal and the exhibit.

Other challenges that may be presented by *R. typus* include stereotypical behavior (e.g., repeated turns in one direction), failure to use the

Figure 8. A whale shark, *Rhincodon typus* (Smith, 1828), being fed underwater by a diver on SCUBA with a food-filled squeeze bottle. Georgia Aquarium, Atlanta, USA.

entire exhibit, negative interactions with tank mates and prolapses (intestine or stomach) into buccal cavity. *R. typus* at the Georgia Aquarium occasionally trapped large bony fishes in their mouth. Although the sharks typically expelled the intruder voluntarily, bony fish could remain trapped for several days and prevent the shark from feeding normally. In these cases, direct intervention was employed whereby a SCUBA diver reached inside the mouth of the shark to extract the bony fish. Thereafter, the *R. typus* was able to resume feeding normally.

Behavioral modification

In addition to feeding management, conditioning strategies were frequently employed at the Georgia Aquarium to manage other *R. typus* behaviors and medical procedures. For example, one specimen exhibited a stereotypical swimming pattern whereby the shark did not use the entire exhibit and repeatedly swam in tight clockwise circles. To interrupt this stereotypical behavior, divers on SCUBA fed the shark underwater. Feeding sessions for the shark were initiated using a unique audio cue (a shaker) wielded by a single diver. The diver offered food to the shark from a squeeze bottle (Figure 8). The shark soon began to track the diver, who incrementally conditioned it, over several months, to swim in increasingly larger circles. Thereafter, a second diver was introduced, who also cued the shark and fed it with a squeeze bottle. By increasing the distance between the divers, it was possible to induce the shark to swim in a straight line, 'passing' the shark back and forth between each of the divers. Over time this strategy extinguished the stereotypical swimming behavior.

Medical examinations

Should restraint of *R. typus* be necessary for medical examinations, the use of stretcher desensitization is recommended to minimize stress on the shark during handling (refer to Coco and Schreiber, this volume). It is also important that the stretcher be large enough to accommodate the shark and readily allow for the necessary clinical work to be conducted. After restraint for a procedure, divers must help the shark exit the stretcher and closely monitor the animal to ensure its behavior returns to normal.

At the Georgia Aquarium, *R. typus* were occasionally restrained for medical procedures, including blood draws, endoscopy, etc. A team of SCUBA divers would use large nets to guide the shark into a suspended vinyl stretcher (Figure 9). Divers had to exercise great care when

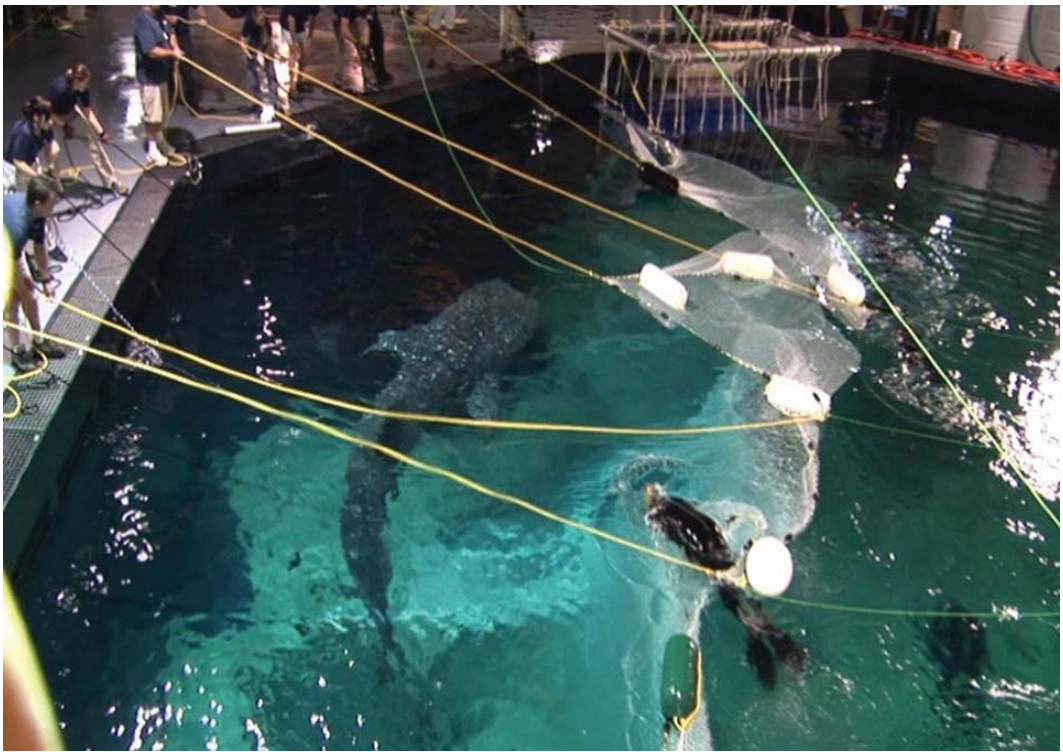

Figure 9. A whale shark, *Rhincodon typus* (Smith, 1828), being directed into a restraint stretcher using nets wielded by divers on SCUBA and other husbandry staff. Georgia Aquarium, Atlanta, USA.

Figure 10. A whale shark, *Rhincodon typus* (Smith, 1828), restrained in vinyl stretcher, prepared for a medical procedure. Note the use of hoses directing oxygen-enriched seawater into the mouth of the shark to aid ventilation. Georgia Aquarium, Atlanta, USA.

operating in close proximity to the shark, exhibit perimeter walls, nets and ropes. Once in the stretcher, oxygenated seawater was directed into the mouth of the shark, towards the outside of the buccal cavity, to maximize flow over the gills (Figure 10). Restrained in this manner, the sharks quickly settled and morphometrics could be obtained, blood draws performed and other clinical procedures conducted.

Underwater feeding of *R. typus* by a diver with a squeeze bottle provided an opportunity to use less invasive techniques for simple medical procedures, administering medications and obtaining morphometrics. For example, it was possible for a diver to draw blood from the pectoral fin of an *R. typus*, using a technique recommended by Yanagisawa (personal communication), while the unrestrained shark was being fed and 'stationed' by another diver (Figure 11).

Figure 11. A whale shark, *Rhincodon typus* (Smith, 1828), under stimulus control by a diver with a food-filled squeeze bottle, allowing a second diver to draw blood from its pectoral fin. Georgia Aquarium, Atlanta, USA.

THE FUTURE

Further work is required to better understand the specific husbandry requirements of *R. typus*. Areas of focus should include habitat design, nutrition, blood chemistry and parasite management, among others. A table detailing medications administered to *R. typus* at the Georgia Aquarium can be downloaded from the online resources for this manual (www4).

Caretakers of *R. typus*, throughout the public aquarium community, should develop an efficient information sharing mechanism to optimize care for this endangered species. Every effort should be expended to ensure that *R. typus* in public displays are used to promote the conservation of wild conspecifics.

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www2 http://www.flmnh.ufl.edu/fish/discover/species-profiles/rhincodon-typus

www3 http://elasmollet.org/Rt/Rt_captive.html

www4 http://www.elasmobranchhusbandry.org

The Elasmobranch Husbandry Manual II: Recent Advances in the Care of Sharks, Rays and their Relatives, pages 99-112.

Chapter 11

Husbandry of freshwater stingrays

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Abstract: The Potamotrygonidae (Garman, 1877), a family of freshwater stingrays naturally occurring in South America, is an increasingly popular taxon for display in public aquaria. Freshwater stingrays have species-specific habitat preferences and require innovative rearing techniques. Potamotrygonids in aquaria require a diverse diet and vitamin supplementation is recommended. Potamotrygonids may be conditioned, or trained, to assist with health management and for enrichment purposes. Freshwater stingray handlers should follow strict protocols to reduce the risk of envenomation. To assist husbandry protocols, individual potamotrygonids should be tagged with transponders to ensure correct identification. Reproduction of potamotrygonids in aquaria is reported in only some species and little is known about the natural history of others. Hybridization of different species of potamotrygonid should be avoided.

INTRODUCTION

Many public aquaria and zoos display the colorful and active freshwater stingrays of the family Potamotrygonidae (Garman, 1877). *Potamotrygon motoro* (Müller and Henle, 1841) is the sixth most common freshwater fish held in institutions accredited by the Association of Zoos and Aquariums (AZA) (www1). Although generally robust, potamotrygonids do require specialized care to thrive. Herein, we discuss the taxonomy, governing regulations, transportation, safe handling, veterinary care, identification marking, nutrition, habitat requirements, and reproduction of this popular and interesting group of elasmobranchs.

TAXONOMY

Potamotrygonids are found only in South America and are the only living family of elasmobranchs completely restricted to fresh water (Carvalho et al., 2003). The family currently contains four genera: *Potamotrygon* (Garman, 1877), which is the most species-rich genus with 26 currently-valid species; *Paratrygon* (Duméril, 1865), a monotypic genus that may see future taxonomic revision (Frederico et al., 2012); *Plesiotrygon* (Rosa et al., 1987), containing two species; and the most recently described genus, *Heliotrygon* (Carvalho and Lovejoy, 2011), which contains two species. The genus *Potamotrygon* contains species that are the most commonly maintained in aquaria. Taxonomic review of the potamotrygonids is ongoing and some scientists expect the number of species to ultimately reach 35 or higher (Carvalho et al., 2011). Fishbase (www2) is an accessible resource for aquarists to remain apprised of recent amendments to potamotrygonid taxonomy.

Identification of freshwater stingrays is difficult, due to high intraspecific variation in color pattern (Araújo et al., 2004), the number of species awaiting formal description, and an incomplete knowledge of definitive species ranges. Complicating the situation further is an enthusiastic production of hybrid potamotrygonids in the ornamental fishes industry and some currently unresolved questions around the potential for polymorphism in dentition. While some species can be distinguished by their dorsal color pattern alone (Carvalho et al., 2011), many cannot. Some specimens may never be positively identified without reliable information about the location of wild collection and/or a postmortem examination of the stingray by a taxonomist.

Considering the likelihood of increased restrictions imposed upon the importation of wild potamotrygonids, and the ease with which various species hybridize, we recommend a precautionary approach to species identification and carefully managed reproduction in aquaria (see below). Further research on the taxonomy and genetics of potamotrygonids will be useful to aquarium managers.

REGULATIONS

Brazil is the largest country overlying the natural range of the freshwater stingrays and has the highest concentration of potamotrygonid species (Araújo et al., 2004). Brazil also has the most regulations related to the wild collection and exportation of potamotrygonids. The Brazilian Environmental Agency (IBAMA) is responsible for developing and enforcing these regulations. In 2008 IBAMA developed a quota system, which allows a limited number of six designated species to be exported from Brazil annually (www3). Institutions sourcing wild potamotrygonids are strongly encouraged to familiarize themselves with updates to regulations and to ensure that importers and exporters have appropriate paperwork for the species in question.

CITES

The discus ray, *Paratrygon aiereba* (Müller and Henle, 1841), the South American freshwater stingray*, Potamotrygon motoro* (Müller & Henle, 1841), and the rosette river stingray*, Potamotrygon schroederi* (Fernández-Yépez, 1958), were all proposed for inclusion in Appendix II of the Convention on International Trade in Endangered Species (CITES) in 2013. All proposals were rejected on the basis of insufficient trade and population status data (www4; www5). Future proposals for CITES listing are likely, so institutions are urged to keep apprised of changes to the conservation status of potamotrygonids.

Regulation in the United States

Trade and transport of potamotrygonids is currently regulated and/or prohibited in the following states and territories: Arizona, California, Florida, Georgia, Oklahoma, Puerto Rico, Nevada, Texas and Utah. Zoos and public aquaria may be granted special permission to transport

and hold potamotrygonids. In general, regulations are frequently subject to change and it is critical to check with local agencies prior to acquiring or de-accessing a potamotrygonid.

TRANSPORTATION

The most common way to transport potamotrygonids is in a sealed bag and insulated box, a technique regularly used for ornamental fishes and described in detail in Smith et al. (2004). This method is appropriate for small- or medium-sized potamotrygonids and may also be used for large specimens, although sourcing appropriately sized insulated boxes may be a challenge. For larger specimens a solid plastic container with a sealed lid and oxygen supply may be a better choice. The ideal size for a shipping container is one that is large enough to allow the ray to swim in a circle and prevent damage to the tail. Potamotrygonids should be double-bagged to reduce the risk of complete water loss resulting from a leak. The inner bag should be half-filled (a third may be sufficient in some cases) with clean aquarium water and the remainder filled with pure oxygen. A layer of newspaper between the bags will reduce the chances of a puncture through both bags and will help absorb water should there be a leak.

The primary consideration for long-duration transports is toxicity from accumulated ammonia. In poorly buffered shipping water, excreted carbon dioxide may cause pH to drop to 6.0 or even lower. This drop in pH can have a protective effect, as most of the ammonia will be in its less harmful ionized form (NH_4^+). Freshwater stingrays appear to tolerate low pH reasonably well for short durations. However, if the shipping water has buffering capacity, the use of an "ammonia-locking" product such as Amquel™ (Kordon, Wilbraham, Massachusetts) is recommended. When using Amquel™ the addition of a pH buffer, such as sodium bicarbonate, is recommended, to counteract the acidifying effects of the chemical. Potamotrygonids should be fasted for a minimum of two days prior to transportation (three or four days for larger specimens).

If cold weather is anticipated, or longer shipment times in cold conditions are expected, crumpled newspaper should be used to fill void spaces in the insulated box and one or more heat packs taped to the inside of the lid to stabilize temperature during transport. Heat packs are also known as "glove warmers" and are available at many camping supply stores (e.g., HotHands®, Uline, Pleasant Prairie, Wisconsin).

Spine cover

A protective vinyl tubing placed carefully, but firmly, on the sharp tip of the venomous spines of the stingray, will help prevent puncturing of the shipping bag. This procedure should be performed, while the stingray is sedated, 2 - 7 days prior to shipping to allow time for recovery. The spine should be checked during this time to ensure the cover does not easily fall off. In some cases, the procedure may need to be repeated. It is also possible to clip the spine to avoid bag punctures, although this option is considered less desirable. Intravenous (IV) administration tubing may be used to cover the spine of juvenile stingrays. Flexible silicone airline tubing is sufficient for the spines of most other stingrays, and slightly larger vinyl tubing may be necessary for larger specimens. If the spine cover does not fit snugly, vetrap bandage tape (3M, St. Paul, Minnesota) may be applied beneath the tubing to improve the fit and facilitate eventual removal. After transportation, it is possible to remove a spine cover while the stingray is acclimatizing in the shipping bag. When removing the spine cover, great care should be exercised so as to not damage the tail of the stingray by pulling too hard. In some cases, it may be necessary to anesthetize the stingray after it recuperates from transport and use scissors or other veterinary tools to remove the spine cover. If in doubt, it is possible to simply monitor the stingray and wait until the spine (and cover) is naturally shed. However, it should be noted that leaving the spine cover in place could lead to localized infection.

HANDLING

Potamotrygonids tolerate handling well if some special considerations are heeded. Nets can entangle the venomous spine, resulting in envenomation of handlers when attempting to disentangle a stressed animal, and/or damage the tail or spine, risking secondary infection. When possible, specimens should be moved in a plastic bin or bag. If a net must be used, rubber landing nets, clear vinyl collecting nets or rubberized or large-mesh nylon nets are recommended. If used, nets must be over-sized and shallow to prevent damage to the long, delicate tail of the stingray. Fabric or other traditional net materials should be avoided.

When handling potamotrygonids, thick neoprene gloves should be worn to reduce the risk of envenomation, especially in situations where hands may be in range of the venomous spine. This recommendation extends to grasping the base of the tail to draw blood, as injury can be sustained from the small, mildly venomous, hook-like denticles that cover the tail of many species. At the John G. Shedd Aquarium (Chicago, USA) and the Vancouver Aquarium (Vancouver, Canada), prior to any clinical procedure, a protective cover is placed over the entire length of the tail of the stingray, once the animal has been sedated. Tail covers are typically fabricated from an appropriate length of rigid polyvinyl chloride (PVC) pipe, or vinyl tubing in the case of pups. If a freshwater stingray has not been sedated, a thick sheet of styrofoam, with a tail-shaped semicircle cut from the bottom edge, can be used to carefully pin the tail of the specimen to the bottom of the holding container. The styrofoam acts as a shield while work is performed on the dorsal side of the stingray.

The practice of clipping the venomous spine of freshwater stingrays has remained more controversial than the equivalent practice in marine stingrays. A survey of public aquaria maintaining potamotrygonids revealed that ~25% of polled institutions clipped spines for medical procedures and/or shipping, and that <10% of institutions clipped spines on a regular basis. Potamotrygonids appeared to be more susceptible to infection following spine clipping than their marine counterparts. Given other available options to reduce envenomation during the handling of potamotrygonids, the practice of spine clipping is discouraged unless the animal is in regular contact with guests or the spine sheath has been severely compromised by trauma or infection.

Envenomation

Envenomation by a potamotrygonid may result in severe injury, typically exceeding the degree of trauma resulting from the barb of a marine stingray. This dichotomy results from the larger number of protein secretory cells located along the entire epidermis of potamotrygonid spines, whereas they are located only in the ventrolateral grooves in marine stingrays (Pedroso et al., 2007). Potamotrygonid envenomation may result not only in severe pain, but also edema, erythema, tissue necrosis, and ulcers, which can take up to three months to heal (Haddad et al., 2004). Some studies (Magalhães et al., 2006) and anecdotal reports indicate that the venom from different *Potamotrygon* species may have different properties, and subsequently, result in differing levels of pain and enduring injury.

In the event of envenomation, the patient should be transferred to a nearby medical facility. The affected area should be immediately immersed in water as hot as tolerable without scalding (~50°C) for 30 - 90 minutes, or until the patient receives professional medical attention (Haddad, 2003; Haddad et al., 2012). If the wound is on a leg or the torso, and not easily immersed, a towel may be placed over the injury and hot water continuously applied to the towel. Hot water provides pain relief, as the venom is thermolabile, but does not seem to reduce the appearance of persistent cutaneous necrosis and chronic ulcers that may result from envenomation (Haddad et al., 2004; Barbaro et al., 2007).

Delayed complications from a potamotrygonid envenomation and the associated laceration can be grave. The wound should be explored, making use of appropriate diagnostic imaging techniques, and surgery may be required to remove fragments of broken dentine. The wound should be thoroughly cleaned and the patient given anti-tetanus prophylaxis and a course of appropriate systemic antibiotics (Haddad et al., 2004). *Pseudomonas* spp. and *Staphlococcus* spp. are frequently associated with potamotrygonid envenomations in South America. *Mycobacterium* spp. may be an additional zoonotic risk from wounds inflicted by potamotrygonids in public or home aquaria.

ENCLOSURE DESIGN

Size

Maximum size, measured as disc width (DW), varies widely between different species of potamotrygonids (i.e., 25 - 100 cm DW) (Carvalho et al., 2003). Freshwater stingrays are very active and enclosures should be large, with consideration given to their predominantly benthic behavior. Wider, longer and shallower tanks are more appropriate than the proportions of a standard aquarium, as they provide an appropriate space on the bottom for the stingrays.

A holding tank of dimensions 1.8 m x 0.8 m x 0.35 m (volume = 0.5 m^3) is sufficient for temporarily maintaining two or three potamotrygonids of 15 - 30 cm DW. A tank of this size can be used to rear pups, or to hold sub-adults, particularly if water quality is rigorously maintained using high-quality

filtration or frequent, high-volume water exchanges. Larger tanks are required for the long-term maintenance and reproduction of potamotrygonids. An enclosure of 2.4 - 3.0 m x 2.0 - 2.5 m x 1.0 m (volume = 4.8 - 7.5 m^3) is sufficient for the long-term maintenance of a breeding group of potamotrygonids consisting of two to four adults, depending upon the maximum size of the species. Potamotrygonids thrive in larger exhibits (e.g., 20 m^3) as long as feeding is unimpeded and husbandry personnel can readily observe the animals. Of surveyed aquaria, the smallest "footprint" for maintaining an individual stingray was 0.75 m^2 and the smallest area for maintaining a group of four animals was 3.0 m^2 (~0.75 m^2 per stingray). This surface area should be considered the absolute minimum for a potamotrygonid. For the long-term maintenance of freshwater stingrays 1.5 - 2.5 m^2 should be provided for each specimen, providing ample space for the animals and minimizing intraspecific aggression.

In addition to adequately sized enclosures, isolated off-exhibit holding is highly recommended, allowing for appropriate health and reproduction management of a potamotrygonid collection.

Filtration

A life support system for potamotrygonids should include a heavy emphasis on mechanical (e.g., pressure sand) and biological (e.g., wet-dry trickle) filtration. Potamotrygonids are active foragers with a high rate of food intake for an elasmobranch. Uneaten food particles will quickly degrade water quality, so easy-to-clean mechanical pre-filtration (e.g., filter floss in surface skimmers) should be employed wherever possible and cleaned regularly. Any residual food debris should be removed from the aquarium shortly after feeding. This recommendation is particularly important for waste material and uneaten food in rearing tanks, or in tanks containing no companion fish that would otherwise consume the fine pieces of food left by the stingrays. The use of ultraviolet sterilization (e.g., Lifegard Aquatics, Cerritos, California, USA) should also be considered, to reduce the concentration of potentially pathogenic organisms within the water column.

In-aquarium filtration or heating equipment may be used, but caution must be exercised. Potamotrygonids may bite and damage filter intake tubes and other life support components, or rest their disc against an exposed heater and sustain a burn. Filtration components should be covered with removable coarse sponge. Some equipment will benefit from reinforcement with a strong material like PVC. Aquarium heaters should be protected with covers made of drilled PVC, plastic mesh (e.g., Conwed Plastics, Minneapolis, Minnesota, USA) and cable-ties, or a similar shielding material.

WATER QUALITY

Parameters

Potamotrygonids occur in a multitude of different habitats, with differing water quality throughout their natural range. Some of the diverse habitats where stingrays can be found include blackwater forest streams, clear fast-flowing rivers, and whitewater main river channels. Although there are some species-specific water quality requirements, most potamotrygonids tend to be tolerant of a range of conditions considered suitable for many neotropical freshwater teleosts. Once the animals are established in a stable aquarium environment, the critical parameters to monitor are temperature, pH and nitrate.

Surveyed public aquaria have successfully maintained potamotrygonids in temperatures of 22.2 - 30.6ºC, a pH range of 5.5 - 8.4, and nitrate concentrations of 0 - 200 mg/L NO_3-N. Most polled institutions (90%) reported nitrate concentrations below 50 mg/L NO_3-N and over 60% reported levels of <25 mg/L NO_3-N. Ross (1999) recommends keeping potamotrygonids at a temperature range of 24 - 27ºC, a pH range of 6.5 - 7.0 and a maximum nitrate concentration of 300 mg/L NO_3 (~ 67 mg/L NO_3-N), with a normal operating concentration of 100 - 200 mg/L NO_3 (~ 22 - 45 mg/L NO_3-N). We endorse these recommendations. Whatever target nitrate level is selected for maintenance or breeding of freshwater stingrays, it should be monitored regularly and water exchanges scheduled accordingly.

Metals

Like other elasmobranchs, freshwater stingrays are very sensitive to metals. Copper, in particular, must be avoided. Most potamotrygonids are maintained in water of a lower pH than marine rays, which may lead to higher levels of free copper available in their water systems. An example of metal toxicity resulting in *Potamotrygon* sp. mortality was traced to overspray water from a misting system containing copper piping. Once the pipe was replaced,

copper concentrations in the water dropped to 0.003 mg/L and no further stingray mortalities were observed.

COMPATIBILITY

A survey of public aquaria revealed that numerous neotropical fish species have been successfully maintained with potamotrygonids. The most common species kept with *P. motoro* and the white-blotched river stingray, *Potamotrygon leopoldi* (Castex & Castello, 1970), include other potamotrygonids, pacu (*Colossoma* spp. and *Piaractis* spp.), catfish (*Brachyplatystoma* spp.), sorubim (*Pseudoplatystoma* spp.), and various South American cichlids (*Geophagus* spp. and *Cichla* spp.) The tiger stingray, *Potamotrygon tigrina* (Carvalho, et al., 2011) and the bigtooth river stingray, *Potamotrygon henlei* (Castelnau, 1855), have been maintained with fewer (or no) other species, but this is likely an artifact of these species being underrepresented in public aquaria.

Companion species maintained in the same exhibit as potamotrygonids may represent a challenge if they actively compete for food intended for the stingrays. This risk may be mitigated through the use of training techniques and the establishment of feeding stations. Companion animals may also represent a challenge by repeatedly biting stingrays and causing trauma, while the stingrays themselves may prey upon smaller companion animals. We recommend selecting larger companion animal species (e.g., those listed above) that are unlikely to be ingested by, or to harangue, the stingrays. The following animals have been maintained with potamotrygonids successfully, but have at times represented a challenge: various loricariid catfishes, arowanas (*Osteoglossum* spp.), pike cichlids (*Crenicichla* spp.), anostomids (*Abramites* spp. and *Leporinus* spp.), and characins (*Hydrolycus* spp., *Hemiodus* spp., and *Boulengerella* spp.). Many geophagines make good companion species because they don't compete for larger pieces of food offered to the stingrays. Instead, geophagines sift the substrate and consume food fragments left behind by foraging stingrays, which would otherwise compromise water quality. Aquarists must experiment with different companion species and be prepared to remove animals if problems are observed. Reptiles may make appropriate companions for potamotrygonids. Over 75% of polled institutions maintaining *P. leopoldi* reported keeping Amazonian turtle species with their rays, including *Podocnemis* spp., *Phrynops* spp. and mata mata, *Chelus fimbriatus* (Duméril, 1806).

DIET AND NUTRITION

Food

Potamotrygonids feed on a wide variety of food items in their natural habitat, including insects, annelids, molluscs, crustaceans, and a wide variety of teleosts (Rosa et al., 2010). Some species, such as *P. motoro*, demonstrate considerable plasticity and variability in their feeding behaviors, while others are better described as specialists. *P. leopoldi* have a diet consisting predominantly of gastropods; discus rays, *Paratrygon aiereba* (Müller and Henle, 1841), feed almost exclusively on fishes; and smooth back river stingrays, *Potamotrygon orbignyi* (Castelnau,1855), are insectivorous (as reviewed by Almeida et al., 2010).

Potamotrygonids typically maintained in aquaria, even those with highly specialized diets in the wild, adapt well to a varied diet consisting of commonly available food items. We recommend a staple diet of small, whole freshwater fishes (bones and organs included), such as rainbow smelt, *Osmerus mordax* (Mitchill, 1814). Other recommended food items include chopped whole rainbow trout, *Oncorhynchus mykiss* (Walbaum, 1792) or other freshwater fishes, prawns or shrimp, clams, squid, krill, capelin, *Mallotus villosus* (Müller, 1776), or Atlantic herring, *Clupea harengus* (Linneaus, 1758). Marine organisms should comprise a smaller proportion of potamotrygonid diet only, to ensure an appropriate fatty acid ratio in the ration. Pre-prepared foods, such as Aquatic Gel (Mazuri®, Purina Mills LLC, Nutrition International LLC, Richmond, USA), are a valuable addition to potamotrygonid diet, ensuring the stingrays get appropriate nutrients and providing an easy method to administer oral medication when necessary.

Food items should be of a size that is readily swallowed by the stingray, yet not rejected because they are undersized. Ejection from the spiracle indicates that food items are either too small to be effectively eaten or are distasteful. Excessive chewing, indicating that a food item is unpalatable or too large, may reduce the nutritional value of the food item and reduce water quality.

Live Foods

Live foods represent an excellent source of behavioral enrichment for potamotrygonids, as well as a great aid for training programs. In addition, live food may be required for conditioning wild-caught stingrays to feed in aquaria, as well as aid in the rearing of pups. Earthworms (e.g., nightcrawler, *Lumbricus terrestris*) are typically available from bait shops, and snails (e.g., red ramshorn snails, pond snails and apple snails) are occasionally available as surplus from other aquarium exhibits. Crayfish (*Orconectes* spp. and *Procambarus* spp.) are available in different sizes and make excellent food. Some species of crayfish may be cultured in aquaria. Blackworms (*Lumbriculus variegatus*), bay ghost shrimp (*Neotrypaea californiensis*) and glass shrimp (*Palaemonetes* spp.) are especially favored by smaller stingrays.

Although live foods are recommended as part of a healthy diet, great care should be taken to ensure that freshwater stingrays do not become dependent exclusively on live feed, reliably eating thawed, frozen foods as well as live food items. The use of live feeder fishes (e.g., goldfish, *Carassius auratus* (Linnaeus, 1758), livebearers, etc.) is discouraged unless the stingray is chronically anorectic or is typically a highly piscivorous species.

Nutritional supplementation

Janse et al. (2004) provided an extensive review of correct nutrition, food preparation, and feeding techniques for elasmobranchs under human care. Little is known about the specific differences in vitamin and mineral requirements for freshwater and marine stingrays, but the natural diet of potamotrygonids does differ significantly from their marine counterparts. In particular, insects play an important role in the diet of many freshwater stingrays, with some species being specialist insectivores (Lasso et al., 1996; Shibuya et al., 2009; Almeida et al., 2010; Moro et al., 2012). Many juvenile potamotrygonids relish thawed, frozen chironomid larvae.

Studies of vitamin degradation during the storage and preparation of frozen seafood suggest that most piscivorous species will benefit from supplements added to thawed food (Bernard and Allen, 1997). A survey of potamotrygonid carers found that 38% used some form of dietary vitamin supplementation for their freshwater stingrays. The majority of carers used Shark and Ray tabs (Mazuri®, Purina Mills LLC, Nutrition International LLC, Richmond, USA). Other products included Thiamin-E paste (Mazuri®, Purina Mills LLC, Nutrition International LLC, Richmond, USA) and Vitafish™ (Marine Enterprises International LLC, Baltimore, Maryland, USA). We recommend nutritional supplementation through the use of these products and/or the inclusion of commercially prepared gel or pellet foods. Paste supplements appear to be more palatable to potamotrygonids and can be readily injected into almost any food in the appropriate proportions, which allows for a regular schedule of safe nutritional supplementation during the rapid growth phase of juveniles. In general, however, efforts to provide the optimum nutrition for potamotrygonids should focus on a wide variety of high-quality food items, rather than relying too heavily on supplementation.

Food quantity

To remain healthy and reproduce successfully, most species of adult potamotrygonids only need to be fed to satiation three to four times per week. A study examining the stomach content of wild freshwater stingrays revealed many to be empty or containing only a small amount of food (Rosa et al., 2010), supporting the notion that daily feeding of potamotrygonids in aquaria is probably unnecessary for mature, healthy animals. Regardless, we recommend feeding potamotrygonid pups a minimum of twice a day to support their rapid growth, especially when converting animals from live to non-live foods. Studies at the John G. Shedd Aquarium showed a significant increase in pup growth when pups were offered food to satiation several times a day, rather than once daily (Hornbrook, unpublished results). Feeding frequency may be decreased as pups grow, but care should be taken to ensure that body condition is maintained. Subadult potamotrygonids should be fed to satiation 4 - 6 times per week, with feeding frequency decreasing as they grow.

Data collected at the Vancouver Aquarium between 2012 and 2013 indicated that an appropriate feeding ration for most age groups of potamotrygonids, as a percentage of body mass (BM), was in the range of 6.8 - 9.2% BM/week. The ration, feeding schedule and varied diet resulted in healthy stingrays, which grew at a reasonable rate and successfully reproduced. By contrast, Janse et al. (2004) recommended that Dasyatid and Myliobatid rays should be offered 4 - 6% BM/week, and Janse and Schrama (2010) offered blue-spotted stingrays, *Neotrygon kuhlii* (Müller and Henle, 1841), 10.1 - 11.3% BM/week.

Signs of poor body condition in a potamotrygonid include a protruding pelvic girdle, on either side of the base of the tail, and/or a shallow indentation between the eyes. Freshwater stingrays in poor body condition should be fed any type and quantity of food they will eat, including live foods. If the animal refuses to eat and becomes increasingly lethargic, assisted feeding is recommended.

Assisted feeding is typically performed while the ray is sedated. A flexible tube is inserted into the stomach, via the mouth, and a slurry of appropriate blended food is introduced through a syringe attached to the tube. Once the freshwater stingray is eating voluntarily, a concerted effort to condition the animal to a greater variety of foods should be the immediate husbandry priority.

Careful and incremental adjustments can be made to a feeding ration to accelerate or slow freshwater stingray growth. However, great care should be used to monitor body condition and growth to ensure the animal(s) are growing at a reasonable rate, and are not tending toward obesity or undernourishment.

SENSITIVE SPECIES

Tiger stingray

P. tigrina is one of the most sensitive and finicky of the freshwater stingrays. This species is very susceptible to shipping and handling stress, and does not readily adapt to dead food. *P. tigrina* may be timid around competitive companion species and can be easily outcompeted for food. In addition, this species can suffer injury to their delicate long tail, so they are not an ideal choice for a busy multi-taxa exhibit.

Experience has shown that female *P. tigrina* become sexually mature at ~48 cm DW in aquaria. Although a maximum size has not been confirmed, *P. tigrina* has reached at least 70 cm DW in aquaria; a suitably spacious enclosure is therefore required for this species.

P. tigrina has been successfully maintained at several AZA institutions, with reproduction achieved at the Vancouver Aquarium and John G. Shedd Aquarium. Water parameter ranges at both these institutions were: a pH of 6.6 - 8.0, a water temperature of 25.0 - 28.5ºC, and a nitrate concentration of 0 - 20 mg/L NO_3-N. In both aquaria *P. tigrina* were maintained on fine sand in water with low mineral content, and were maintained with appropriate companion fishes (see above).

Antennae stingray

The antennae stingrays, genus *Plesiotrygon*, currently contains two species, long-tailed river stingrays, *Plesiotrygon iwamae* (Rosa et al., 1987), and black-tailed antenna rays, *Plesiotrygon nana* (Carvalho and Ragno, 2011). *P. iwamae* is imported into the US with some regularity, but *P. nana* is less frequently available. The husbandry team at the John G. Shedd Aquarium was able to successfully maintain a young female *P. nana*. The pup was maintained in water of pH 6.5 - 7.0, while it was slowly converted from a preferred food of tadpoles to live *L. variegatus*, *N. californiensis*, and ultimately (frozen and thawed) seafood. As the *P. nana* grew, it was acclimated to a higher pH of 7.5 and appeared to suffer no ill effects.

P. nana in aquaria are notorious for losing pieces of their long tail to physical damage, entangling it in tank décor or having it bitten by companion animals. A specimen maintained at the John G. Shedd Aquarium lost a portion of its tail. However, once healed, and the risk of infection had abated, foreshortening of the tail did not appear to pose a problem to the general wellbeing or behavior of the animal.

In general, great care should be exercised when considering the maintenance of *P. nana* with companion fishes. *P. nana* appear to have a small mouth, relative to other potamotrygonids, so offered food items should be proportionately smaller. The maximum size of *P. nana* has been reported as 25 cm DW, however the specimen maintained at the John G. Shedd Aquarium reached 38 cm DW.

Other freshwater stingrays

The genera *Paratrygon* and *Heliotrygon* are rarely maintained in aquaria. *P. aiereba* presents husbandry challenges related to its large size, with a maximum recorded disc width of 80 cm DW (Lasso et al., 1996), its fastidious feeding preferences and its sensitivity to handling.

P. aiereba demonstrates strong piscivorous tendencies both in the wild and in aquarium settings (Lasso et al., 1996; Shibuya et al., 2009). Husbandry personnel at the John G. Shedd Aquarium were unable to successfully convert *P. aiereba* to dead food, despite numerous attempts. In addition, Aquarium staff had an unexpected challenge moving *P. aiereba* within the facility, with

two out of three healthy individuals dying shortly after transfer.

Staff at the John G. Shedd Aquarium observed a curious and distinct behavior in *P. aiereba*, whereby specimens attached their undersides to exhibit window or walls and remained stationary for long periods of time. This behavior was not observed in other potamotrygonids. Three *P. aiereba* pups were stillborn at the Aquarium, resulting from reproduction within the facility. No hybridization with other potamotrygonids was observed.

VETERINARY CARE

Health examinations

Some institutions do annual or biannual examinations of their animals, while others only investigate when overt signs of illness are observed. Health examinations are an important tool for assessing the status of individual stingrays. Health exams provide an opportunity to obtain updated morphometric data and to ensure identification transponders are still functioning (see below). Blood can be collected and, as more baseline blood values become available from healthy animals, analyzed and assessed for general health indication.

Quarantine

Quarantine of potamotrygonids should follow accepted industry practices of a 30-day period of isolation from specimens already in the existing collection. During quarantine, skin scrapes, fecal samples and other samples may be collected to assess parasite loads and treatments prescribed where appropriate. Salt treatments of 3 - 4 g/L may be applied during quarantine if the epidermis has been damaged in transit, particularly if water mold (see below) or other signs of secondary infection appear. Fish lice (*Argulus* spp.) are common on recently imported wild potamotrygonids, and can usually be removed with forceps even before the stingray is introduced into the quarantine tank.

At the end of quarantine, a physical exam should be performed to obtain baseline blood values and morphometric data. In addition, a transponder should be inserted into the stingray to allow for reliable identification of individual specimens. Pharmaceuticals and associated dosages used with potamotrygonids have been summarized by Mylniczenko and Clauss (this volume).

Transponders

Transponders, or microchip tags, are a useful tool for identifying individual stingrays within a collection. When tagging potamotrygonids, husbandry personnel should comply with *Guidelines for Transponder Placement and Recording* (www6) and place the microchip on the left-hand side of the dorsal surface of the ray. Before tagging with a transponder, the stingray should be anesthetized using 75 - 100 mg/L buffered MS-222 (Finquel, Argent Chemical Laboratories Inc., Redmond, Washington, USA).

Because elasmobranch skin is tough and elastic, it may be helpful to make a “guide hole” at the site of tag placement. This procedure should be performed above the water by inserting a sterile 16-gauge needle into the skin, bevel side up, and advancing it in a cranial direction two to three times the length of the microchip. The needle used to make the “guide hole” is then removed and the microchip needle inserted into the hole until the bevel is no longer visible, at which point the microchip can be injected. The injection site is then closed with sterile tissue glue or a suture.

It is good practice to pre-load the microchip insertion needle and syringe, sterilize the entire assembly, and confirm microchip function, before commencing the procedure. Once a stingray has been tagged, or “chipped”, it is recommended that the chip be checked several weeks later to ensure that it has not been shed (Poll, personal communication).

Water mold

Water mold is an infectious freshwater, fungus-like microorganism of the class oomycota, the most widespread belonging to the order saprolegniales. Any anorectic potamotrygonid, especially a healthy specimen showing signs of interest in food, but not eating, should be checked for water mold in the buccal cavity. The teeth and mouth of the stingray may have a yellowish fuzzy appearance, symptomatic of water mold. Excess shedding of teeth, lethargy, and/or loss of body condition may also indicate a water mold infection. A diagnosis of water mold can be verified by taking a small sample from the buccal cavity using forceps and examining it under a microscope.

Continuous exposure to salt (NaCl) at a dosage of 3.0 - 4.5 mg/L, for a minimum period of 4 - 6 weeks, will reduce and eventually eliminate water mold, depending on the severity of the infection. A prolonged water mold infection, deep in the

tissue, may require a longer treatment for complete remission.

ENRICHMENT

Basic enrichment, which is sufficient for many potamotrygonids, includes a complex habitat (sand, driftwood, rocks, etc.), a variety of companion species and a diverse diet. If stereotypic behavior (e.g., swimming in circles, repeated swimming at the surface, habitual swimming at the tank perimeter) is observed, relief may be provided by adding conspecifics, changing habitat structure, varying food and feeding, and by the use of training techniques (Scott et al., 1999).

Environmental enrichment

Araújo et al. (2004) noted that potamotrygonids occur in diverse habitats, including sandy beaches, flooded forests and small creeks with mud or stone substrates. The behavior of different stingray species is correspondingly varied. *P. tigrina* may be distressed when maintained in aquaria without fine sand and without darkened areas for refuge. Other species, such as *P. leopoldi*, rarely use the protective covering of sand, are more active throughout the day, and tend to be inquisitive about their surroundings and available enrichment items.

All potamotrygonids benefit from environmental enrichment, which can be provided by irregular exhibit topography, pieces of driftwood, and piles of rubble, where food can be secreted to encourage foraging behavior. Stingrays will hide within, or swim through, short lengths of large-diameter PVC pipe, providing topographic enrichment. A lighting gradient is also recommended within the habitat, as it allows stingrays access to darkened areas should they desire.

While a variety of substrates may be used for potamotrygonids, silica filter sand appears to be the most commonly used and desirable substrate. Newborn stingray pups exhibit calmer behavior when they have fine silica sand available for cover. A sandy bottom allows stingrays to bury themselves if desired and helps prevent the formation of contact abrasions, which can occur in bare fiberglass aquaria. Substrate choice is important, as abrasive sand can irritate the ventral disc of potamotrygonids and lead to secondary infection. It should be noted that once acclimated and eating well, many species of freshwater stingray (with the exception of *P. tigrina*) could live comfortably on a bare-bottomed glass or acrylic exhibit.

Dietary enrichment

Potamotrygonids can learn to recover food hidden within enrichment items, including the commonly available rubber “Kong” dog toy (The Kong Company, Golden, Colorado, USA). Live or frozen food can be placed inside the dog toy to slow the rate at which stingrays find and ingest the food, as well as provide foraging enrichment. This technique may be used to distract a larger voracious stingray, allowing more time to focus on feeding smaller or undernourished specimens. PVC pipes of varied length and diameter can serve a similar function. Other feeding enrichment techniques include the introduction of food within a tray of rocks, which encourages foraging behavior, scatter feeding, or changing the location of targeted food introduction.

Training

Operant conditioning, or training, provides benefits to husbandry personnel, the viewing public, and the stingray. Potamotrygonids are highly motivated by food and have been readily trained using techniques described by Sabalones et al. (2004). Training provides cognitive stimulation for the stingrays and it allows greater control of individual stingray diet. In addition, training allows husbandry personnel to capture and restrain stingrays, with a minimum of stress to the animal, and it can facilitate medication administration.

Association with a target, or target training, is a powerful foundation for other desired behaviors. If tongs are employed to feed stingrays, the animals will quickly associate the tongs with food. The tongs thus become the “target”. Using the target, a stingray can be led onto or into an object of choice (e.g., a husbandry device) by a process of successive approximation. At the Pittsburgh Zoo and PPG Aquarium, stingrays were trained to swim against the viewing window of their aquarium to facilitate examination of their ventral disc. Stingrays at the Vancouver Aquarium were trained to feed from PVC poles, allowing much greater control of food ration within multi-species exhibits.

REPRODUCTION IN AQUARIA

Priority species

The AZA Freshwater Fish Taxon Advisory Group (FFTAG) actively manages a potamotrygonid

studbook and has designated four stingray species as a priority for breeding: *P. henlei*, *P. leopoldi*, *P. motoro* and *P. tigrina*. Breeding is discouraged in other species, so that all available resources can be directed toward the priority species. The FFTAG makes breeding recommendations to ensure that the aquarium metapopulation maintains a high degree of genetic diversity, remains demographically healthy and does not outgrow available space within AZA institutions.

Population management and hybridization
At present, there are no accepted methods for surgical or hormonal birth control in potamotrygonids. As a result, non-priority potamotrygonid species should be maintained in single sex populations to avoid unwanted reproduction. Exclusively female populations tend to work well as they are less prone to intraspecific aggression. However, exclusively male populations can also be maintained if there is sufficient space and the temperament of individual animals allows. In this case, an area to separate animals is highly recommended to allow for the management of intraspecific aggression. Enrichment techniques described above will also facilitate the management of an exclusively male stingray population.

In aquarium situations, the possibility of interbreeding between different species of potamotrygonids is assumed and should be actively avoided. Hybrid animals displace priority species and there is a risk that hybrids could be introduced into, and compromise, managed populations. We recommend keeping opposite genders of different species separated, especially once they reach sexual maturity.

Monitoring pregnancy and ultrasonography
Pregnancy in potamotrygonids can frequently be detected by direct observation and can be confirmed by ultrasonography. Most pregnant potamotrygonids visibly swell on one or both sides of the dorsal surface, near the base of the tail. The size and degree of swelling varies between species and can be indicative of the stage of pregnancy and/or the number of pups. Careful observation of a stationary gravid female stingray may reveal distinctive ripple-like or jerky movements, which are also a sign of pregnancy.

Ultrasonography is a relatively noninvasive diagnostic technique. The ultrasound transducer can be attached to a long pole, allowing staff to maintain a safe distance from the stingray during examination. In many cases the stingray can remain in the aquarium for a reading. Another useful ultrasound technique is to place the gravid female in a shallow tub filled with water. Ultrasound gel is applied directly to the underside of tub and a reading taken from underneath, when the stingray is stationary and on the bottom. If the embryonic pup(s) is horizontal, gill arches, disc margin and vertebrae can all be observed.

Gestation time
Fecundity and gestation time varies significantly between different species of potamotrygonid, with a gestation time of 3 - 11 months and a litter size of 1 - 21 pups (Rosa et al., 2010). Potamotrygonid reproduction in their natural habitat appears to be closely tied to the seasonal hydrologic cycle (Charvet-Almeida et al., 2005). The effect of aquarium conditions, particularly food intake and seasonal cues (e.g., photoperiod and temperature), on the timing and frequency of reproduction and gestation times, remains uncertain. The gestation time in aquaria for *P. motoro* is ~3 months (Ross, 1999; Ross, 2004), while *P. tigrina* and *P. leopoldi* have a gestation time in aquaria of ~4 months.

Management of gravid females
Once a potamotrygonid is confirmed to be gravid, it should be separated from other stingrays, especially males, as intraspecific aggression could cause the female to abort. Moving pregnant females should be done with care, as this process could also lead to spontaneous abortion. Removing non-gravid conspecifics from the aquarium, or the use of in-exhibit barriers, may be a preferred technique to segregate animals.

If more than one gravid female shares an aquarium, it is helpful to separate them so that the provenance of newborns is known. Should a litter be discovered in an aquarium where more than one gravid female was present, it is possible to determine the new mother through the use of ultrasound (i.e., the new mother is no longer pregnant).

Male potamotrygonids have been observed biting and killing newborn pups. On other occasions, males and newborns have been maintained together without incident. As the risk of aggression is dynamic and dependent on a variety of factors—species, population dynamics, habitat complexity, etc.—population management deci-

sions should be made conservatively and on a case-by-case basis.

Care should always be taken to ensure that pregnant females are receiving an adequate food ration, consistent with the energetic and nutritional demands of both the mother and the pups.

Rearing pups

Most newborn potamotrygonids are relatively robust. When provided with a fine sand substrate, clean water of high quality and appropriate food items, representing an appropriate ration, newborn stingrays will flourish. Separating newborns into a rearing tank minimizes competition for food and allows the baby stingrays to be carefully monitored. Experience has shown that leaving pups with their parents tends to decrease their survivorship.

Food should be offered to newborns within a day of birth as, *in utero*, pups have been relying on both their yolk sac and histrotroph for nutriment (Conrath and Musick, 2012). For this reason, the first fecal deposits of newborn pups are typically whitish in color (Reynolds, 2013). Careful observation of fecal deposits in the rearing tank can therefore be used as a diagnostic tool to assess the nutritional status of young pups.

Newborn pups of some species (e.g., *P. motoro*) may immediately eat *Mysis* spp., bloodworms (*Glycera* spp.) or finely chopped seafood. Initiating feeding in other species (e.g., *P. tigrina*) may prove to be more difficult and may require up to six months to acclimate specimens to dead food. Conditioning young stingrays to dead and prepared foods should be one of the main goals of rearing; an over-reliance on live food places the stingrays at risk of insufficient caloric intake and/or nutritional deficiency. However, live food should be provided, as needed, while conditioning pups to dead food, ensuring they maintain good body condition and exhibit expected growth rates.

Live *N. californiensis* is one of the most consistently accepted food items for potamotrygonid pups. Glass shrimp (*Palaemonetes* spp.) and finely chopped *L. terrestris* or redworm (*Eisenia fetida*) are also frequently accepted. Small freshwater snails, often considered a nuisance species in many aquaria, are attractive to newborn potamotrygonids and can be offered as a first food.

Conditioning wild-caught or aquarium-born stingray pups to new food is most readily achieved by changing one aspect of the food at a time. Stingray pups are initially conditioned to eat dead food by feeding freshly killed or thawed food items that they will reliably eat when offered live. Once conditioned to dead food, new food items can then be introduced. New food should be offered before any other regular food, to entice the stingray pups to try the novel food items. New food may also be rubbed with the scent of a favored food item, or stuffed, as pieces or a paste, into a favored food item. This approach works well for chopped nightcrawler worm, which can be used as a "sausage casing" and stuffed with small pieces of thawed shrimp, fish or squid. The amount of nightcrawler worm in the food ration is progressively decreased over a number of feedings until the stingrays are eating the new food items only. Once pups are reliably eating a diverse range of thawed frozen foods, live food should be discontinued. Live foods may be reintroduced as a nutritional and behavioral enrichment once a stingray pup is reliably and regularly feeding on dead food items, but care should be taken to ensure that a reliance on live food does not redevelop.

CONCLUSION

Field research on potamotrygonids is challenging, because of the intense seasonal hydrologic cycle within, and the remoteness of, their natural range. Aquarists working with potamotrygonids are in a unique position to collect data on this spectacular and relatively poorly understood taxon. As such, aquarists should make every effort to keep thorough records related to their collection, to stay well informed about developments in the field of freshwater ray husbandry and to share any new information about potamotrygonids with aquarium colleagues and field researchers.

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Chapter 12

Elasmobranch quarantine

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Abstract: Quarantine reduces the risk of introducing infectious diseases into collections and allows close monitoring while animals adjust to new environmental conditions and diets. Common infectious diseases that represent a risk to aquarium collections include monogeneans, copepods, leeches, and coccidia. Bacterial and fungal infections are more likely to be a problem in individual animals, and are often secondary to trauma or other stressors. Emerging infectious diseases include Scuticociliatida-like ciliates and Chlamydiales. Common non-infectious problems in quarantine include inappetence and trauma. Some species, particularly aquarium-bred animals, present few challenges during quarantine, while others frequently require intensive nutritional support or medical management. Good record keeping is essential during quarantine and allows evaluation of management, diagnostic and treatment plans.

INTRODUCTION

The primary goal of quarantine is to prevent the introduction of significant infectious diseases into living collections. This goal is accomplished through isolation of new specimens, close monitoring and appropriate treatments. Capture and transport stress can make subclinical diseases present clinically, and quarantine keeps animals isolated and accessible through this critical post-transport time period. Quarantine also allows feeding in a controlled environment, as well as the collection of baseline data on animal physiology and behavior. Multiple variables affect quarantine success, including adaptability of the species to captivity, source, shipping conditions and the available infrastructure.

This chapter focuses on institutions with multiple recirculating systems and an established animal collection, where quarantine is an essential management tool. There are some situations where quarantine is less critical–e.g., an institution with flow-through aquarium systems or a research laboratory with a single population. In some instances, quarantine is precluded by the size of the new animal or by a lack of suitable space. However, there are reports of metazoan parasites being introduced into collections because quarantine had to be abbreviated for practical reasons (Poynton et al., 1997; Dove and Clauss, 2008; Kik et al., 2011). A well-planned quarantine period is always recommended.

PLANNING

A designated quarantine area, isolated from collection animals, with multiple independent systems, will allow for the segregation of different

quarantine groups. This situation should be considered a minimum requirement for a modern public aquarium or research institution. System design and associated life-support should dictate the species (including numbers and sizes) that can be quarantined. It is essential to provide elasmobranchs appropriate space for normal swimming, gliding and turning, to reduce stress and the risk of trauma. Large, oval tanks are preferred for many pelagic shark and ray species. Some species require cover for hiding, or sand/crushed gravel substrate; others do better with no obstructions. Non-abrasive tank walls and floors are recommended, and any fiberglass surfaces should be checked for cracking or peeling. It may be necessary to mark pale tank walls with dark patterns to help animals see and negotiate the aquarium. Windows in the side of the tank and good overhead lighting help with the monitoring of specimens. Jump barriers are required and should be inspected regularly for efficacy. Ozone and/or ultraviolet disinfection can reduce pathogen burden in the system.

Each quarantine system should have dedicated equipment (e.g., nets, barriers, feeding poles) to minimize the risk of cross-contamination. It is ideal to have a dedicated quarantine staff that does not work with animals within the main collection. If this level of staffing is not possible, adjusting workflow to allow for the servicing of quarantine systems at the end of the day is recommended. Other biosecurity measures include ready access to gloves (some with full sleeves), hand-washing or showering stations, and suitable cleaning and disinfection protocols. A hoist system for appropriately managing large animals (e.g., moving, weighing) is highly recommended. A work area should be provided near quarantine systems, where data recording and animal examinations can take place, and an additional isolated space should be available for performing necropsies.

Each animal transport should be carefully planned to ensure sufficient experienced personnel are available. Animal acquisitions prior to major holidays should be avoided. Plans should include acclimation protocols, décor and substrate needs, animal diets, animal behavioral needs, and potential diagnostics and treatments. These plans are particularly important for species that are not routinely housed at the institution or for species that have shown morbidity or mortality in the past. For animals coming from other institutions, it is important to obtain their husbandry and medical history. Quarantine systems should be run as ‘all in/all out’ groups. For elasmobranchs, it may be best to limit quarantine tanks to a single species. Elasmobranchs and bony fishes should not be quarantined together as this limits the medications that can be used to manage parasite outbreaks. Quarantine systems should be clearly labeled with species, number of animals and quarantine status. Good record-keeping is essential and should include: (1) clear identifiers; (2) dates of arrival and significant events; (3) feeding ration and status; (4) behavior and changes to physical appearance; (5) water quality target parameters and results; (6) examinations, diagnostics, and treatments; (7) mortalities, gross necropsy and histology results, as well as any ancillary tests.

Minimum quarantine duration should be based on the time required for significant infections to manifest and be diagnosed, although this time is often unknown for elasmobranchs. Many institutions use the 30-day minimum recommended by the Association of Zoos and Aquariums (AZA), although some use up to 90 days for quarantine (AZA; Hadfield and Clayton, 2011). Longer quarantine periods may be warranted for recently wild-caught animals. Contingency plans for rare events (e.g., major life support system failure, power-outage, disease outbreak that significantly extends the quarantine period) should be in place before quarantine commences.

Animal identification

Possible external identifiers include physical markings, scars, animal size, sex, fin clips, and external tags (e.g., Floy, Peterson, or Rototags) (Kohler and Turner, 2001; Marshall and Pierce, 2012). Passive internal transponder (PIT) tags are routinely used. The AZA recommends intramuscular placement of PIT tags on the left hand side and typical tag sites are the epaxial muscle in sharks or pectoral fin in batoids. Multiple transponder types exist (e.g., ISO-conforming Duplex in Canada and Europe, Trovan and Avid in USA); a universal reader is useful where animals are coming in from other institutions.

Water quality

Water quality guidelines are provided in Table 1. It is helpful to test transport water (particularly temperature, pH, and dissolved oxygen) to document potential stressors in transit and to help determine acclimation needs. Freezing a liter sample of transport water can allow further testing in case of health issues following transport. Water testing frequency for quarantine systems will vary, based on the history of the system (particularly if systems are used intermittently), the biological filtration and bio-load, as well as any

Table 1. Recommended water quality parameters for elasmobranch quarantine systems showing suggested testing frequency and typical target values.

Parameter	Testing frequency	Typical target value	Comments
Temperature	Once to twice daily	Species-dependent	More frequent testing for smaller systems, particularly if different from ambient temperature
Dissolved oxygen	Daily	95-100%	Should also be monitored during and after any immersion treatment
pH	Daily to weekly	Species-dependent; marine = 7.8-8.4	
Salinity	Daily to weekly	Species-dependent; marine = 28-35 g/L	
Ammonia (unionized)	Daily to weekly	<0.10 mg/L	
Nitrites	Daily to weekly	<0.10 mg/L	
Nitrates	Prior to arrival	<20 mg/L	Rarely a concern in quarantine systems because of high water and animal turnover
Alkalinity	Prior to arrival	Species-dependent; marine > 200 mEq/L	
Hardness	Prior to arrival	Species-dependent; marine > 200 mEq/L	
Ionized copper	Prior to arrival	< 0.06 mg/L	
Ionized chlorine	Prior to arrival	0.0 mg/L	
Total gas pressures	Where indicated	< 102%	

observed concerns related to the animals. A complete cation panel may be evaluated periodically to ensure suitable levels. Life-support systems should be checked at least daily. Automated sensors for water level, temperature and/or dissolved oxygen can be helpful. Ultraviolet and ozone disinfection reduce the number of some water-borne pathogens. The goitrogenic effects of ozone use and high nitrates are unlikely to be an issue in short-term quarantine systems, but diets should include routine supplementation of iodide (Morris et al., 2012).

FOOD AND FEEDING

Establishing stable feeding patterns can be challenging, particularly for animals recently wild-caught. It is essential to know the diet and feeding methods for the species in the wild, as well as the feeding history of the animal(s) during any time post-capture. If an animal is showing a poor feeding response, it is important to review the husbandry, particularly temperature, water quality, water flow, number and types of refugia, social hierarchy if any, and feeding history (e.g., food types and sizes, food preparation techniques, frequency and timing of feeding, antecedents to feeding, and food presentation). Medical reasons for inappetence should also be considered. Various techniques may be used to encourage food intake in recalcitrant animals, including:

- Offering a wide variety of foods: fish, crustaceans, mollusks, and cephalopods;
- Offering fresh foods rather than frozen and thawed foods;

- Adding fish blood to the water prior to feeding;
- Crushing or cutting stripes into the food prior to feeding;
- Using a rod, grabber, or line to move the food in the water;
- Offering live foods that have cleared quarantine;
- Feeding at different times, ideally to match feeding times in the wild (many animals are crepuscular feeders) or at the previous institution;
- Using antecedent feeding practices employed at the previous institution;
- Avoiding potentially aversive stimuli (e.g., people leaning over the aquarium, loud noises, etc.); and,
- Isolating animals in suitable systems or parts of the system to reduce competition for food.

Subtle improvements in feed response (e.g., orienting towards, touching, or mouthing food) may be noted prior to the initiation of feeding. Many elasmobranchs chew their food, and close monitoring is needed to ensure they do not subsequently reject the chewed food item.

Various supplements and medications have been used to encourage feeding, including oral or injectable vitamin B complex, vitamin C, vitamin E, garlic, diazepam, mirtazapine, megestrol acetate, low-dose dexamethasone, dronabinol extract and levothyroxine. These supplements have ususally shown no effect.

Assisted feeding

Assisted feeding may be needed for animals that are not eating well. The decision to use assisted feeding should be based on the species, life stage, and condition of the animal. Body mass can be a useful decision-making tool, particularly where incoming mass is available for individually-identified animals. Body condition can also be used as a diagnostic tool. In general, epaxial muscles and the muscles over the pelvic girdle should be rounded and firm, and the coelomic contours should be flat to slightly convex. Ultrasound can be used to evaluate liver size and echogenicity. A well-fed animal should have a large, lipid-laden liver (Mylniczenko, 2012; Grant et al., 2013). When animals are in a negative energy balance, the liver becomes smaller and denser (Mylniczenko, 2012; Grant et al., 2013). Grant et al. (2013) used an ultrasound-guided technique to measure the distance between the caudal margin of the liver and the cranial margin of the pelvis, in combination with an external measurement of coelom length, to calculate a liver-to-coelom ratio. This technique may be useful for evaluating lipid stores over time. Some blood values may help to determine nutritional status. In the authors' experience has shown that urea and total proteins tend to decrease with inappetence, although fasting studies have shown a more variable response (Leech et al., 1979; Kajimura et al., 2008; Wood et al., 2010). Glucose and electrolytes are generally well regulated during prolonged fasting (Kajimura et al., 2008; Wood et al., 2010).

Assisted feeding typically involves gavage feeding with a blended slurry (e.g., Atlantic herring, *Clupea harengus* (Linnaeus, 1758), capelin, *Mallotus villosus* (Müller, 1776), and Atlantic mackerel, *Scomber scombrus* (Linnaeus, 1758), mixed with water or an electrolyte solution, and often with supplemental fish oils). Commercial products (e.g., Mazuri Shark/Ray Gel or Meal, Purina Feed, USA; Emeraid Piscivore diet, Lefeber's, USA; A/D Canine/Feline Critical Care, Hill's Pet Nutrition, USA; Tomlyn Nutri-Stat, Vetoquinol, USA) may be offered in combination or as an alternative. Various recipes have been used. Thomas (2005) reports a recipe that is practical and rich in calories.

Force-feeding inappetent animals with normal prey items may also be used. Food fishes are easier to force feed to inappetent animals than invertebrate prey items, particularly partially thawed larger fishes, such as Atlantic butterfish, *Peprilus triacanthus* (Peck, 1804), Atlantic bonito, *Sarda sarda* (Bloch, 1793), *C. harengus*, or *S. scombrus*. A good rule of thumb for a starting food ration, measured as a percentage of body mass (BM), is 2 - 4% BM per feeding. Frequency of assisted-feeding depends on the condition of the animal, as well as how often it is possible and practical to catch and restrain the animal. In general, juvenile (or small) stingrays and skates, require aggressive nutritional support, daily to every other day (Thomas, 2005). Fast-swimming rays and sharks also require frequent feeding. Larger stingrays can be fed twice weekly. Demersal and slow-swimming sharks and cold-water elasmobranch species can often be managed with gavage or force-feeding once every one to two weeks. In some cases, months of assisted feeding are required before an elasmobranch starts to show adequate food intake. Many elasmobranchs will start eating voluntarily while being assisted. Intermittent voluntary food intake should not exclude further assisted feeding, particularly in thin animals.

Gastritis and enteritis are common in elasmobranchs under significant stress, and ulcerative lesions have been observed in the stomach and proximal intestine (Garner, 2013). Theoretically, this phenomenon may be more common in elasmobranchs that secrete gastric acid continuously while fasting, i.e., in species that normally feed frequently (Papastamatiou and Lowe, 2005). Elasmobranchs may benefit from gastroprotectants, such as ranitidine or sucralfate, while on nutritional support.

BEHAVIORAL CONDITIONING

Behavior analysis investigates the universal laws of behavior change due to experience. People routinely underestimate the impact of experience on behavior, and instead use concepts of 'instinct' (e.g., "the species does that") or 'personality' (e.g., "that animal is like that"). Behavior analysis is critical for understanding how a specific behavior emitted by an individual animal is learnt and maintained through interactions with the environment (Friedman and Haug, 2010). Applied behavior analysis (ABA) takes the learning history of an individual animal and the current environmental conditions into account when investigating the purpose (i.e., function) that a behavior serves. The process of feedback modifying future behavior happens constantly (Friedman et al., 2006; Chance, 2009).

Behavior analysis and ABA are the foundation for positive reinforcement training–i.e., the deliberate process of increasing specific behavior. While positive reinforcement may be used to purposefully train behavior, it is functioning at all times, not just during 'training sessions'. Improved understanding of ABA allows staff to effectively and efficiently train desired behaviors, avoid training undesirable behaviors, and ameliorate problem behaviors. Introducing deliberate positive reinforcement training during quarantine helps develop basic husbandry-related behaviors–e.g., feeding from a pole, feeding at a specific target or swimming into a stretcher (Corwin, 2012; Schluessel and Bleckmann, 2012; Guttridge and Brown, 2014; Kimber et al., 2014). More advanced husbandry behaviors are also possible, such as stationing for voluntary blood samples or ultrasound imaging (Corwin, 2012). Animals that will be in 'touch exhibits' may benefit from positive reinforcement training for, and desensitization to, touching or handling.

In the authors' experience, staff instruction in ABA and positive reinforcement training techniques has improved their ability to manage animals with potentially conflicting behavioral needs and to train desired husbandry behaviors in a quarantine setting. Husbandry behaviors have been readily maintained, even in the face of significant environmental changes (e.g., new staff, animal movement to new systems). Animals with a foundation in trained husbandry behaviors have demonstrated a reduction in overt behaviors typically associated with stress.

COMMON PROBLEMS

Infectious Diseases

Monogeneans are common, particularly on the skin and gills. Parasite loads can increase rapidly in aquaria and monogeneans are often associated with significant morbidity and mortality (Cheung et al., 1982; Poynton et al., 1997; Bullard et al., 2001; Chisholm et al., 2004; Justine et al., 2010). Common monogoneans are the monopisthocotylean Monocotylidae (e.g., *Dendromonocotyle* spp.); Capsalidae (e.g., *Benedeniella* spp., *Entobdella* spp., and *Neoentobdella* spp.); Microbothriidae (e.g., *Dermophthirius* spp. and *Neodermophthirius* spp.); and the polyopisthocotylean Hexabothriidae (e.g., *Erpocotyle* spp. and *Heterocotyle* spp.) (Poynton et al., 1997; Bullard et al., 2000; Bullard et al., 2001; Benz and Bullard, 2004). Host specificity varies. Many monogeneans are species-specific in the wild but have wider host ranges in aquaria (Frasca et al., 2001; Benz and Bullard, 2004). Treatment typically involves praziquantel immersion, organophosphate immersion, or temperature or salinity changes (Thoney, 1990; Chisholm and Whittington, 2002; Benz and Bullard, 2004; Chisholm et al., 2004). Treatment plans must take into account the eggs (i.e., resistance to treatment, hatch period, etc.), host range, and host sensitivity to treatment (Chisholm and Whittington, 2002; Benz and Bullard, 2004; Chisholm et al., 2004).

Bacterial diseases, particularly sepsis, are also common and are often secondary to transport, trauma, or poor water quality (Garner, 2013). Common bacterial agents include *Vibrio* spp., *Photobacterium damselae*, *Aeromonas* spp., *Pseudomonas* spp., *Citrobacter* spp., *Flavobacterium* spp., *Streptococcus agalactiae*, and *Serratia marcescens* (Grimes et al., 1984; Grimes et al., 1985b; Briones et al., 1998; Borucinska and Frasca, 2002b; Bowater et al., 2012; Camus et al., 2013a). While some species, particularly *Vibrio* spp. and *Photobacterium* spp., can be au-

tochthonous flora, positive blood cultures in sick animals may be significant and warrant a response (Grimes et al., 1985a; Mylniczenko et al., 2007). Treatment with antibiotics should be based on bacterial culture and sensitivity results, where available. Chlamydiales have been associated with branchial epitheliocystis lesions, with subsequent morbidity and mortality (Borucinska and Frasca, 2002b; Polkinghorne et al., 2010; Camus et al., 2013b). Mycobacteriosis appears to be rare in elasmobranchs and has only been reported in long-term aquarium animals (Anderson et al., 2012a; Janse and Kik, 2012; Clarke et al., 2013; Garner, 2013).

Nematodes are common in animals that were wild-caught or fed fresh food (Benz and Bullard, 2004). Adults, larvae, and ova may be found within the gastrointestinal tract, but can also be found in cysts, granulomas, or blood vessels in any tissue (Benz and Bullard, 2004; MacLean et al., 2006; Bullard et al., 2012; Borucinska and Adams, 2013; Garner, 2013). Nematodes are often commensal, but larval nematodes from the orders Spirurida and Dracunculoidea have been linked to vasculitis, branchitis, pancreatitis, meningoencephalitis, oophoritis, and metritis (Credille et al., 1993; Borucinska and Frasca, 2002a; Borucinska and Adams, 2013; Garner, 2013; Hadfield et al., 2013). If nematodes are found, the decision to treat is not straightforward due to the low safety index of common dewormers (Myers et al., 2007; Hadfield et al., 2013; Mylniczenko, personal communication).

Copepods from the orders Siphonostomatoida and Poecilostomatoida are common external parasites, found primarily around the head and in the buccal and branchial cavities, and occasionally on the fins, cloaca, and claspers of elasmobranchs (Benz and Bullard, 2004). They can be associated with morbidity and mortality, but are principally an aesthetic issue in exhibits (Kik et al., 2011). Treatment may involve physical removal, organophosphates, or chitin inhibitors (Kik et al., 2011). The life cycle of some copepods can be broken in simple quarantine systems and this may be sufficient for management. Branchiura, namely *Argulus* spp., are reported in dasyatid and potamotrygonid rays (Caira and Healy, 2004; Mylniczenko, 2012). Treatment may involve physical removal, organophosphates, or chitin inhibitors.

Leeches from the family Piscicolidae are rare but can be potentially serious pathogens. They are often hidden in the buccal and branchial cavities and have potentially wide host ranges. Leeches can cause severe morbidity and mortality in naïve animals, if the parasites are transferred out of quarantine (Dove and Clauss, 2008; Marancik et al., 2012; Yamauchi and Ota, 2012). Treatment may involve physical removal, organophosphates or chitin inhibitors.

Fungal infections may be seen in quarantine, but are typically associated with individual animals. Dermatitis due to *Fusarium solani* is the most commonly reported fungal disease (Muhvich et al., 1989; Smith et al., 1989; Crow et al., 1995, Boylan et al., 2014). *Exophiala pisciphila*, *Mucor circinelloides*, *Paecilomyces lilacinus* and water molds (fungus-like microorganisms of the class Oomycota) have also been reported (Marancik et al., 2011; Mylniczenko, 2012). Fungal diseases are often secondary to transport or trauma, and morbidity and mortality may be seen within days to weeks of animal acquisition (Crow et al., 1995; Marancik et al., 2011). Early and prolonged treatment with voriconazole is recommended, but treatment is often unrewarding (Davis et al., 2007).

Coccidia in elasmobranchs is most commonly from the genus *Eimeria*. The most problematic species remains *Eimeria southwelli* in the coelom and gastrointestinal tract of myliobatid rays (Stamper and Lewbart, 1998). These parasites can be commensal, but are capable of causing significant morbidity and mortality secondary to stress, and should be avoided in collections where possible. Treatment may include sulfadimethoxine, clindamycin, toltrazuril, or ponazuril, but elimination of the parasite is difficult.

Protozoal diseases are less common in elasmobranchs than in bony fishes, but they can be significant pathogens (Hadfield, 2012). Invasive Scuticociliatida-like (*Uronema*-like) ciliates have been associated with morbidity and mortality. Protozoan parasites have been reported in the skin, gills, brain, and liver of skates and demersal sharks (Stidworthy et al., 2011; Garner, 2013). *Amyloodinium*-like dinoflagellates have been associated with morbidity and acute mortality during the quarantine of sharks maintained with bony fishes (Boylan et al., 2014; Tuttle, personal communication). Other ciliates and dinoflagellates have been identified in elasmobranchs (Borucinska and Frasca, 2002b; Garner, 2013).

Cestodes, myxozoa, and microsporidia may be seen in quarantine and are generally considered incidental. Cestodes from the orders Tetraphyllidea,

Diphyllidea, Lecanicephalidea, and Trypanorhyncha often reside within the spiral valve and may be noted in fecal samples from animals that were recently wild-caught or fed fresh fish (Borucinska and Frasca, 2002b). Cestodes are not usually considered a significant concern in healthy elasmobranchs (Benz and Bullard, 2004). Myxozoa are most commonly seen within the biliary tract and are considered incidental (Borucinska and Frasca, 2002b; Benz and Bullard, 2004; Garner, 2013). Microsporidia may be found in any tissues and are typically incidental, although morbidity and mortality have been reported (Diamant et al., 2010; Garner, 2013).

Non-infectious Diseases

Traumatic lesions are common, in particular bite wounds and abrasions. Bite wounds may be associated with feeding, breeding, territorial behavior or inappropriate stocking density, and are typically on the caudal edge of the pectoral fins in rays and at the base or tip of the tail in skates. Abrasions in rays may develop on the eyes, ventrum, tail tip or the leading edges of the pectoral fins. Abrasions in pelagic sharks are particularly common on the rostrum. Closed tail fractures may be seen in skates and rays, and usually heal uneventfully. Traumatic lesions are primarily managed by correcting environmental issues. Correction may involve increased feeding, substrate modifications, addition or removal of physical or sensory barriers (e.g., bubble curtains), changes to water flow, adjustment to lighting conditions or moving animals. Oral or injectable antibiotics, antifungals, anti-inflammatories, supplemental oral vitamin C and/or surgical correction may be used for treatment.

Trauma from animal capture (e.g., from hook and line fishing) may need to be addressed, particularly in species that swallow prey whole. Swallowed hooks are typically found within the distal esophagus or gastric wall and may perforate the liver or pericardium; hooks are less commonly found within the spiral valve (Borucinska and Frasca, 2002b; Borucinska et al., 2002; Lucifora et al., 2009; Lecu et al., 2011). Species that cut their prey into pieces before ingestion are more likely to have hooks in the buccal or branchial cavities (Lucifora et al., 2009). Endoscopic retrieval or surgery may be indicated for the removal of hook foreign bodies.

Other non-infectious diseases reported in quarantine are often associated with life-support problems, such as hypoxia, supersaturation, loss of water, or exposure to ammonia, chlorine, chloramine or copper. These risks are mostly preventable with good planning, system maintenance and monitoring.

MONITORING AND DIAGNOSTIC TESTING

Regular visual examinations are essential during quarantine and should be carried out at least twice daily. Important factors to monitor include:

- Changes in behavior, posture or swimming pattern (e.g., erratic movements, ‘flashing’, rubbing, pelagic species resting on the substrate or demersal species swimming constantly);
- Aggression or displacement;
- Changes in the respiratory pattern (e.g., increased effort or rate);
- Skin lesions (e.g., hyperemia, vesicles, erosions, ulcerations, focal discoloration, pallor, visible parasites);
- Ocular lesions (e.g., discoloration, visible parasites); and
- Changes in the feeding response or body condition.

Life-support systems and water flow should also be checked twice daily at the time of visual examinations.

Hands-on physical examinations are commonly performed on elasmobranchs in quarantine (Hadfield and Clayton, 2011). These procedures may be under chemical or manual restraint, depending on species, staff safety and experience, and risks to the animal (particularly the risk of trauma and capture myopathy) (Henningsen, 1994; Mylniczenko et al., 2003; Mylniczenko, 2012). In some cases, hands-on examinations may be limited to a subset of animals within a population. Hands-on examinations may be avoided in species that are typically unproblematic for a given institution, animals that will remain isolated from the established collection or where the risks associated with handling are considered too high.

During physical examinations, bright illumination helps with visualization of the buccal cavity, olfactory sacs, spiracles, cloaca and claspers. An endoscopic or laryngoscopic examination of the buccal and branchial cavities can help identify parasites and may be useful when assessing wild-caught animals. Sexual maturity and body condition should be determined. Morphometrics, such

as body mass (BM), total length (TL), precaudal length, snout-to-vent length and/or disc width (DW), should be taken. Markings used as identifiers may be photographed and tags may be placed.

Visible parasites should be removed using forceps or scrapes and examined under microscopy. Images should be saved for future reference. Parasites can be fixed (e.g., in 95% ethyl alcohol) if further identification is needed (Benz and Bullard, 2004). Gill biopsies are less commonly performed in elasmobranchs than in bony fishes because of a potential for hemorrhage and the lower diagnostic value (Benz and Bullard, 2004; Hadfield and Clayton, 2011). Gill biopsies may be primarily carried out in wild-caught specimens. It is recommended to perform the gill biopsy under direct visualization (e.g., with an endoscope, otoscope or laryngoscope), but it can be done blindly. Biopsy forceps help with the collection of small gill samples.

Fecal samples are sometimes collected to identify gastrointestinal parasites such as helminths and coccidia (Hadfield and Clayton, 2011). Fecal samples may be collected from the water or by cloacal wash, typically for direct analysis and flotation. Coelomic aspirates or flushes are recommended in cownose rays, *Rhinoptera bonasus* (Mitchill, 1815), to look for coccidia (Stamper and Lewbart, 1998).

Blood can be collected for hematology, biochemistry, blood gases, lactate and ancillary tests (e.g., macronutrient, vitamin, or hormone assessment). Skin preparation prior to phlebotomy may involve a flush with sterile saline, or a single swipe of a swab with 70% alcohol; elasmobranch skin is extremely sensitive to more rigorous disinfection (Mylniczenko et al., 2007). Phlebotomy sites include the ventral tail vein, posterior cardinal sinuses, and wing or radial veins (Alexander, 1991). Venipuncture site affects the packed cell volume. The ventral tail vein appears less affected by the secondary vascular system than the posterior cardinal sinus (Mylniczenko et al., 2006). It is likely that white blood cell count is also affected by collection site. Dry heparin is the preferred anticoagulant for most hematology and biochemistry tests (Hahn et al., 2011). Blood gases, electrolytes, and lactate are useful point-of-care tests for animals that are ill (Gallagher et al., 2010; Hyatt et al., 2012; Naples et al., 2012).

The modified Natt-Herrick technique is used for the manual white blood cell count. The count must be carried out within a few hours of sampling to prevent sample degradation, which may negatively impact results, although the cells may be preserved in formalin for later evaluation (Arnold, 2005; Arnold et al., 2014). Granulocyte nomenclature and descriptions vary, but the authors recommend the descriptive nomenclature reported in Arnold (2005).

In elasmobranchs, total dissolved solids are approximately twice the total proteins. Sodium, chloride, urea and osmolality are high in marine-adapted elasmobranchs, and their serum or plasma may require dilution, depending on the linear range of the blood chemistry analyzer. Blood cultures are not taken routinely on healthy fishes in quarantine (Grimes et al., 1985a; Mylniczenko et al., 2007). Interpretation of standalone blood results is limited, so there is value in collecting samples to provide comparisons in individual animals and cohorts over time. A summary of blood values reported in the literature is provided in electronic form at the elasomobranch husbandry web site (www1).

Ultrasonography can be used to assess liver size and echogenicity to help evaluate nutritional status. Free fluid in the coelom may be indicative of inappropriate salinity, inanition, coelomitis, or bacterial or parasitic infection. Ultrasound examination of the buccal and branchial cavities may show larger parasites. Ultrasound examination of the female reproductive tract is essential to check gestational status, identify any pathology, and to obtain baseline images of the ovaries and oviducts (Carrier et al., 2003; Daly et al., 2007). Radiography allows the identification of hook foreign bodies and has been recommended to evaluate the vertebral spine in sand tiger sharks, *Carcharias taurus* (Rafinesque, 1810) (Preziosi et al., 2006; Lecu et al., 2011).

It is important that any animal that dies receives a full necropsy with squash preps and cytology and, where possible, cultures (often aerobic and fungal) and histopathology by a pathologist familiar with elasmobranch tissues (Crow, 2004). Tissue samples can be frozen for subsequent molecular testing. Necropsies should be done as soon as possible, but even if severely autolyzed a gross necropsy can still shed light on organ involvement and trauma.

COMMON ANTIPARASITIC TREATMENTS

The following is a brief overview of common prophylactic and reactive treatments used during elasmobranch quarantine. The pharmacology

chapter (Mylniczenko and Clauss, this volume) covers treatments in more detail.

Before using therapeutics that are novel to the institution or the species, the authors recommend a thorough review of the literature, discussion with colleagues and biotesting on a subset of a group. Immersion treatments, in particular, can have unpredictable effects on biological filtration systems and dissolved oxygen concentrations, as well as the animals, and must follow relevant legislation on use and disposal.

The most commonly reported prophylactic treatment in elasmobranchs is the use of repeated praziquantel baths for monogeneans (Chisholm and Whittington, 2002; Hadfield and Clayton, 2011). Manual dissolution of praziquantel is more common than chemical dissolution, because of the potential effects of ethyl alcohol on biofilter bacterial loads and dissolved oxygen. Regardless of dissolution method, dissolved oxygen should be monitored during and after immersion treatment. Where possible, praziquantel levels in the water should be monitored because the drug is rapidly degraded in some systems (Innis, 2012). Praziquantel testing is available at the Georgia Aquarium Veterinary Services Water Quality Laboratory (Atlanta, Georgia, USA).

Salinity changes may be used for routine parasite control. Stenohaline and pelagic species can be intolerant of salinity changes, but may handle slow decreases to not less than 20 g/L. Some marine species can tolerate freshwater dips (where the water is matched to temperature and pH, with normal to high levels of dissolved oxygen), but adverse effects have been reported (Cheung et al., 1982; Chisholm and Whittington, 2002; Chisholm et al., 2004; Justine et al., 2010).

Organophosphates, particularly trichlorfon, are used prophylactically in some institutions to control monogeneans, copepods and leeches (Hadfield and Clayton, 2011). Possible side effects include skin changes, inappetence and mortalities (Thoney, 1990). Species that appear to be particularly sensitive to organophosphates include eagle rays (*Aetobatus* spp.) and guitarfish (*Rhinobatos* spp. and *Rhina* spp.). Animals may be medicated with atropine prior to treatment to reduce the risk of adverse side effects. Appropriate human personal protective equipment (PPE) is required when using organophosphates. Chitin inhibitors, particularly lufenuron or diflubenzuron, may be used for copepods and leeches (Kik et al., 2011).

Most institutions avoid the use of copper during elasmobranch quarantine due to concerns over toxicity (Grosell et al., 2003; Hadfield and Clayton, 2011). Long-term immersion of copper sulfate has, however, been used successfully in lemon sharks, *Negaprion brevirostris* (Poey, 1868), infected with the monogenean *Neodermophthirius harkemai*, and *R. bonasus* infected with the monogenean *Benedeniella posterocolpa* (Poynton et al., 1997; George, personal communication).

Fenbendazole use has been reported, but subsequent mortalities have been seen, in zebra sharks, *Stegostoma fasciatum* (Hermann, 1783), yellow stingrays, *Urobatis jamaicensis* (Cuvier, 1816), *C. taurus*, *R. bonasus* and freshwater stingrays, *Potamotrygon* spp. (Myers et al., 2007; Hadfield and Clayton, 2011; Mylniczenko, personal communication). Side effects may be more common when the medication is gavage fed, potentially because no medication is lost on dosing (Mylniczenko, personal communication). Pyrantel and febantel were associated with the death of whitetip reef sharks, *Triaenodon obesus* (Ruppell, 1837), in quarantine, from the inflammatory response associated with the death of histozoic nematodes (Hadfield et al., 2013). Nematode treatments should be carried out with caution.

REVIEW BY TAXONOMIC DIVISIONS

Odontaspididae

C. taurus (sand tiger shark) are generally a hardy species. Animals collected in the western Atlantic are commonly infected with copepods, particularly around the distal fin edges and mouth (e.g., *Anthosoma crissum*, which can be associated with deep gingival erosions and tooth loss) (Benz and Bullard, 2004). Therapeutics may be considered, but the authors have seen clearance of three copepod species after 14 weeks of quarantine with no recurrence in three years, so the parasite life cycle may be broken in simple quarantine systems. Juvenile porkfish, *Anisotremus virginicus* (Linneaus, 1758), which have cleared quarantine, can be used as “cleaner fish” to remove copepods from *C. taurus* (George, personal communication). Heavy loads of *Amyloodinium*-like dinoflagellates on the gills were associated with acute mortalities in *C. taurus* during quarantine. Treatment with chloroquine immersion resolved the infection (Tuttle, personal communication). Two possible viral diseases have been noted in *C. taurus* during quarantine: diffuse, crusting lesions on the skin that resolved over approximately one month, and black, raised, polyploid lesions

that expanded and coalesced forming mucinous gray lesions that resolved over the course of a year (George, personal communication). Lesion recurrence was not seen (George, personal communication). Other infectious diseases appear to be rare during quarantine of *C. taurus*. Inflammation of the ampullae of Lorenzini has been observed in *C. taurus*, but the etiology is unknown (Mylniczenko, personal communication).

Scoliosis has been reported in several collections of *C. taurus*. Predisposing factors may include pound net capture of small animals and insufficient space for normal glide distances in systems; it is critical that the size and shape of quarantine aquaria allow for normal swimming behavior (Anderson et al., 2012b). Radiographs of the spine, cranial to the dorsal fin, should be considered, both to identify affected animals and to provide baseline images (Preziosi et al., 2006; Anderson et al., 2012b). If caught by hook and line, *C. taurus* may show perforation or laceration of the esophagus, stomach, liver, or pericardium, and radiographs should be considered to check for hook foreign bodies (Lucifora et al., 2009). Ultrasonography of the coelom is often limited in *C. taurus* due to air swallowing by the species. It is important to monitor animals after handling, as it may be necessary to decompress or insufflate the stomach. Gastric prolapse through the mouth or gill slit has been reported in *C. taurus*, but has not been seen in quarantine (Tuttle et al., 2008; Tuttle, personal communication).

Carcharhinidae

Sandbar sharks, *Carcharhinus plumbeus* (Nardo, 1827), also called brown sharks, are generally hardy. Wild-caught *C. plumbeus* from the western Atlantic may be infected with copepods (e.g., *Alebion* spp. and *Pandarus* spp.) (Benz and Bullard, 2004). The eggs of the nematode *Huffmanela carcharhini* create characteristic black tracks in the skin of *C. plumbeus* (MacLean et al., 2006; Bullard et al., 2012). Clinical signs have been noted in wild *C. plumbeus* and in an aquarium specimen six months after collection (MacLean et al., 2006; Bullard et al., 2012). Lesions have been cleared successfully using levamisole (MacLean et al., 2006). Clinical signs are not limited to *Carcharhinus* spp. *Huffmanella markgracei* has recently been reported causing characteristic black tracks within the buccal cavity of a wild-caught Atlantic sharpnose shark, *Rhizoprionodon terraenovae* (Richardson, 1836) (Ruiz and Bullard, 2013). *C. plumbeus* are particularly prone to rostral and pectoral fin trauma. The size and shape of quarantine aquaria must be sufficient to accommodate the glide portion of their normal swimming behavior. Self-limiting prolapses of the cloaca and valvular intestine have been seen in quarantine in carcharhinids (Crow et al., 1990; Boylan, personal communication). Hematology values have been reported for *C. plumbeus* in aquaria (Arnold, 2005).

Blacktip reef sharks, *Carcharhinus melanopterus* (Quoy & Gaimard, 1824), are considered sensitive to environmental stressors. However, if transport is uneventful and further stressors are avoided, *C. melanopterus* typically do well in quarantine. *C. melanopterus* may be infected with *Dermophthirius melanopteri* or *D. penneri* monogeneans causing focal dermatitis (Bullard et al., 2000; Frasca et al. 2001; Benz and Bullard, 2004). Prolapses of the cloaca and valvular intestine have been observed shortly after acquisition in wild-caught juvenile and adult *C. melanopterus* (Crow et al., 1991). Mortalities associated with conspecifics biting prolapsed tissue were observed 8 - 173 days following the arrival of the sharks (Crow et al., 1991). *C. melanopterus* are prone to skin erosions. The size and shape of quarantine systems must be appropriate to accommodate swimming behavior.

Blacknose sharks, *Carcharhinus acronotus* (Poey, 1860), have shown a high prevalence of intravascular nematodiasis, associated with vascular occlusion, necrosis, and inflammation in the gills and brain (Garner, 2013).

N. brevirostris (lemon sharks) are generally a hardy species of shark. *N. brevirostris* commonly harbor monogeneans, particularly *Dermophthirius nigrellii* and *Neodermophthirius harkemai* on the gills and skin (Cheung et al., 1982; Poynton et al., 1997). Signs of *Dermophthirius* spp. include rubbing, erratic swimming, gray plaques and ulcers (Cheung et al., 1982). The most effective treatment for *Dermophthirius* spp. was trichlorfon immersion (Cheung et al., 1982). Signs of *N. harkemai* infection include rubbing, dark bands of hemorrhage around the mouth and increased mucus production particularly around the head (Poynton et al., 1997). The most effective treatment for *N. harkemai* was isolation and copper sulfate immersion, but when animals were returned to their exhibit the parasites and lesions recurred (Poynton et al., 1997). *N. brevirostris* tend to be more aggressive than other commonly displayed carcharhinid sharks.

Wild-caught *T. obesus* (whitetip reef sharks) may carry the copepod *Paralebion elongatus* (Benz,

1992). *T. obesus* have shown morbidity from gill nematodes while in quarantine, with a loss of all five sharks in the five months following treatment with pyrantel and febantel, due to a severe inflammatory response to the death of the nematodes (Hadfield et al., 2013). *T. obesus* are pack hunters and conspecific aggression has been reported (Tuttle, personal communication).

Triakidae

Leopard sharks, *Triakis semifasciata* (Girard, 1855), may be aquarium-bred and generally present few problems in quarantine. Common parasites in wild-caught *T. semifasciata* include *Erpocotyle* spp. monogeneans on the gills and copepods on the skin (Benz and Bullard, 2004). Morbidity and mortality have been reported from microsporidial parasites in captive *T. semifasciata* (Garner, 2013). Chlamydiales have been associated with branchial hyperplasia in a *T. semifasciata* that died during quarantine (Polkinghorne et al., 2010). Focal areas of depigmentation have been seen in *T. semifasciata* during quarantine, but the lesions resolved over time (Murray, personal communication). Papillomaviruses were identified in similar lesions (Garner, 2013; Murray, personal communication). Conspecific aggression, particularly associated with breeding, may be noted in *T. semifasciata* during quarantine if maintained in groups (Tuttle, personal communication). "Spyhopping" behavior has been observed in *T. semifasciata* in smaller quarantine systems (Murray, personal communication). The banded houndshark, *Triakis scyllium* (Muller & Henle, 1839), is closely related to *T. semifasciata* and is commonly displayed in China and Japan (AES, 2008).

Dusky smooth-hound, *Mustelus canis* (Mitchill, 1815), also called smooth dogfish, have variable survival rates in quarantine. Some individuals do well from the start, while others do not adapt well. An iridovirus that causes intravascular hemolysis (viral erythrocytic necrosis) has been identified in wild and captive *M. canis*, as well as *T. semifasciata*, and was associated with mortalities in younger animals (Leibovitz and Lebouitz, 1985; Garner, 2013). A herpes virus that causes ulcerative dermatitis has been isolated from *M. canis*; the lesions resolved over time (Leibovitz and Lebouitz, 1985). An adenovirus was associated with ulcerative dermatitis, epithelial and gill hyperplasia, and ultimately mortalities, in seven juvenile wild-caught *M. canis* during a three-month quarantine (Bowman et al., 2008). Intralesional ciliates and septicemia were also observed and appear to be common in *M. canis* (Bowman et al., 2008; Garner, 2013). A high prevalence of biliary myxosporeosis and pancreatic nematodes (*Pancreatonema americanum*) were identified in *M. canis* wild-caught in the northwestern Atlantic (Borucinska and Frasca, 2002b). *M. canis* are prone to traumatic lesions from tank wall abrasions. Quarantine system design should accommodate the swimming behavior of the species. Hematology and biochemistry values have been reported for captive *M. canis* (Persky et al., 2012).

Scyliorhinidae

The most commonly displayed species of scyliorhinid is the lesser spotted dogfish, *Scyliorhinus canicula* (Linnaeus, 1758); other common species are the chain catshark, *Scyliorhinus retifer* (Garman, 1881), nursehound, *Scyliorhinus stellaris* (Linnaeus, 1758), cloudy catshark, *Scyliorhinus torazame* (Tanaka, 1908), and swell shark, *Cephaloscyllium ventriosum* (Garman, 1880) (AES, 2008). *S. canicula* are often aquarium-bred and are generally hardy. Invasive Scuticociliatida-like ciliates have been found on the skin, gills, brain and liver of *C. ventriosum* (Garner, 2013). *C. ventriosum* may take several hours to deflate their stomachs after handling. It is helpful to keep the sharks submerged at all times during physical exams so that water is swallowed rather than air.

Sphyrnidae

Bonnethead sharks, *Sphyrna tibur*o (Linnaeus, 1758), especially juveniles, are often difficult to maintain. *S. tiburo* are prone to *Fusarium solani* dermatopathy (Muhvich et al., 1989; Smith et al., 1989; Crow et al., 1995, Boylan et al., 2014), suboptimal environmental temperatures and trauma increased the risk. Clinical signs include white pustules or ulcers along the lateral line system and cephalofoil. The lesions are progressive, with local and sometimes systemic invasion. The disease is typically fatal, but there is one report of resolution in an adult female using voriconazole and temperature modification (Davis et al., 2007). *Erpocotyle tiburonis* monogeneans are common on the gills of *S. tiburo* (Frasca et al., 2001; Benz and Bullard, 2004; Boylan et al., 2014). In one report, proliferative gill lesions and higher parasite loads were observed, with mortalities seen within 28 - 156 days of arrival at the aquarium (Bullard et al., 2001). *Huffmanella* spp. nematodes have caused characteristic black tracks in the skin of infected *S. tiburo* (Dove, personal communication). *S. tiburo* are often inappetent post-transport and emaciation is a common postmortem finding (Garner, 2013). Aggressive nutritional support is recommended for *S.*

tiburo until they are eating reliably. *S. tiburo* are sensitive to environmental stressors such as poor water quality, small changes in salinity or pH, and suboptimal housing. Mortalities have been observed a few weeks into quarantine, with no previous clinical signs or apparent stressors and no gross or histologic lesions (Clauss, personal communication). Similar mortalities have also been observed in scalloped hammerheads, *Sphyrna lewini* (Griffith & Smith, 1834), a few weeks into quarantine (Jones, personal communication). *S. tiburo* are prone to cephalofoil and ocular trauma during shipping or quarantine, as well as bite wounds from tank mates, and should be quarantined in isolation or in small groups. Hematology, biochemistry and some nutrient and vitamin analyses have been reported for wild *S. tiburo* (Harms et al., 2002; Haman et al., 2012).

Heterodontidae

Horn sharks, *Heterodontus francisci* (Girard, 1855), are often aquarium-bred and are generally hardy. Invasive Scuticociliatida-like ciliates have been associated with severe inflammation in the skin, gills, brain and liver of *H. francisci*, Japanese bullhead sharks, *Heterodontus japonicus* (Miklouho-Maclay & Macleay, 1884), and Port Jackson sharks, *Heterodontus portusjacksoni* (Meyer, 1793) (Stidworthy et al., 2011; Garner, 2013). Clinical signs included lethargy, inappetence and acute mortality (Stidworthy et al., 2011). Breeding aggression may be noted from male heterodontids if maintained in groups during quarantine. Refugia or "hides" are necessary for heterodontids, but tight spaces have been associated with entrapment and severe abrasions (Murray, personal communication). *H. francisci* have prominent spines on the dorsal fins, which are a potential hazard during handling.

Ginglymostomatidae

Nurse sharks, *Ginglymostoma cirratum* (Bonnaterre, 1788), are hardy and rarely have issues during quarantine. Heavy loads of *Amyloodinium*-like dinoflagellates on the gills have been associated with acute mortalities in *G. cirratum* during quarantine; chloroquine immersion treatment resolved the signs (Tuttle, personal communication).

Hemiscylliidae

Epaulette sharks, *Hemiscyllium ocellatum* (Bonnaterre, 1788), whitespotted bamboosharks, *Chiloscyllium plagiosum* (Bennett, 1830), and brownbanded bamboosharks, *Chiloscyllium punctatum*, (Muller & Henle, 1838), are hardy and rarely have issues in quarantine. *H. ocellatum* and *C. plagiosum* are routinely bred in aquaria, while *C. punctatum* are less commonly bred. *H. ocellatum* wild-caught on the Great Barrier Reef have shown a 100% prevalence of gnathiid isopod larvae on the skin, particularly around the cloaca and gills, but no morbidity was observed despite heavy loads (Heupel and Bennett, 1999; McKiernan et al., 2005). *H. ocellatum* and *C. punctatum* have evolved to tolerate low dissolved oxygen concentrations (Wise et al., 1998; Chapman and Renshaw, 2009; Chapman et al., 2011) and are relatively tolerant of freshwater dips.

Stegostomatidae

S. fasciatum are often aquarium bred and unproblematic in quarantine. A wild-caught *S. fasciatum*, following an abbreviated quarantine, was considered the source of *Lepeophtheirus acutus* copepods identified in *S. fasciatum*, *T. obesus*, and a giant shovelnose ray, *Glaucostegus typus* (Bennett, 1830) (Kik et al., 2011). The parasites were typically on, or around, the eyes and mouth, and were associated with flashing and severe ulcerative keratitis. Trichlorfon and dilflubenzuron immersion were effective treatments for the parasites (Kik et al., 2011). Invasive Scuticociliatida-like ciliates were associated with necrotizing vasculitis and meningoencephalitis in juvenile *S. fasciatum* (Stidworthy et al., 2011; Garner, 2013). The ciliates were identified by PCR as *Philasterides dicentrarchi* (syn. *Miamiensis avidus*) (Stidworthy et al., 2011).

Orectolobidae

Commonly displayed orectolobids include the spotted wobbegong, *Orectolobus maculatus* (Bonnaterre, 1788), Japanese wobbegong, *Orectolobus japonicus* (Regan, 1906), and ornate wobbegong, *Orectolobus ornatus* (De Vis, 1883) (AES, 2008). A review of elasmobranch histopathology showed that *Orectolobus* spp. had a particularly high prevalence of septicemia (Garner, 2013). Orectolobids require refugia or "hides" and a complex substrate to reduce stress. In addition, orectolobids can be difficult to transfer from a live to frozen food diet, and they tend to prey on other exhibit fishes. Wild-caught female orectolobids are often gravid and ultrasound of the reproductive tract should be part of the quarantine exam. Leukocyte morphology and biochemistry values have been reported for wild orectolobids (Old and Huveneers, 2006; Otway et al., 2011). Orectolobids can be difficult to manually restrain as they have a strong rolling response.

Squalidae

Spiny or piked dogfish, *Squalus acanthias* (Linnaeus, 1758), have shown variable success in quarantine. *S. acanthias*, wild-caught in the northwestern Atlantic, showed a high prevalence of biliary myxosporeosis and pancreatic nematodes (*Pancreatonema americanum*) with histologic signs of chronic pancreatitis (Borucinska and Frasca, 2002a; Borucinska and Frasca, 2002b). These sharks also showed a high prevalence of mononuclear inflammation of the ampullae of Lorenzini (Borucinska and Frasca, 2002b). *S. acanthias* are prone to traumatic lesions from tank wall abrasions and quarantine systems should be designed to accommodate the swimming behavior of the species. Hematology, biochemistry, and some nutrient and vitamin analyses results have been reported for wild *S. acanthias* (Haman et al., 2012).

Rhinidae and Rhinobatidae

The bowmouth guitarfish, *Rhina ancylostoma* (Bloch & Schneider, 1801), is hardy, whereas other common species, such as the Atlantic guitarfish, *Rhinobatos lentiginosus* (Garman, 1880), common guitarfish, *Rhinobatos rhinobatos* (Linnaeus, 1758), and shovelnose guitarfish, *Rhinobatos productus* (Ayres, 1854), can be more problematic in quarantine. Leeches (e.g., *Pontobdella* spp.) are often found on wild-caught rhinobatids and can be present on animals that come from other aquarium collections (Murray, personal communication). Observed parasite loads have been low and mechanical removal is often sufficient (Murray, personal communication). Inappetence is common in rhinobatids during quarantine and can sometimes be resolved by offering uncommon food items such as lobster or crab (Clauss, personal communication; Mylniczenko, personal communication). Traumatic lesions are common following collection and shipping of rhinobatids, particularly ulcerative lesions on the plates and eyes (Clauss, personal communication; Mylniczenko, personal communication). Quarantine systems for rhinobatids need to be large, with smooth walls and soft substrate.

Rajidae

Common Pacific species of rajids in aquaria include big skates, *Raja binoculata* (Girard, 1855), and longnose skates, *Raja rhina* (Jordan & Gilbert, 1880). Common Atlantic species of rajids include clearnose skates, *Raja eglanteria* (Bosc, 1800), thornback skates, *Raja clavata* (Linnaeus, 1758), and little skates, *Leucoraja erinacea* (Mitchill, 1825) (AES, 2008). Some species breed well in aquaria. Rajids are prone to skin lesions, particularly on the tail, claspers, or rostrum (Innis, personal communication; Murray, personal communication; Tuttle, personal communication). These lesions can result from unsuitable tank design or intraspecific aggression, and may be infected with filamentous bacteria (*Tenacibaculum*-like), *Fusarium* spp., and Scuticociliatida-like parasites (Haulena, personal communication). In the authors' experience, lesions developed 5 - 8 weeks into quarantine. The filamentous bacteria were successfully treated with trimethoprim and sulfadiazine immersion, but *Fusarium* spp. and Scuticociliatida-like parasites were invariably fatal once lesions developed. A sand or fine gravel substrate in quarantine may help reduce stress in rajids. The small charge produced by the electric organs of *L. erinacea* and *R. clavata* cannot be felt by humans (Morson and Morrissey, 2007).

Dasyatidae

Commonly displayed species of dasyatid include southern stingrays, *Dasyatis americana* (Hildebrand & Schroeder, 1928), roughtail stingrays, *Dasyatis centroura* (Mitchill, 1815), common stingrays, *Dasyatis pastinaca* (Linnaeus, 1758), Atlantic stingrays, *Dasyatis sabina* (Lesueur, 1824), and pelagic stingrays, *Pteroplatytrygon violacea* (Bonaparte, 1832) (AES, 2008). These species are hardy and *D. americana* and *D. sabina* are commonly bred in aquaria. Less common dasyatids include bluespotted stingrays, *Neotrygon kuhlii* (Muller & Henle, 1841), ribbontail stingrays, *Taeniura lymma* (Forsskal, 1775), round ribbontail rays, *Taeniurops meyeni* (Muller & Henle, 1841), and honeycomb stingrays, *Himantura uarnak* (Gmelin, 1789). These species of dasyatid are typically more difficult to maintain in aquaria.

Dasyatids commonly carry *Monocotyle*, *Dendromonocotyle*, *Entobdella* spp., and *Neoentobdella* spp. monogeneans (Chisholm et al., 2001; Benz and Bullard, 2004; Chisholm and Whittington, 2004; Chisholm et al., 2004; Pulido-Flores and Monks, 2005; Whittington and Kearn, 2009). There is a report of heavy loads of *Neoentobdella taiwanensis* and *Dendromonocotyle pipinna* in *T. meyeni* wild-caught off New Caledonia, seen 24 days after arrival. A freshwater dip removed ~2,000 of the monogeneans, although no follow-up information was provided (Justine et al., 2010). *D. americana* and *D. sabina* appear relatively tolerant of freshwater dips. *N. kuhlii* may present with leeches (Boylan, personal communication). *D. pastinaca* in the Mediterranean have

shown morbidity and mortality from microsporidial parasites (Diamant et al., 2010). In a review of elasmobranch histopathology *D. americana* and *N. kuhlii* showed a high prevalence for septicemia (Garner, 2013).

Non-infectious diseases of dasyatids include conspecific aggression, often related to breeding behavior or dominance. It is useful to have facilities available to isolate animals if needed. Ultrasound of the female reproductive tract is indicated for dasyatids, as these species are often gravid and can present with pathology of the reproductive tract in quarantine (Mylniczenko, personal communication). *N. kuhlii* and *T. lymma* may come into an institution emaciated and are often inappetent in quarantine. Mortalities within the first few weeks have been high without aggressive nutritional support (Thomas, 2005; Boylan, personal communication). Tail trauma is common in *P. violacea*, *T. lymma* and *H. uarnak*. Dasyatid rays may benefit from substrate, but if not available the tank floor must be smooth to avoid erosions developing on the ventral surface of the pectoral and pelvic girdles. Dasyatid rays have one or more venomous spines on the dorsal tail. These spines are typically trimmed, removed or covered prior to restraint. Many dasyatids have a large disc width, making it difficult to turn them over during physical exams and leading to the possibility of overlooking cryptic parasites in the buccal cavity. Endoscopy under chemical restraint may be considered for wild-caught dasyatids. Many dasyatids secrete abundant mucus and frequent water changes, or a flow-through water system, may be required during physical exams. The mucus is particularly thick in leopard whiprays, *Himantura leoparda* (Manjaji-Matsumoto & Last, 2008) (Clauss, personal communication). Biochemistry values have been reported for wild-caught *D. americana* (Cain et al., 2004).

Myliobatidae

Spotted eagle rays, *Aetobatus narinari* (Euphrasen, 1790), and ocellated eagle rays, *Aetobatus ocellatus* (Kuhl, 1823), are considered difficult species to maintain in aquaria. These species commonly have heavy loads of gill monogeneans, particularly *Dendromonocotyle* spp. (Chisholm and Whittington, 2004; Chisholm et al., 2004), which can be extremely hard to eliminate, despite prolonged treatment rotations. Treatment often needs to continue for as long as the animals are in the collection (e.g., regular praziquantel or freshwater dips). Freshwater dips (if matched to temperature and pH) are well tolerated by otherwise healthy eagle rays. Chlamydiales have been associated with branchial epitheliocystis lesions in two *A. narinari* (Camus et al., 2013b). Clinical signs started on days 53 and 139 of quarantine, and included lethargy, increased respiratory rates and abnormal swimming patterns (Camus et al., 2013b). Treatment of one animal with chloramphenicol and oxytetracycline was ineffective, and both animals died (Camus et al., 2013b). *Eimeria southwelli* has been identified in the spiral valve of both asymptomatic and thin animals (Stamper and Lewbart, 1998; Clauss, personal communication).

Myliobatids can be hard to convert to frozen-thawed food and often need aggressive nutritional support in quarantine. Dental plate overgrowth can develop in quarantine if whole clams and other bivalves are not provided in the diet (Clauss, personal communication). Erosive lesions can develop rapidly on the leading edge of the pectoral fins, as well as the ventral surface of the pectoral and pelvic girdles. Myliobatid rays have one or more venomous spines on the tail.

R. bonasus (cownose rays) are hardy but often carry significant parasites. *R. bonasus* are commonly infected with *Benedeniella posterocolpa* monogeneans, particularly on the ventral skin surface. These parasites are hard to eliminate and can be present on animals that come from other aquaria (Langan, personal communication). Treatments may involve repeated praziquantel or organophosphate immersion, or long-term copper therapy (Thoney, 1990; Pulido-Flores and Monks, 2005; George, personal communication). *R. bonasus* may be infected with the leech *Branchellion torpedinis*, particularly within the buccal cavity. *R. bonasus*, following an abbreviated quarantine, were considered the source of leeches that caused morbidity and mortality in demersal and pelagic elasmobranchs in a multitaxa exhibit (Dove and Clauss, 2008). Clinical signs in naïve animals included anemia, hypoproteinemia, inappetence, lethargy, secondary infections and mortalities (Dove and Clauss, 2008). Endoscopy of the buccal cavity is recommended for this species, but some institutions will prophylactically treat with organophosphates. *R. bonasus* wild-caught from the western Atlantic Ocean and Gulf of Mexico are known to carry *Eimeria southwelli* coccidia in the coelom and gastrointestinal tract (Stamper and Lewbart, 1998). While often commensal, the parasite can cause significant morbidity and mortality secondary to stress (Stamper and Lewbart, 1998). Coelomic aspirates or flushes show the highest sensitivity for *E. southwelli* diagnosis and screening of incoming animals is warranted (Stamper and

Lewbart, 1998). Treatment of the parasite with sulfonamides, clindamycin, toltrazuril, or ponazuril can reduce symptoms, but rarely clears the infection. Side effects of treatment have been reported, including cloacal prolapses and mortalities (McDermott et al., 2013; Harms, personal com-munication). *R. bonasus* can be hard to handle under manual restraint and better success may be seen with chemical restraint or training for handling (e.g., feeding as they swim through a shallow stretcher). The thin tail may limit blood volume available from the ventral tail vein and the wing or radial veins are useful secondary sites for venipuncture (Alexander, 1991). Hematology and biochemistry values have been reported for captive *R. bonasus* (Ferreira et al., 2010).

Bullnose eagle rays, *Myliobatis freminvillei* (Lesueur, 1824), and bat eagle rays, *Myliobatis californica* (Gill, 1865), are less hardy than *R. bonasus*. *M. freminvillei* and *M. californica* are commonly infected with *Dendromonocotyle* monogeneans (e.g., *Dendromonocotyle californica* in *M. californica*) and freshwater baths appear to be effective and well-tolerated by *M. californica* (Olsen and Jeffries, 1983; Chisholm et al., 2004; Murray, personal communication). Leeches may be found on wild-caught *M. californica* (Murray, personal communication). *E. southwelli* has been identified in *M. freminvillei* showing poor body condition and skin pallor (Harms, personal communication). *M. freminvillei* and *M. californica* are prone to tail trauma and to ventral erosions if no substrate is provided (Hyatt, personal communication; Murray, personal communication).

Urotrygonidae

U. jamaicensis (yellow stingrays) are generally hardy. *Dendromonocotyle octodiscus* monogeneans are reported from *U. jamaicensis* in aquaria and in the wild (Chisholm et al., 2004; Pulido-Flores and Monks, 2005). *D. octodiscus* can cause morbidity and mortality, and has an unusually wide range of hosts, including dasyatids (Chisholm and Whittington, 2004). Inappetence is common early in quarantine of *U. jamaicensis* and aggressive nutritional support may be needed (Boylan, personal communication). Females are often gravid and ultrasonography of the reproductive tract is warranted. Urotrygonids have one or more venomous spines on the tail.

Gymnuridae

The Japanese butterfly ray, *Gymnura japonica* (Temminck & Schlegel, 1850), and the spiny butterfly ray, *Gymnura altavela* (Linnaeus, 1758), can be difficult to maintain, particularly large *G. altavela*. These species often require prolonged nutritional support before they start eating on their own (Henningsen, 1996; Boylan, personal communication). Some gymnurids have one or more venomous spines on their tail.

Potamotrygonidae

The most commonly exhibited potamotrygonid is the South American freshwater stingray, *Potamotrygon motoro* (Müller & Henle, 1841) (AES, 2008). Many potamotrygonids, such as the *P. motoro* and the white-blotched river stingray, *Potamotrygon leopoldi* (Castex & Castello, 1970), are aquarium-bred and unproblematic in quarantine. Potamotrygonids can be prone to water mold infections on the skin and around the dental plates and treatment may include prolonged low-dose salt immersion (Mylniczenko, 2012; Haulena, personal communication). Branchiuran *Argulus* spp. may be present on wild-caught potamotrygonids (Mylniczenko, 2012). Physical removal is often sufficient as a treatment for *Argulus* spp. Coccidia and nematodes are often identified in potamotrygonids, but have not been associated with morbidity or mortality (Mylniczenko, personal communication). Emaciation was a common postmortem finding in potamotrygonids in a review of elasmobranch histopathology (Garner, 2013). Potamotrygonids have one or more venomous spines on the tail and the venom is more potent than that of marine stingrays (Pedroso et al., 2007).

Chimaeridae

Spotted ratfish, *Hydrolagus colliei* (Lay & Bennett, 1839), are relatively common in aquaria, but other chimaerids are not (AES, 2008). *H. colliei* are often infected with copepods (e.g., *Acanthochondria* spp. on the claspers), and leeches (e.g., *Branchellion* spp.) (Johnson and Horton, 1972). In the authors' experience, mechanical removal has been a successful treatment for copepods and leeches. Wild-caught *H. colliei* have a high prevalence of *Gyrocotyle* spp. flatworm in the gastrointestinal tract, but the parasites have not been associated with any pathology (Johnson and Horton, 1972; Benz and Bullard, 2004). Lesions from physical trauma may be seen in chimaerids after capture or transport, and quarantine tank surfaces should be smooth as their integument is easily damaged. Chimaerids do well in darkened quarantine systems with strict temperature control. Chimaerids have a venomous dorsal spine and should be handled with care.

CONCLUSIONS

The most important aspects of elasmobranch quarantine are thorough planning, managed isolation, and close monitoring of the animals and life support systems. Good record keeping allows for comparison with previous quarantine groups to identify common issues and evaluate management, diagnostic and treatment plans. Critical evaluation of quarantine plans is particularly important for species that are not routinely maintained at the institution or that have shown morbidity or mortality in the past. In these cases, it is the responsibility of the receiving institution to gather as much information as possible from the literature and colleagues in the field to establish robust management plans. Effective quarantine can reduce risk to the existing collection and ensure greater success with newly acquired animals.

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INTERNET RESOURCES

www1 www.elasmobranchhusbandry.org

The Elasmobranch Husbandry Manual II: Recent Advances in the Care of Sharks, Rays and their Relatives, pages 135-146.

Chapter 13

Elasmobranch Mineral and Vitamin Requirements

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Abstract: Proper nutrition for elasmobranchs is a key component of preventative medicine programs for zoological institutions. The optimal diet for elasmobranchs managed under human care consists of essential nutrients, including vitamins and trace minerals, found in comparative wild diets. A lack of understanding of the mineral and vitamin composition of diets in the wild, and the utility of these nutrients for elasmobranchs, makes supplementing diets in aquaria a challenge. Current dietary vitamin and trace mineral recommendations for elasmobranchs are frequently based on requirements determined from teleost fishes. A variety of commercial supplements are available for elasmobranchs. Minimal dietary supplementation with iodine, thiamin (B1), and vitamin E is recommended. Additional nutrient supplementation with calcium, zinc, manganese, vitamin C, vitamin A and other nutrients is dependent upon diet composition, life history stage and disease state. Diets should be formulated and evaluated regularly.

INTRODUCTION

Proper nutrition is the cornerstone of good health and is considered a key part of a preventative medicine program for zoological institutions. A nutritionally-appropriate diet for elasmobranchs under human care consists of the essential nutrients (i.e., proteins, lipids, carbohydrates, minerals, vitamins, fatty acids, amino acids) normally found in the wild diet. A lack of understanding about what elasmobranchs are consuming in the wild, combined with a paucity of information about the nutrient composition of prey items, makes managing diets in aquaria challenging. Vitamin and mineral supplementation has become routine practice in elasmobranch husbandry. However, there are large gaps in our understanding of dietary requirements, appropriate dosing schemes, and the threshold for deficiency and/or toxicity. Many of our current practices are extrapolations from studies on teleost fishes, or the result of research on a limited range of elasmobranch species. The goal of this chapter is to summarize what is known about mineral and vitamin requirements for elasmobranchs, make recommendations on supplementation and identify areas where further research effort is needed.

DIET COMPOSITION IN THE WILD

Research on wild elasmobranchs has focused primarily on measuring energy content and gross nutritional profiles (i.e., protein, lipid, carbohydrates) of teleost prey items, for the calculation of energy requirements. While extensive research has been undertaken with teleosts used in aquaculture, few comprehensive studies have been done to understand dietary mineral and vitamin consumption for elasmobranchs. A recent study examining the nutritional composition of lemon shark, *Negaprion brevirostris* (Poey, 1868), prey items in the Bahamas, found that the daily intake of zinc (Zn) in this species exceeded the minimum requirements for teleosts, while daily intake of iron (Fe), copper (Cu), and manganese (Mn) were much lower than the minimal requirements for other fishes, suggesting a deficiency (Pettitt-Wade et al., 2011). Daily intake of macro and trace nutrients were calculated as a percentage of total

diet at 6.6% calcium (Ca), 1.1% sodium (Na), 1.0% potassium (K), 0.3% magnesium (Mg), 0.008% Zn, 0.005% Fe, 0.0007% Cu and 0.0001% Mn. Ca was the highest of the minerals consumed in the diet, although the significance of this amount is unclear given that dietary Ca may play a negligible role in the Ca metabolism of fishes (Flik et al., 1995; Pettitt-Wade et al., 2011).

DIET COMPOSITION IN AQUARIA

Wild elasmobranchs consume a large variety of prey that can change seasonally and differ throughout their development. Variation of diet items in aquaria is recommended to ensure that essential nutrients are consumed and mineral and vitamin deficiencies are minimized. Vitamin and/or mineral supplementation may be necessary to fill nutritional holes in the diet resulting from an animal consuming a limited or inappropriate diet, or from the loss of nutrients during food transport, storage and leaching during thawing.

Minerals

The essential macro and trace minerals for elasmobranchs are presumed to be the same as those determined for teleosts, although minimum dietary requirements may differ. It is difficult to study the dietary mineral requirements for elasmobranchs, given the potential for mineral uptake from both the diet and from seawater, and from the large number of mineral-mineral and mineral-vitamin interactions (Dallinger et al., 1987). For example, Cu requirement is dependent on the concentrations of water-borne minerals, such as Zn, Fe, and molybdenum (Mo), which can interfere with Cu absorption (NRC, 2011).

Six minerals, including Ca, chloride (Cl), Mg, phosphorus (P), K and Na, are commonly recognized as essential macrominerals in fish nutrition (Table 1). Trace minerals are required at much lower concentrations than the macrominerals, yet are important components of hormones and activators for a variety of enzymes and chemical processes. The most commonly recognized trace minerals are chromium (Cr), cobalt (Co), Cu, iodine (I), Fe, Mn, selenium (Se), and Zn (Table 1) (NRC, 2011). Generally, fishes take in minerals from dietary sources preferentially over uptake from seawater (Dallinger et al., 1987; Bury et al., 2003). Very little is known about mineral metabolism in elasmobranchs, and more research is needed in this area.

Table 1. Minerals, deficiency signs, and requirements per kg of dry diet for fish. Sources of data: [a]NRC (2011); [b]Chow and Schell (1980).

Mineral	Deficiency Signs	Requirement per kg dry diet
Calcium	Impaired growth and hard tissue mineralization	2 - 15[a] g
Chloride	Impaired growth	1 - 5[b] g
Magnesium	Muscle convulsion and weakness	0.5 - 0.7[a] g
Phosphorus	Impaired growth, reduced hard tissue mineralization, lipid gain	5 - 10[a] g
Potassium	Convulsions	2 - 3[a] g
Sodium	Impaired growth	1 - 3[b] g
Copper	Impaired growth	1.5 - 5[a] mg
Cobalt	Anemia	5 - 10[b] mg
Chromium	Impaired glucose metabolism	trace[a]
Iodine	Goiter	0.6 - 1.1[a] mg
Iron	Impaired growth, anemia	30 - 200[a] mg
Manganese	Impaired growth, skeletal abnormalities	7 - 13[a] mg
Molybdenum	Reduced enzyme activity	trace[a]
Selenium	Impaired growth, anemia	0.1 - 0.7[a] mg
Zinc	Impaired growth, cataracts, skeletal abnormalities	20 - 150[a] mg

The most common mineral deficiency that has been noted for elasmobranchs has been I deficiency, which can result in goiter (Stoskopf, 1993; Janse, 2003). Oral I supplementation at 10-30 mg/kg body mass (BM)/week is recommended in systems where levels are below those found in natural seawater (0.06 mg/L) and nitrate levels are >10 mg/L (Crow, 2004). Commercially-available supplementation tablets for elasmobranchs meet these requirements (Table 2).

Ca and P play a major role in proper growth and development of bone, teeth and cartilage, muscle contraction and blood clotting in marine teleosts. Generally, the preferred ratio of Ca to P in the diet falls between 1:1 and 2:1 (Lall and Lewis-McCrea, 2007). Inverted ratios can lead to skeletal malformations, poor growth and muscle disorders. The role of dietary Ca in elasmobranch nutrition is not completely understood. However, in marine teleosts the role of dietary Ca is thought to be minimal in meeting daily requirements, as fishes have access to ionic Ca present in natural seawater (Flik et al., 1995; Lall and Lewis-McCrea, 2007). Supplementation with Ca may be appropriate if fish filets or squid constitute a large portion of the diet fed to elasmobranchs, or if Ca levels are low in the exhibit water (<10 mmol/L). Diets that include whole bony fishes generally meet minimum Ca:P ratios of 1:1. A few institutions have reported supplementing the diets of elasmobranchs with Ca (range 77 - 360 mg/week; Janse, 2003) when feeding both chopped and whole fishes.

Vitamins

Fifteen different vitamins are recognized as essential for normal fish growth, reproduction and health (Table 3; NRC 2011). In mammals, the absence of vitamins leads to characteristic symptoms of deficiency and eventually disease, but in aquatic species, such as fishes, symptoms of deficiency can be inconsistent between species, and therefore less easily identified (Table 3). Unfortunately, many vitamin deficiencies in elasmobranchs are detected at the time of necropsy and subsequent histopathology.

Nutritional deficiency (especially vitamin C and Zn) has been suggested as a causative cofactor of spinal deformity in sand tiger sharks, *Carcharias taurus* (Rafinesque 1810) (Berzins et al. 1998; Berzins et al. 2002). In a study by Anderson et al. (2012), serum collected from healthy aquarium *C. taurus* had significantly higher levels of K, vitamin C, vitamin E and Zn, compared with the serum of specimens affected with spinal deformity. However, the sample sizes of affected sharks in the study was small, so caution should be used when interpreting the results. Both vitamin C and Zn are cofactors involved in aspects of collagen synthesis and cartilage development across various taxa. Both vitamin C and Zn are cofactors involved in aspects of collagen synthesis and cartilage development across various taxa. Plasma vitamin C, vitamin E and Zn values for wild *C. taurus* in Delaware Bay were similar to, or lower than, values reported in aquarium *C. taurus* with spinal deformity (Hoopes, unpublished results), suggesting either that requirements of these nutrients may be higher for smaller animals susceptible to spinal trauma during capture and transport, or that there is no connection between these nutrients levels and spinal deformity. Supplementation with high levels of vitamin C showed no resolution in spinal deformity of affected *C. taurus* (Tollefson, personal communication). Anderson et al. (2012) noted that most multivitamins formulated for elasmobranchs lack K and encouraged the supplementation of diets with vitamin C, vitamin E, K, and Zn; although no levels of supplementation were offered.

While most teleost fishes studied are not able to synthesize vitamin C, and thus require it in the diet, limited work with elasmobranchs suggests that some species possess the enzyme for synthesis in their kidneys (Mæland and Waagbø, 1998). Vitamin C can safely be supplemented to elasmobranchs at a dose of 500-2000 mg/kg of dry diet.

Vitamin E is an antioxidant, and an important nutrient for the proper function of many organs, nerves, and muscles. Vitamin E also protects polyunsaturated fatty acids and other lipids from oxidizing. Vitamin E levels in frozen fishes decline over time through the storage process and therefore elasmobranchs fed thawed fishes are susceptible to vitamin E deficiency (Crissey et al., 1999). The requirement for vitamin E in elasmobranchs may increase if a high lipid diet is fed, or if the diet is deficient in vitamin C or Se (Hambre, 2011). The recommended level for vitamin E supplementation is 50-200 mg/kg dry diet (Halver, 1989; NRC, 2011). In high doses, vitamin E may have an anticoagulant effect resulting from interaction with vitamin K. However, in the case of vitamin E (and vitamin C), impairment in health and/or immunity from deficiency is of greater concern than supplementation in excess (Waagbø, 2006).

Table 2. Nutrient comparison of commercially-available mineral and vitamin supplements for elasmobranchs. [[a]PMI Nutrition International, USA; [b]Pacific Research Laboratories, USA; [c]International Zoo Veterinary Group, UK]

	Mazuri® Vita-Zu® shark/ray tabs I (5M24)[a]	Mazuri® Vita-Zu® shark/ray tabs II (5MD8)[a]	Sea Tabs® BTF&S[b]	Pike et al., 1993	AQUAVITS®[c]	ELASMOBRANCH TABLET®[c]
	1.5 g tab per 227 g fish	1.5 g tab per 227 g fish	Based on veterinary recommendation	Formulation per kg BM/week	1 tab per 20 kg BM/week	1 tab per 250 g fish
Vitamin A, IU	7,350	5,270	1,000	3,570	15,000	4,000
Vitamin D3, IU	655	655	20	150	2,500	450
Vitamin E, IU	51	98	50	37.5	200	40
Vitamin C, mg	500	475	10	37.5	125	400
Thiamin (B1), mg	80	80	50	210	250	65
Vitamin B2, mg	1.4	1.4	0.25	0.39		
Vitamin B6, mg	1.4	1.4	0.15	0.23		1
Vitamin B12, µg	3.5	3.5	2	900	50	3.75
Niacin, mg		2.4	0.15	0.6		0.01
Pantothenic Acid, mg	3.9	3.9	1.5	0.6	9	3
Folic Acid, mg		0.71	0.1	Trace	0.05	
Biotin, µg		59	2		200	
Choline, µg			5	Trace	10,000	
Inositol, µg			5	Trace	5,000	
Taurine, µg			5			
Calcium, mg	70	38				
Phosphorous, mg	25					
Iodine, mg	230	235	0.007			250
Iron, mg		0.25	1	11.25	50	
Copper, mg			0.1			
Cobalt, mg		0.3				
Magnesium, mg			0.5			
Zinc, mg		8	0.5			
Manganese, mg		3	Trace			
Kelp, mg			1	0.018	50	

Table 3. Vitamins, signs of deficiency and requirements per kg of dry diet for fishes. Sources of data: NRC (2011).

Vitamin	Signs of deficiency	Requirement (units/kg dry diet)
A	Acites, anorexia, spinal deformities, edema, erosion, eye pathology, lethargy	6,600 - 13,000 IU
C	Abnormal swimming, acites, anorexia, erosion, spinal deformities, eye pathology, lethargy, loss of equilibrium, spinal curvature	100 - 500 mg
D	Fatty liver, spasms	400 - 2,400 IU
E	Acites, edema, fatty liver, spinal curvature, muscle weakness, spasms	25 - 200 mg
K	Hemorrhage, prolonged blood clotting	0.5 - 2 mg
Thiamin (B1)	Hyperirritability, loss of equilibrium, spasms	1 - 10 mg
Riboflavin (B2)	Abnormal swimming, spinal deformities, erosion, eye pathology, hyperirritability, lethargy	3 - 20 mg
Niacin (B3)	Abnormal swimming, spinal deformities, edema, lethargy, muscle weakness	12 - 150 mg
Pantothenic Acid (B5)	Abnormal swimming, erosion, hyperirritability	10 - 50 mg
Pyridoxine (B6)	Hyperirritability, lethargy, spasms	1 - 20 mg
Cyanocobalamin (B12)	Anemia and other hematological changes, anorexia	0.01 - 0.05 mg
Biotin	Abnormal swimming, hyperirritability, fatty liver, lethargy, spasms	0.05 - 2.5 mg
Folic Acid	Hematological changes, lethargy	1 - 10 mg
Choline	Fatty liver	400 - 3,000 mg
Myoinositol	Erosion, fatty liver, lethargy	166 - 500 mg

Thiamin (B1) is an essential water-soluble vitamin that is integral for carbohydrate metabolism, digestion, growth, fertility and neurological function in fishes (NRC, 2011). Thiaminases are enzmyes found in the raw flesh and viscera of certain fishes and shellfish. When ingested these enzymes split thiamin, rendering it inactive. Thiamin also can be lost by holding diet items too long in storage. Wet or frozen diets risk thiamin deficiency, as moisture content increases the chance of thiaminase hydrolysis and subsequent destruction of thiamin (Halver, 1980; Crissey, 1998). Capelin, *Mallotus villosus* (Müller, 1776), Atlantic herring, *Clupea harengus* (Linnaeus, 1758), European smelt, *Osmerus eperlanus* (Linnaeus, 1758), Atlantic mackerel, *Scomber scombrus* (Linnaeus, 1758), clams, shrimp, and mussels all contain thiaminase (Greig and Gnaedinger, 1971), making supplementation of thiamin a necessity when feeding frozen thawed fishes and shellfish.

Deficiencies in thiamin (and other B vitamins) can result in a number of neurological symptoms that include convulsions, ataxia, muscle spasms, “spinning”, and difficult or jerky movements (Janse et al., 2004; NRC, 2011). Serum samples collected from six unsupplemented aquarium bonnethead sharks, *Sphyrna tiburo* (Linnaeus, 1758), displaying neurological symptoms (“spinning”), revealed significantly lower thiamin levels (mean 11.6 ng/mL) compared to levels measured from six wild counterparts (mean 24.8 ng/mL) (Mader, personal communication). While no other B vitamins were tested in the serum, newly introduced *S. tiburo* given a diet supplemented with thiamin presented no recurrence of the problem (Mader, personal

communication). Since thiamin and other B vitamins are water-soluble, there is low risk in dietary supplementation of thiamin at 10-20 mg/kg dry diet, or injectable B complex at 5 mg/kg BM intramuscularly (IM).

Fat-soluble vitamins A and D are essential dietary vitamins for teleosts and presumably elasmobranchs. Concern for supplementing these vitamins to elasmobranchs in aquaria arises from the potential for accumulation in the liver and adipose tissue when supplemented in excess. Some teleosts can tolerate relatively high levels of vitamin D in the diet (Hilton and Ferguson, 1982), but excess supplementation with vitamin A causes slowed growth, anemia, abnormal vertebral growth and mortality (Hilton, 1983; NRC, 2011). Toxic levels for vitamin A have not been determined in elasmobranchs. However, many elasmobranch species are top predators in the food chain, similar to terrestrial carnivores, and are likely have a tolerance to high levels of vitamin A. Dietary requirements for vitamin A and D are likely met in aquarium elasmobranchs that consume whole fatty fish (e.g., *C. harengus*, *S. scombrus*), as vitamin A and D levels are high in the liver of these species. Elasmobranchs that consume a diet consisting largely of mollusks, crabs and/or other crustaceans may require vitamin A and/or D supplementation, as these prey items are generally low in these fat-soluble vitamins.

Dosing

Elasmobranch vitamin and mineral supplementations and dosing schedules vary greatly between institutions. Some commercial supplements provide instructions for dosing based on BM. However, there is the chance for over/under supplementing large animals that are weighed infrequently. Other supplements provide instructions for dosing based on the amount of diet offered, but adjustment is required as diets change and over/under supplementation can still occur should food consumption rates vary. A survey of European aquaria by Janse (2003) found that 80% of institutions were supplementing their elasmobranch collection, although the quantity and quality of supplements were variable. A few aquaria chose to supplement additional iodine (60%) or calcium (20%) over and above preformulated commercial supplements (Janse et al., 2004). A more recent survey of 71 aquaria in the United States (US) found that the majority (75%) were dosing supplements for their elasmobranchs based on diet weight, or were offering a precise number of tablets per day, while a few institutions (7%) offered no supplementation at all (Mazzaro et al., personal communication). Whichever route of supplementation is chosen, periodic evaluation is suggested to account for dietary and food quality variability, and as animals grow and develop.

DIET FORMULATION

There is great value in understanding the nutrient formulation of diets for elasmobranchs in aquaria. By comparing diet formulations for elasmobranchs to known vitamin and mineral requirements for teleosts, potential deficiencies or excesses in the diet may be identified (Table 4). This exercise, paired with routine blood sampling for nutritional profiles, may help to elucidate aspects of the nutritional health of elasmobranchs under human care (Table 5).

Table 4 shows the nutrient profile of a typical diet for a 5 kg and 80 kg elasmobranch, both unsupplemented and supplemented with a commercially available tablet dosed to diet weight. Dietary values for Mn, I and thiamin in the unsupplemented diet are below the recommended requirements, but these values improve with supplementation. However, with supplementation, I values in the diet fall outside the upper recommendation range for all species. Little is known about I toxicity in fishes (including elasmobranchs), but excessive I intake in mammals can produce goiter and inhibit the production of thyroid hormones (Penglase et al. 2013). While supplementation of I, in excess, is an important factor to consider, I deficiency is of far greater concern because of difficulties in maintaining I in exhibit water and ensuring that supplements are consumed. In addition, I deficiency has been detected in elasmobranchs without any visible sign of goiter (Tollefson, personal communication), making detection more difficult. Further research is needed in this area of nutrition.

Nutrient values for Zn, vitamin C, and vitamin E in the unsupplemented diet met recommendations. However, in light of the potential for spinal disease development in *C. taurus*, additional supplementation may be beneficial. Vitamin A levels in the unsupplemented diet were elevated compared to recommended values. Most commercial vitamin supplements formulated for elasmobranchs contain some

Table 4. Example of dietary nutrient profile for a 5 kg and 80 kg elasmobranch (on a dry matter basis), both unsupplemented (UN) and supplemented (S), with a commercial tablet dosed to diet weight. Nutrient recommendations for elasmobranchs, Atlantic salmon, *Salmo salar* (Linnaeus, 1758), and marine teleosts are listed for comparison. Source of data: [a]Janse et al. (2004), [b]NRC (2011).

Nutrients	5 kg Elasmobranch[1]		80 kg Elasmobranch[2]		Recommendations		
	(UN)	(S)	(UN)	(S)	Elasmo.[a]	*Salmo salar*[b]	Mar. teleosts[b]
Dry Matter (%)	24.72	25.19	27.04	27.53			
Crude Protein (%)	69.95	68.21	72.34	70.56		40-45	50-55
Crude Fat (%)	18.68	18.22	20.24	19.74			
Carbohydrates (%)	4.13	6.51					
Gross Energy (kcal/kg)	5,850	5,710	5,990	5,840			
Digestible Energy (kcal/kg)	4,270	4,168				4,200-4,400	4,000-4,200
Ca (%)	1.2	1.21	2.12	2.13			
P (%)	1.19	1.19	2.06	2.01		0.6-0.93	0.55-1.09
Mg (%)	0.13	0.13	0.13	0.12		0.06-0.07	
K (%)	1.24	1.20	1.46	1.42			
Na (%)	0.65	0.64	0.61	0.6			
Fe (mg/L)	73.31	75.48	134.87	135.49	50-100	30-200	150-199
Zn (mg/L)	51.9	126.59	65.79	188.98	15-100	26-29	
Cu (mg/L)	23.26	22.68	3.75	3.66	1-4	5-10	4-6
Mn (mg/L)	4.24	50.78	2.97	48.88	20-50	12-13	
I (mg/L)	1.72	3732.4	1.27	3,679.5	100-300	0.6-1.1	
Se (mg/L)	1.76	1.71	3.52	3.43	0.15-0.38	0.15-0.38	0.7
Thiamin (mg/L)	1.42	1317.1	2.46	1299.6	1-10	10	11
Vitamin A (IU/kg)	72,900	94,550	1,837,400	1,878,350	2,000-2,500	7,500	9,000-31,000
Vitamin C (mg/L)	88.13	880.38	189.72	968.34	100-1100	20	15-54
Vitamin E (IU/kg)	50.97	1,828.68	45.03	1,644.99	30-50	50-60	31-115

[1]Based on diet composed of 25% Atlantic herring, *Clupea harengus* (Linnaeus, 1758), 25% Capelin, *Mallotus villosus* (Müller, 1776), 25% shrimp, 25% squid

[2]Based on diet composition of 40% *C. harengus,* 45% Atlantic bonito, *Sarda sarda* (Bloch, 1793), 15% Atlantic mackerel, *Scomber scombrus* (Linnaeus, 1758)

Table 5. Summary of mean blood nutrient values for aquarium and wild elasmobranchs. Source of data: [a]Hoopes, unpublished results; [b]Sullivan et al., 2012 and Valdes and Sullivan, unpublished results; [c]Anderson et al., 2012; [d]Haman et al., 2010 ; [e]Persky et al., 2012. Diet composition, feeding frequency and . . . . . .

Species	Sample Size	Sample Type[1]	Captive or Wild	Vitamin A (ng/mL)	Vitamin C (mg/dL)	Vitamin E (µg/mL)	Ca (mg/dL)	P (mg/dL)	Mg (mg/dL)
Aetobatus narinari	14[a]	P	C	306.2		12.5			
	14[a]	W	C						
	19[b]	S	C	139.1		9.5	15.7	4.5	2.7
Carcharhinus acronotus	4[a]	P	C	207.5		8.3			
Carcharhinus melanopterus	6[a]	P	C	183.2		30.5			
	6[a]	W	C						
Carcharhinus plumbeus	4[a]	P	C	162		16.7			
	11[a]	P	C	98.9		2.7			
Carcharias taurus	11[a]	W	C						
	23[c]	S	C, healthy	187.5	0.622	7.89	13.91	6.4	
	10[c]	S	C, spinal	140.7	0.412	4.12	13.63	5.9	
Cephaloscyllium ventriosum	6[a]	P	C	62.2		4.9			
	6[a]	W	C						
Chiloscyllium plagiosum	6[a]	P	C	117.5		11.6			
Chiloscyllium punctatum	5[a]	P	C	143.3		8			
Dasyatis americana	10[a]	P	C	144.4		10.6			
	10[a]	W	C						
	64[b]	S	Semi-C	216.3		22.9	15.6	4.7	3.6
Dasyatis centroura	13[a]	P	C	185.4		6.1			
	13[a]	W	C						
Eucrossorhinus dasypogon	3[a]	P	C	161.7		7.3			
	3[a]	W	C						
Glaucostegus typus	1[b]	S	C	159		14.3	15.9	5.8	3.5
Hemiscyllium ocellatum	6[a]	P	C	119.5		8.1			
Himantura fai	1[a]	P	C	167		24.1			
	1[a]	W	C						
Himantura jenkinsii	1[a]	P	C	215		20.5			
	1[a]	W	C						
Himantura uarnak	3[a]	P	C	129.7		8.3			
	3[a]	W	C						
Himantura undulata	2[a]	P	C	183.5		9.8			
	2[a]	W	C						
Manta birostris	20[a]	P	C	123		2.7			
	20[a]	W	C						
Mobula hypostoma	9[a]	P	C	438.3		1.9			
	9[a]	W	C						
	4[b]	S	C	262.5		2.5	14.7	4.1	2.4
Mustelus canis[3]	20[e]	S	C		0.43, 0.49	12.3, 9.3	16.8, 17	5.4, 4.5	
Orectolobus maculatus	18[a]	P	C	99		20			
	18[a]	W	C						
Pristis microdon	10[a]	P	C	231.1		13.1			
	10[a]	W	C						
Pristis zijsron	1[a]	P	C						
	1[a]	W	C						
Rhina ancylostoma	6[a]	P	C	169.5		5.8			
	6[a]	W	C						
Rhincodon typus	10[a]	P	C	115.5		3.4			
	10[a]	W	C						
Rhinoptera bonasus	14[a]	P	C	154.2		9.7			
	14[a]	W	C						
	21[b]	S	C	178.9		11	16.4	4.3	2.5
Rhizoprionodon terraenovae	30[d]	P	W[4]	280		2.05	18.3	7.15	
Rhynchobatus djiddensis	4[a]	P	C	186.3		8			
	4[a]	W	C						
Sphyrna mokarran	3[a]	P	C	383.3		14.4			
	3[a]	W	C						
Sphyrna tiburo	20[a]	P	C	235.8		70.1			
	20[a]	W	C						
	31[d]	P	W[4]	111		2.69	18.9	7.3	
Stegostoma fasciatum	9[a]	P	C	110.3		5.9			
	9[a]	W	C						
Squalus acanthias	30[d]	P	W[4]	20		1.6	13.6	4.4	
Taeniurops meyeni	6[a]	P	C	122.7		7.7			
	6[a]	W	C						
	1[b]	S	C	201		4.2	15.3	3.4	3.1
Triakis semifasciata	1[a]	P	C	181		55.9			
Trygonorrhina fasciata	2[a]	P	C	200		17.3			
Urogymnus asperrimus	5[a]	P	C	206		8.6			
	5[a]	W	C						

. . . . . supplementation amount differed between species, although most elasmobranchs in aquaria did receive some type of vitamin supplementation. [1]Plasma (P), whole blood (W), serum (S); [2]Indicates different sample size. [3]Mean values listed separately for males and females. [4]Median values listed.

Co (ng/mL)	Cu (µg/mL)	Fe (µg/dL)	I (ng/mL)	Mn (ng/mL)	Mo (ng/mL)	Zn (ug/mL)	Se (ng/mL)	As (µg/dL)	Pb (µg/dL)	Hg (µg/dL)
39.9	0.6	56.8	64.5 (6)[2]	78.8	6	10.5	762			
							850.5	360.3	84.5	54.3
8.9	0.7	48.5		28.5	7.7	12.4	930.9			
41.7	0.5	31		8.3	0.5	0.6	241.3			
184.1	0.4	24.3		59.2	0.5	0.7	391.7			
							3,019.00			
260.2	0.6	50	272.7 (3)	16.8	1.2	1	703.3			
23.2	0.4	20.3		47.3	1.4	1.6	262.8			
							548.8	948.3	12	428.8
						0.76				
						0.47				
82.7	1	19.5	709.5 (2)	403	0.9	0.7	281.2			
							328.2	102		
272.3	0.8	57.2	690.3 (3)	46.9	21.2	0.7	437.8			
286.5	0.9	65.6	1883 (1)	52.3	6.8	0.4	464.4			
1165.7	0.6	69.6	1328 (5)	44.6	0.5	15.8	365			
							3,745.40	735.8	6	79
1,062.00	0.6	54.3		64.7	2	21.8	548.2			
12.4	0.3	44.1	82.5 (2)	53.8	0.5	7.5	316.3			
							676.8	9,237.70	16.5	241
10.4	0.2	41.7	69 (2)	261.4	1.1	0.3	117.7			
							119.5	1,178.50		151.5
11.2	0.7	37		32.3	1.3	30.8	1140			
206.4	0.5	81.2		27.7	3.7	0.9	248.3			
39	0.5	64		9	0.9	21.3	715			
							7,667.00	604	16	146
14.8	0.3	43		3	1	1.3	503			
							472	445	<5	26
84.7	0.3	28	90 (1)	80.9	1.2	13.6	305.3			
							8,556.50	3,168.50	9	17
334.7	0.4	23.5		364.8	0.5	19.4	464.5			
							5,928.00	568	32	6
491.8	0.5	19.1	167 (4)	84.2	0.9	3	273.1			
							776.3	563	25.5	8
359.9	1.3	<10	206 (3)	17.9	0.6	9	623.5			
							1,621.30	2,726.90	7	
18.1	1.1	137.5		39	2.9	10.8	447			
71.6, 73.1	0.81, 0.59	71, 32		13.7, 6.1	0.80, 0.74	0.9, 1.0	230, 347			
42.2	0.3	43.3	88.4 (5)	123.8	2.1	0.5	294.2			
							331.7	348	6	52.4
789.1	0.6	29	1,619.8 (4)	121.1	0.4	21.9	420			
							411.4	96.4	8.5	
24.4	0.9	<10		5.5	0.5	10.2	308			
							279	389	7	254
118.6	0.4	12.8		115.8	0.4	13	187			
							575.5	389	40	218
221.1	0.7	14.4	278.3 (6)	57.3	3	0.3	312.1			
							928.3	6,053.50	55.5	60.75
465.3	0.4	27.6	266.2 (5)	96.5	0.9	2.5	273.6			
							1,255.00	2,574.00		34
23.1	0.6	64.1		46	2.4	3.6	343.5			
10	0.34	0.88			3	0.79	840			
48.7	0.5	22		11.7	0.5	9.6	303.7			
							523.5	266		10
347.1	0.5	65.3		18.5	0.9	0.9	790.7			
							3,469.70	4,089.00		143
515.1	0.4	40.3	2,512.6 (5)	29.2	1.5	0.9	289.1			
							6,094.50	14,998	15.3	74
40	0.34	0.82			3	0.94	1030			
184.8	0.8	26.9	348.3 (4)	96.3	5.1	0.6	570.2			
							1,682.80	434.5	8	98
20	0.23	0.44			1	0.37	220			
124	0.4	34.8	213.5 (2)	63.2	0.8	5.5	328.3			
							1,096.40	1,853.20	17.5	53.2
15.2	0.4	25		2.3	<0.05	9.6	469			
10.1	0.5	<10		8.1	1.3	0.3	222			
468.1	0.3	13		14.6	1.2	10	302			
26.8	0.2	58.8	55 (1)	91.1	1	7.5	301			
							6,878.00	2,538.50	13.5	

level of vitamin A (Table 2). However, whole fishes contain high levels of vitamin A and D (e.g., 2.5 - 130 and 0.75 - 1.0 IU/kg, respectively, in *S. scomber* (Halver, 1980). Similar to other marine piscivores (e.g., marine mammals), elasmobranchs that eat whole fishes may not require added levels of vitamin A in their diet.

NUTRIENT VALUES IN ELASMOBRANCH BLOOD

Understanding normal blood nutrient parameters for elasmobranchs may be valuable in identifying changes in their nutritional health and potential dietary deficiencies while under human care. Ideally, blood nutrient values from wild counterparts should be used to help identify reference ranges, and a few studies have begun to address these knowledge gaps (e.g., Haman et al., 2012; Hoopes, unpublished results). Blood values from aquarium elasmobranch collections are also useful in gauging the health of the collection and monitoring potential changes in individuals over time. Collated blood nutrient values from both aquarium and wild elasmobranch species are provided in Table 5.

Differences in plasma vitamin A are largely influenced by diet and/or level of vitamin supplementation, and plasma or serum values of vitamin A (retinol) are good indicators of vitamin A status (Crissey et al., 1999). Vitamin A levels measured in aquarium elasmobranchs are comparable to the few recorded values from wild specimens, although there is great variability within, and between, species.

Differences in plasma vitamin E (a-tocopherol) in aquarium elasmobranch species may be directly related to diet and/or level of vitamin supplementation (Hamre, 2011). There can be large differences in vitamin E levels among species and between individuals and changes in supplementation should not be based on a single sample (Crissey et al., 1999). While we do not understand the level at which deficiency occurs, values for elasmobranchs under human care are generally higher than those reported in wild species.

Serum Ca levels are tightly regulated and remain constant over a wide range of intakes. Low levels generally only occur after prolonged Ca deprivation or from some other pathology. However, plasma or serum P levels are a good determinant of evaluating phosphorous status. In addition to deficiency, excessive dietary Ca and inadequate supplies of vitamin D can affect phosphorous levels. Plasma/serum levels of Mg are reflective of dietary status, while K may not be reflective of dietary status given its role in fluid exchange and acid-base balance (Crissey et al., 1999).

Given our lack of understanding of mineral metabolism in fishes, the blood values listed in Table 5 should be used as rough guide for comparison and a starting point for evaluating the nutritional health of an individual elasmobranch. In particular, because some minerals are sensitive to changes in the quantity of other minerals and vitamins, as well as environmental stressors, variation in animal age, activity level and health status (Crissey et al., 1999).

MANAGEMENT RECOMMENDATIONS

At minimum, dietary supplementation with I, thiamin (B1) and vitamin E is recommended, given husbandry and water quality concerns for I and degradation of vitamins in thawed previously frozen food items. Additional nutrient supplementation with Ca, Zn, Mn, vitamin C, vitamin A and other nutrients, is largely dependent upon diet composition and consumption, life-history stage and disease state. Diets should be formulated and evaluated regularly.

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Chapter 14

Development of a body condition scoring tool for the spotted eagle ray, *Aetobatus narinari*

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Abstract: In an effort to advance the care of spotted eagle rays, *Aetobatus narinari* (Euphrasén, 1790), the husbandry team at Disney's The Seas (Orlando, Florida, USA) developed a standardized scoring tool to objectively assess specimen body condition. Through an iterative process, a five point body condition score (BCS) was determined to provide the best compromise between detection of meaningful changes in body condition and minimizing inter-category ambiguity. When tested on ten reviewers, average inter-observer BCS agreement was 90% for all viewing aspects, with the lowest inter-observer agreement of 87% for the ventral aspect and highest inter-observer agreement of 93% for the lateral aspect. The BCS tool was implemented at Disney's The Seas and became invaluable for assessing the well-being of *A. narinari*, providing an early warning detection system for potential health challenges.

INTRODUCTION

As the aquarium industry has grown so has the desire to improve animal welfare, encouraging staff to expand upon traditional husbandry methods to better manage and benefit the animals within their care. One way to assess animal welfare is through the use of body scoring tools. One of the earliest methods of body scoring was the body mass index (BMI) for humans, developed by Adolph Quetelet in 1832. Still in use today, BMI compares the height and weight of a person and is used as an index of general health (Eknoyan, 2008). BMI does not translate well for use with non-human animals, due to a high variability in animal size, body mass and morphology. As an alternative, body condition scores (BCS) have been developed for a number of different animal species, especially livestock (www1).

Animal BCS systems have been used by small and large veterinary practices to assess companion animals, while agricultural workers have used BCS systems to manage their livestock. These systems provide an easy way to evaluate body condition, which can be an indicator of general health (Clingerman and Summers, 2012). An objective scoring rubric minimizes subjective opinion and imprecise assessments (e.g., statements that an animal is "too thin" or "obese"), and decreases the introduction of personal biases into management decisions. Since BCS tools rely on a process of non-invasive observation, animals under managed care can be monitored from a distance without disturbance. This absence of physical manipulation reduces potential stressors, which can lead to adverse physiological responses, particularly in elasmobranchs (Piiper and Baumgarten, 1969; Piiper et al., 1972; Cliff and Thurman, 1984; Smith, 1992; Smith et al., 2004; Stevens, 1994; Mandelman and Skomal, 2009; Hyatt et al., 2011).

Spotted eagle rays, *Aetobatus narinari* (Euphrasén, 1790), are a highly charismatic species and are becoming increasingly common in public aquaria. Despite growth of husbandry and medical management information for the species, there is no information about assessment of *A. narinari* body condition. As a pelagic species, with a finely balanced energy budget, it is not always easy or prudent to regularly capture *A. narinari* to measure body mass (BM). As an alternative, descriptors of body condition, such as "good" or "poor", are vague and allow for inconsistencies in assessment, especially if more than one observer is involved. The inherent risk is that personal biases and a lack of measurable data can lead to husbandry personnel overlooking subtle shifts in BM and/or body condition, which can be an early indicator of a serious health challenge. Early detection of these changes provides a necessary tool for assessing the current status, and future needs, of an animal.

A detailed body condition scoring tool can be used routinely, in conjunction with behavioral observations and food intake, to provide an indirect but powerful indication of the health status of an animal. BCS systems are based on a set of standardized images (diagrams, drawings, photos, etc.) and employ a numerical scoring system to quantify animal status. A score of "1" typically represents an extremely thin or emaciated body condition, with the scale increasing over multiple numerals to represent the entire spectrum of body conditions (Henneke et al., 1983; Clingerman and Summers, 2012). The optimum BCS for a species generally falls in the midrange of the numerical sequence (Clingerman and Summers, 2012). Hereafter we describe a system for assessing body condition of *A. narinari* using a BCS developed at Disney's The Seas (Orlando, Florida, USA).

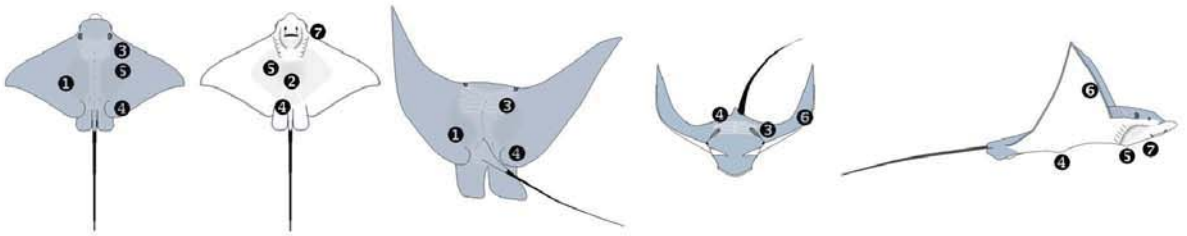
METHODS

A total of 200 photographs of *A. narinari* were evaluated to determine aspects or views that would best provide an objective assessment of animal body condition. The images represented a wide range of body shapes and sizes, taken at a variety of angles. Five different aspects of *A. narinari* were determined to provide the most robust set of views for BCS assessment: (1) dorsal; (2) ventral; (3) lateral; (4) anterior or "head-on"; and (5) posterior or "rear". Anatomical features used for the BCS were also standardized and reflected seven discrete body regions, including: (1) dorsal coelomic surface; (2) ventral coelomic surface; (3) gill arches; (4) pelvic girdle; (5) pectoral girdle; (6) wing; and (7) head (Figure 1).

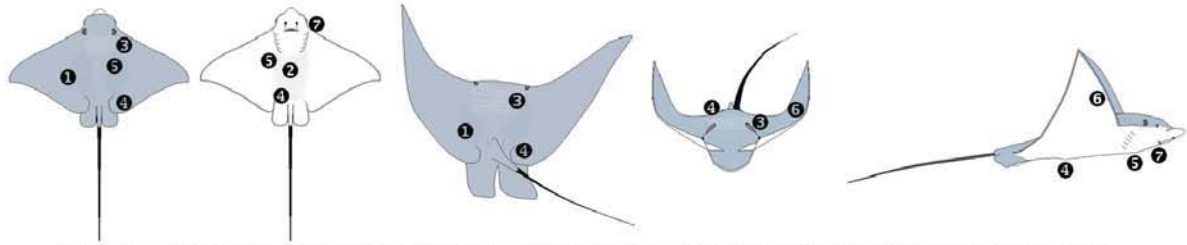
The 200 photographs were ranked into five discrete groups based on relative body condition. The groups were categorized on a sliding scale of "1 to 5", representing a spectrum from an emaciated to an obese *A. narinari*. The possibility of using a higher number of scores (e.g., 1 - 9) was abandoned as anatomical changes were deemed to be too subtle to differentiate when category granularity was increased beyond five.

Each of the seven anatomical features was then sketched and a written description generated to better define each of the five BCS categories, making objective assessment more possible. Some descriptions were binary (e.g., gill arches were categorized as "visible" or "not visible"), while other descriptions expressed a spectrum of possible states (e.g., coelomic surface was categorized on a scale from "severe concavity" through "flush with body wall" to "severe convexity") (Figure 1).

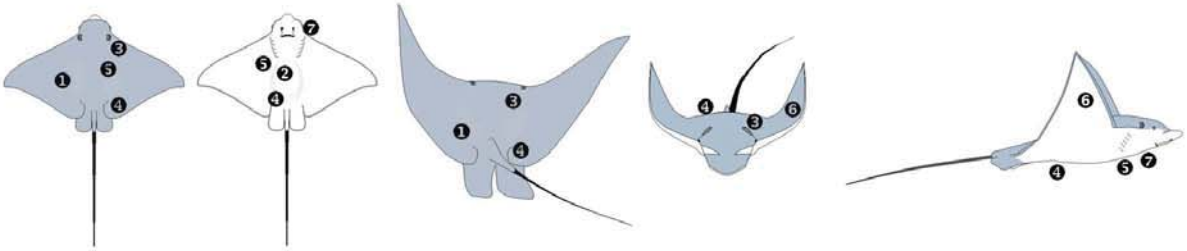
Once the model BCS tool was established, it was tested using a pool of ten observers. Each observer was shown five photographs of an individual *A. narinari*, from all five aspects, and asked to assign a BCS. Evaluated images included photographs of *A. narinari* in the wild and in aquaria. Inter-observer reliability was evaluated (using % agreement) and the results were used to



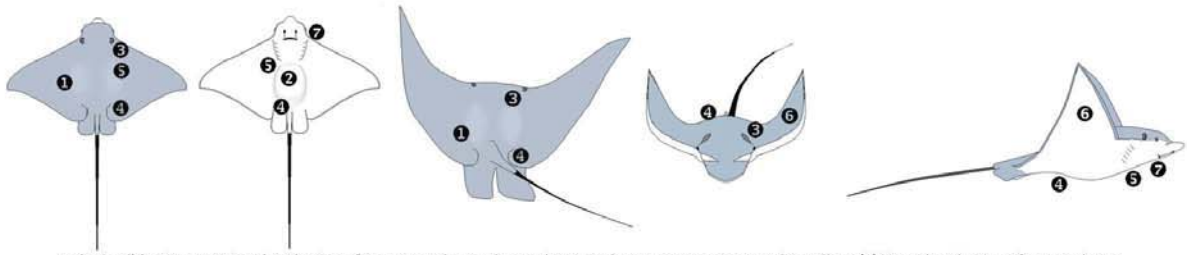
a. **Body Condition Score 1:** (1) Dorsal coelomic surface: pectoral musculature shows concavity nearest midline; (2) Ventral coelomic surface: moderate to severe concavity (sunken appearance); (3) Gill arches: visible; (4) Pelvic girdle: visible dorsally and ventrally; (5) Pectoral girdle: visible dorsally and ventrally; (6) Wing: no thickening; (7) Head: skin around eye and mouth show concavity; skin around mouth may appear stretched.



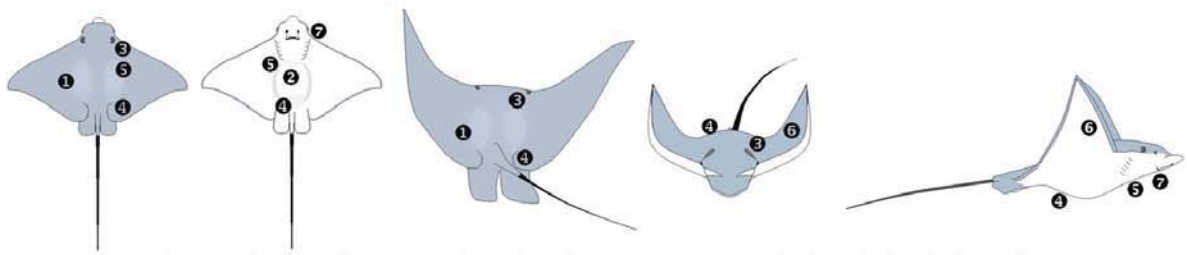
b. **Body Condition Score 2:** (1) Dorsal coelomic surface: pectoral musculature shows slight concavity or flush nearest midline; (2) Ventral coelomic surface: mild concavity or flush with body wall; (3) Gill arches: mildly visible; (4) Pelvic girdle: mildly visible dorsally and ventrally; (5) Pectoral girdle: mildly visible dorsally and ventrally; (6) Wing: thickening mild nearest the midline; (7) Head: normal (no concavity noted in skin near eyes or mouth).



c. **Body Condition Score 3:** (1) Dorsal coelomic surface: pectoral musculature shows mild convexity (distension) nearest the midline; (2) Ventral coelomic surface: no concavity; mild convexity starting past the last gill slit; (3) Gill arches: not visible; (4) Pelvic girdle: not visible; (5) Pectoral girdle: not visible; (6) Wing: thickening mild; most noticeably nearest midline; (7) Head: normal (no concavity noted in skin near eyes or mouth).



d. **Body Condition Score 4:** Dorsal coelomic surface: pectoral musculature shows moderate convexity nearest the midline; (2) Ventral coelomic surface: moderate convexity; (3) Gill arches: not visible; (4) Pelvic girdle: not visible; (5) Pectoral girdle: not visible; (6) Wing: thickening moderate; can be seen throughout the wing musculature; (7) Head: normal (no concavity noted in skin near eyes or mouth).



e. **Body Condition Score 5:** (1) Dorsal coelomic surface: pectoral musculature shows severe convexity nearest the midline; (2) Ventral coelomic surface: severe convexity; (3) Gill arches: not visible; (4) Pelvic girdle: not visible; (5) Pectoral girdle: not visible; (6) Wing: thickening severe; extreme cases may have wing thickening to the wingtips; (7) Head: normal (no concavity noted in skin near eyes or mouth).

Figure 1. Body condition scoring (BCS) tool for spotted eagle rays, *Aetobatus narinari* (Euphrasén, 1790), showing each of the five viewing aspects (i.e., dorsal; ventral; lateral; anterior or "head-on"; and posterior or "rear") and a description of the seven anatomical features for each of five discrete scoring levels.

fine-tune the BCS—i.e., the five viewing aspects, the descriptions of the seven anatomical features, and the sliding scale.

RESULTS AND DISCUSSION

Analysis of 200 photographs of *A. narinari* afforded a better understanding of the spectrum of possible animal body conditions. This process also helped determine appropriate viewing aspects to assess and score each animal. Dorsal and lateral aspects are often sufficient for assessing the body condition of terrestrial animals, however accurate assessment of *A. narinari* (and likely other pelagic rays) required three additional aspects to ensure evaluation veracity: ventral, anterior ("head-on"), and posterior ("rear"). By including all five aspects, it was possible to generate a set of standardized guidelines to accurately evaluate a ray, regardless of the position of the animal or the human evaluator.

A comparison of BCSs from ten test observers examining photographs of *A. narinari,* from each of the five viewing aspects, yielded an average inter-observer agreement of 90%. The lowest and highest inter-observer agreement was 87% for the ventral aspect and 93% for the lateral aspect, respectively. These results indicated a high degree of inter-observer reliability, as well as the utility of the BCS tool to aid husbandry decision-making. Ground-truthing the BCS tool at Disney's The Seas enabled fine-tuning of the descriptions of the seven anatomical features, as well as the "1" to "5" rating scale. The rating scale was determined to be sufficiently fine to detect meaningful changes in *A. narinari* body condition, yet sufficiently quantized to minimize ambiguity between individual BCS scores.

Once established, a BCS of "3", the middle score, was deemed ideal for *A. narinari* in aquaria. However, it should be noted that many wild *A. narinari* scored a BCS of "2". This difference is reflective of *A. narinari* in aquaria having access to a regular and highly nutritional diet, and being generally more robust than conspecifics in the wild. A BCS of "2" in an aquarium setting would indicate that an *A. narinari* was underweight. In general, animals with a slightly elevated BCS have a better ability to tolerate modest shifts in BM and better withstand environmental challenges (e.g., water quality shifts, inter- and intra-specific competition, etc.), as well as offering a health advantage when the animal is challenged by an active disease state (Henneke et al., 1983).

Once established, and in routine use, the BCS tool became invaluable for assessing the well-being of *A. narinari* maintained at Disney's The Seas. Each ray was evaluated weekly using the BCS tool. A database of assessed BCSs was established and individual animal body condition tracked over time, providing an early detection system for potential health challenges.

An example of BCS tool utility was demonstrated by tracking a female *A. narinari* that had been introduced into the exhibit at Disney's The Seas. Regular BCS assessments highlighted a dramatic decline in body condition (from a score of "3" to "1") within three months of introduction to the aquarium. Declining condition was coupled with an increased pallor. As a consequence of the observed trend intervention was deemed appropriate. The *A. narinari* was given a series of anthelmintic immersion treatments using praziquantel, as there had been a history of other animals suffering infestation with the parasitic flatworm *Decacotyle floridana*. As suspected, analysis of treatment water revealed the presence of the parasite. In addition to treatment with praziquantel, the diet of the *A. narinari* was augmented to accelerate improvement of specimen body condition. Within two months of intervention the body condition of the *A. narinari* had recovered to a BCS of "2" and continued to improve thereafter.

The BCS tool was also used to monitor potential pregnancies in *A. narinari*. Once baseline BCSs were established for each ray, increasing scores, in the absence of dietary changes, indicated a possible pregnancy. When this phenomenon was observed and marked, ultrasound imaging was prescribed for confirmation of pregnancy.

The von Bertalanffey and Gompertz equations allow calculation of BM or disc width (DW) when only one of the values is known—e.g., in cownose rays, *Rhinoptera bonasus* (Mitchill, 1815), (Neer and Thompson, 2005). However, obtaining weight or morphometric information typically requires capture and handling of the animal, which can be stressful (Piiper and Baumgarten, 1969; Piiper et al., 1972; Mazeaud et al., 1977; Cliff and Thurman, 1984; Wood, 1991; Smith, 1992; Stevens, 1994; Mandelman and Skomal, 2009; Hyatt et al., 2011), and may not be possible for wild specimens. The described BCS tool may be further developed to yield an estimate of BM, DW and/or age, without

direct physical manipulation or measurement of the animal. Preliminary results from application of the BCS as an estimate tool, using six *A. narinari* at Disney's The Seas, yielded promising results, representing an area for future study.

Ideally, a BCS tool would cater to other species of pelagic or free-swimming rays with similar body forms to *A. narinari*, such as R*. bonasus* and bat eagle rays, *Myliobatis californica* (Gill, 1865). However, the usefulness of the BCS tool must be validated for each species before implementation. Although mobulids (e.g., giant manta, *Manta birostris* (Walbaum, 1792) are considered pelagic, like myliobatids, differences in overall body shape between the two families would necessitate modification of BCS criteria before the tool could be employed to assess the body condition of mobulids.

The BCS concept described above provides a valuable framework for the development of improved husbandry tools, aiding the growing aquarium industry in advancing best practices for the management of the animals in their care.

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INTERNET RESOURCES

www1 http://www.staywell.co.uk/Intl/UK/You-and-Your-Pet/Recommendations/BMI-or-Condition-Score

The Elasmobranch Husbandry Manual II: Recent Advances in the Care of Sharks, Rays and their Relatives, pages 153-157.

Chapter 15

Preliminary evidence for a biennial feeding strategy related to reproduction in female sand tiger sharks, *Carcharias taurus* (Rafinesque, 1810)

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Abstract: It has been known for some time that female sand tiger sharks, *Carcharias taurus* (Rafinesque, 1810), have a biennial reproductive cycle (Grant et al., 1983). Feeding behavior of female *C. taurus* (n = 2) maintained at Manly SEA LIFE Sanctuary (Sydney, Australia) displayed a pattern aligned with their biennial reproductive cycle; increasing food intake between September and February every second year. Food consumption for female *C. taurus* increased by a factor of four during a reproductive year, when compared to food intake during a non-reproductive year. A matured male *C. taurus* (n = 1) also displayed a distinct feeding pattern, aligned with an annual preparation for breeding season. The majority (90%) of annual food intake for the male *C. taurus* occurred between November and March every year.

INTRODUCTION

Sand tigers, *Carcharias taurus* (Rafinesque, 1810), are a large-bodied, inshore mackerel shark (Last and Stevens, 2009). *C. taurus* are threatened globally (Pollard et al., 1996; Otway et al., 2003), with the eastern Australian population considered critically endangered (Pollard et al., 1996). The various global sub-populations of *C. taurus* face on-going threats from fishing efforts (Bansemer and Bennett, 2010), bather protection programs and habitat disturbance, placing the species at considerable risk. *C. taurus* generally do well in aquaria, possibly as a result of their preference for near shore environments (Otway et al., 2003; Last and Stevens, 2009), with some individuals living up to 25 years in human care.

C. taurus employ a unique reproductive strategy that involves oophagy and intra-uterine cannibalism. In the best-case scenario, mature female *C. taurus* give birth to two pups (one from each uterus) every second or third year (Grant et al., 1983). A recent study showed that the mass of the liver increases during the period leading up to a reproductive event in female *C. taurus* (Davidson and Cliff, 2011), suggesting a changing nutritional uptake over the reproductive cycle.

As with many shark species, *C. taurus* copulation can be aggressive and result in damage to both female and male sharks. Male sharks typically bite and grasp the pectoral fin or flank of the female to facilitate insertion of the clasper (Gordon, 1993). The whole process of copulation can take up to 10 minutes and causes damage to the

female in the form of superficial and deeper lacerations (Gordon, 1993; Lucifora et al., 2002). It is unknown how many mating attempts a female shark experiences in the wild before she becomes gravid. However, at least two mating events must occur if both uteri are to be fertilized (Grant et al., 1983). Furthermore, analysis of pre-cannibalism embryo paternity indicates polyandry is common in the species (Chapman et al., 2013).

In most *C. taurus* populations, evidence exists for sexually dimorphic migration patterns (Lucifora et al., 2002). Male and female *C. taurus* come together to mate, then females segregate to gestate and pup, possibly at two different sites (Bansemer and Bennett, 2009). While *C. taurus* can migrate vast distances (up to 1,550 km) (Otway and Ellis, 2011), they spend much of their time at aggregation sites; typically <50 m deep and close to shore (Otway et al., 2003; Otway and Ellis 2011).

C. taurus have been kept in public aquaria worldwide since at least 1896 (Townsend, 1928). Throughout this time only four aquariums have recorded mating, gestation and birth of *C. taurus* (Choromanski, personal communication; Willson, personal communication), and only a handful of others have recorded births from wild-caught gravid females (Choromanski, personal communication).

Reproduction of *C. taurus* in aquaria is challenging for a variety of reasons, space being a key factor. In an aquarium environment, where it may be difficult to segregate sharks, a female *C. taurus* may be subjected to repeated and excessive copulation attempts. In one example, copulation was attempted 15 times with a single female in one season. In some cases, these repeated copulation attempts have proven fatal for the female shark (Townsend, unpublished results; Willson, personal communication)

Despite these setbacks, aquaria are increasingly investing in dedicated breeding programs for *C. taurus*. These efforts have redoubled as threats to *C. taurus* wild populations have grown, as our understanding of *C. taurus* reproductive biology has improved and as the need for sustainable aquarium populations has increased (Choromanski, 2004). It is hoped that efforts to reproduce *C. taurus* in aquaria will ultimately aid the recuperation of wild populations through information gleaned from reproductive biology studies, through the development of reproduction technologies and through opportunities to increase public awareness about anthropogenic threats to the species.

Hereafter we report on the observed feeding patterns of reproductive age male and female *C. taurus* in aquarium conditions at Manly SEA LIFE Sanctuary (Sydney, Australia).

METHODS

C. taurus at Manly SEA LIFE Sanctuary were maintained in a “donut”-shaped Oceanarium exhibit of 3,000 m^3 volume, representative of the Pacific Ocean, containing a variety of temperate elasmobranchs, teleosts and sea turtles. The exhibit operated as a semi-closed recirculating system with a daily blowdown rate of 1,382 m^3/day or 46% of prefiltered “make-up” water drawn from Sydney Harbour. System water was also recirculated through a bank of rapid sand filters at a rate of 25% exhibit volume/hour.

Lighting for the Oceanarium was provided by a combination of metal halide and light emitting diode (LED) fixtures, controlled by a photo switch to mimic natural photoperiods. The Oceanarium was not temperature conditioned and generally remained at 0.5ºC above ambient harbor temperature, ranging from 15.5 - 25.5ºC (mean = 20.0ºC).

A total of 13 *C. taurus* were maintained in the Oceanarium between 1980 and 2013, with an average resident population of eight individuals. The last founder was introduced in 1995, and five of the original founders remain in the collection to this day. As much as possible, a male to female ratio of 1:1 was maintained.

Reproductive activity of *C. taurus* in the Oceanarium was recorded every year and followed a similar temporal pattern to that observed in wild populations. Females in the Oceanarium only bred every second year. A number of successful *C. taurus* births were recorded from multiple females (refer Henningsen et al., this volume; Willson and Smith, this volume). Some stillborn and premature births were also recorded.

C. taurus were offered food three times per week *ad libitum*. Food items included a variety of locally caught, seasonally available fishes. Of the food fishes offered, 90% constituted flathead grey mullet, *Mugil cephalus* (Linnaeus, 1758), Australian bonito, *Sarda australis* (Macleay,

1881), and silver trevally, *Pseudocaranx georgianus* (Cuvier, 1833). The size of food fishes offered to the sharks was based on the size of prey typically taken by wild *C. taurus* (Smale, 2005)—i.e., 300 - 450 mm total length (TL), with an average mass of 1.25 kg. The number (note: not mass) of fishes consumed per month was recorded for each *C. taurus* over a five-year period.

RESULTS

It was not uncommon for individual *C. taurus* in the Oceanarium to go for a period of up to five months without accepting offered food. Juvenile and subadult *C. taurus* of both sexes (n = 3) showed the least variation in monthly feeding rates over the annual cycle, with a slight increase in food intake over the late spring and summer, between September and February (Figure 1a). A matured male *C. taurus* (n = 1) displayed a distinct annual feeding pattern, with 90% of its annual food intake consumed between November and March (Figure 1b). Mature female *C. taurus* (n = 2) demonstrated a distinctive biennial feeding pattern, consuming only a quarter of the food fishes, in a non-reproductive year (mean ± standard error = 38 ± 8 fishes), compared to intake during a reproductive year (148 ± 16 fishes) (Figure 1c). In general, annual consumption rates were highest in mature females during a reproductive year, feeding more consistently throughout the year than adult males.

DISCUSSION

The preliminary result of this study—i.e., that female *C. taurus* had an increased food intake

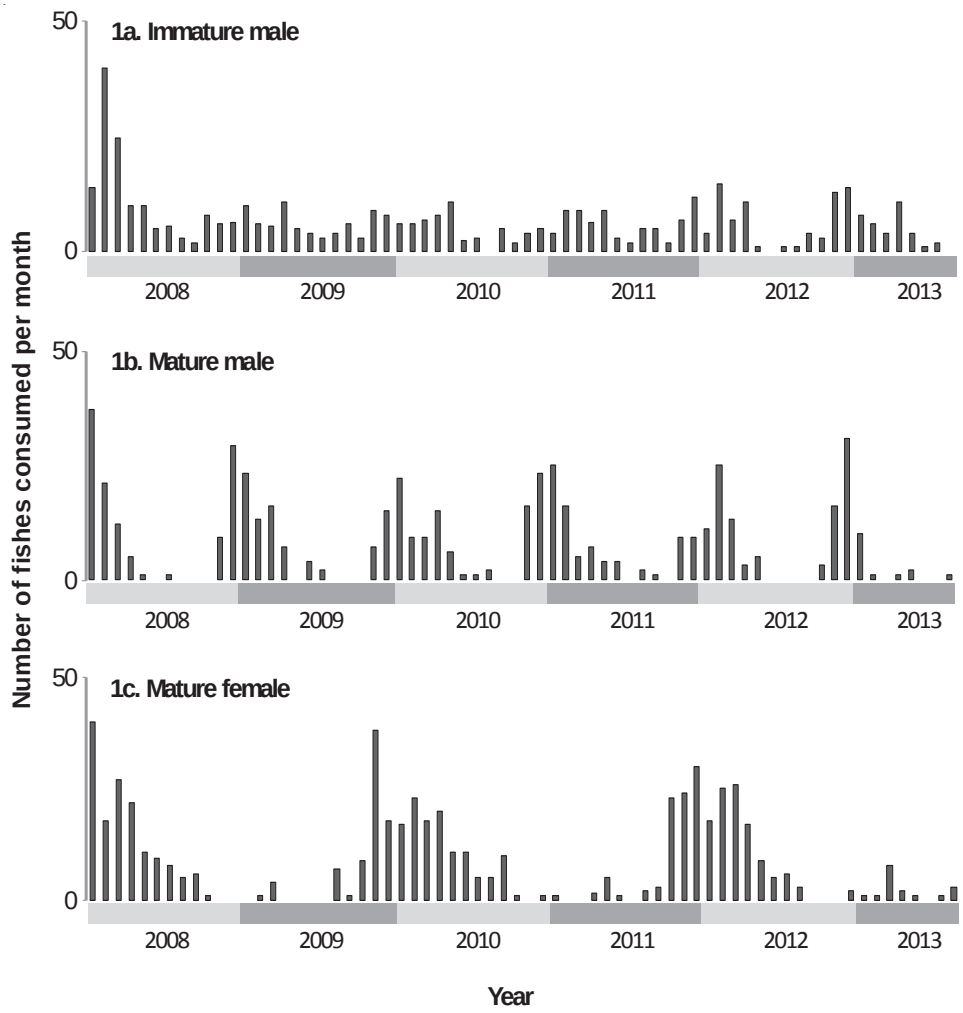

Figure 1. Feeding activity for three sand tiger sharks, *Carcharias taurus* (Rafinesque, 1810), maintained at the Manly SEA LIFE Sanctuary (Sydney, Australia), showing examples of monthly food intake from 2008 to 2013 for: (**a**) an immature male; (**b**) a mature male; and (**c**) a mature female.

during a reproductive year—is consistent with the finding by Davidson and Cliff (2011) that liver mass increases during the period leading up to a reproductive event. Increased food intake may help prepare females sharks for the rigors of the breeding season, as well as the substantial parental investment of nutriment for large, precocious offspring, consistent with the K-selected life history strategy of *C. taurus.*

The annual cycle of augmented food consumption in male *C. taurus*, prior to breeding season, may be related to the production of sperm and/or provisioning in preparation for the challenges of reproductive competition. However, changes to food intake may also be, in part or wholly, a response to changing water temperature.

With an increasing interest in the propagation of *C. taurus* in aquaria worldwide, including the growing possibility of assisted reproduction strategies, the careful tracking of food consumption and its relationship to seasonal breeding patterns may provide a useful non-invasive diagnostic tool.

Limitations of the study

It should be stated clearly that the results of this study should be interpreted with extreme caution. The sample sizes of different *C. taurus* age classes and genders were extremely small, placing the data outside the realm of statistical analysis. Inter- and intra-observer reliability questions arise from the nature in which the data was collected and recorded. The mass of individual food items was not recorded, but rather the number of fishes consumed was used as the unit of measurement. Recorded food intake did not account for any fishes taken by *C. taurus* from the living collection maintained within the same exhibit, a common occurrence in aquaria supporting this piscivorous species.

Future direction

Despite the limitations noted above, the preliminary findings of this work are intriguing and suggest a worthy avenue of study for verification. A framework of standardized food intake should be developed and rigorously implemented at other aquaria through an inter-institutional collaboration. If at all possible, such a study would include some mechanism for normalization of water parameters, lighting conditions, exhibit dimensions, energetic challenges to the sharks and *C. taurus* sex ratios.

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Chapter 16

Effects of noise and vibration on the behavior and feeding activity of whale sharks, *Rhincodon typus* (Smith, 1828), in Osaka Aquarium Kaiyukan

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Abstract: Changes in swimming and feeding behavior were observed in two whale sharks, *Rhincodon typus* (Smith, 1828), maintained at the Osaka Aquarium Kaiyukan and were concluded to result from changes in environmental noise and vibration, firstly as a result of construction sound resulting from Aquarium renovations and, secondly, attributed to the magnitude 9.0 Great East Japan Earthquake and associated aftershock activity.

INTRODUCTION

Sound generated in aquatic environments propagates five times faster than in air, resulting in far-reaching impacts (Myrberg, 2001; Weilgart, 2007; Boyd et al., 2008; Gutscher et al., 2011). There has been a growing concern about *in situ* marine organisms suffering from a variety of anthropogenic noises, including seismic exploration, industrial activities and construction, offshore wind farms, ships and sonar (Thomson et al., 2006; OSPAR, 2009; Popper and Hastings, 2009a). Studies have been conducted on the auditory threshold of teleosts (Park and Iida, 1998; Akamatsu et al., 2003) and the impact of anthropogenic noise on fishes and fisheries (Popper and Hastings, 2009b; Hawkins and Popper, 2012), yet very little is known about how elasmobranchs may be impacted by, or respond to, environmental noise. Indeed, very few studies have reported on the impact of noise and vibration on marine organisms maintained in aquaria (Anderson, 2009; Leong et al., 2009; Gutscher et al., 2011). Sound generated by visitors, sound or vibration from mechanical plant, and sound from divers and/or other animals in the aquarium could all be possible stressors for elasmobranchs.

Changes in swimming behavior and feeding activity were observed in two whale sharks,

Rhincodon typus (Smith, 1829), maintained at the Osaka Aquarium Kaiyukan (Osaka, Japan). These changes were attributed to stress induced by: (1) construction noise during a renovation of the Aquarium commencing in 2010; and (2) vibration resulting from a magnitude 9.0 earthquake, which occurred off the Pacific coast of Tohoku, Honshu Island, Japan on March 11th, 2011.

METHODS AND RESULTS

Environment

R. typus were maintained at the Osaka Aquarium Kaiyukan in the cross-shaped Pacific Ocean exhibit (maximum horizontal dimension = 34 m; depth = 9 m; and volume = 5,400 m^3). The exhibit operated as a semi-closed recirculating system with an average monthly blow-down rate of 3,500 m^3/month or ~7.8 times per annum. System water was recirculated through a closed filtration system at a rate of 37% exhibit volume/hour and water temperature was maintained at 25.0°C. *R. typus* were exhibited with a variety of other marine species, including 1,308 teleosts divided into 43 species, and 98 elasmobranchs divided into 25 species.

Food and feeding

R. typus were fed twice a day, once in the morning and once in the afternoon. The maximum amount of food fed during the cases described in this study was 10.1 - 11.7 kg/day. *R. typus* diet consisted of different proportions of: opossum shrimp (Family: Mysidae); Antarctic krill, *Euphausia superba*; sakura shrimp, *Sergia kishinouyei*; Japanese anchovy, *Engraulis japonicus*; and Mazuri Shark/Ray Gel # 5C8X (Mazuri®, Purina Mills LLC, Nutrition International LLC, Richmond, USA; www1). Food ration was supplemented with Mazuri Vita-Zu Shark/Ray Tablet # 5M24 (Mazuri®, Purina Mills LLC, Nutrition International LLC, Richmond, USA; www2).

R. typus feeding sessions were initiated using an audio and visual cue—i.e., by slapping the water surface with a long-handled food ladle, or by scooping up and pouring water from the ladle.

Table 1. Swimming behavior ethogram for whale sharks, *Rhincodon typus* (Smith, 1828), at the Osaka Aquarium Kaiyukan, Japan. Swimming behavior was observed for one hour, three times a day, at 09:00, 10:45, and 15:15.

Swimming behavior	Description
Surface swimming	Duration of time (min) with part of body above the water surface.
Swimming depth	Duration of time (min) in the upper 4.5 m of the exhibit.
Transition	Frequency of times moving between the upper 4.5 m ('floating') and lower 4.5 m ('submerging') of the exhibit.
Swimming direction	Duration of time (min) swimming in a clockwise direction.
Turning	Frequency of turns from a clockwise to anticlockwise direction, and anticlockwise to clockwise direction.
Lap time	The duration of time (min) required to circumnavigate the exhibit.
Accidental contact	Frequency of contact with exhibit wall or acrylic panel.
Mouth open	Frequency of mouth fully open (>30 cm) while swimming.
Mouthing	Frequency of mouth opening and closing while swimming.

Table 2. Feeding behavior ethogram for whale sharks, *Rhincodon typus* (Smith, 1828), at the Osaka Aquarium Kaiyukan, Japan.

Feeding behavior	Description
Swimming behavior before feeding	Noteworthy swimming behavior before feeding (e.g., increased speed, approaching feeding station).
Foraging behavior before feeding	Frequency of foraging behavior at water surface prior to feeding.
Approaching food ladle	Intensity of approach when cued to feed with food ladle.
Response to food ladle	Speed of reaction to food ladle.
Food sucking	Intensity with which food is sucked into the mouth.
Ladle avoidance (case study 2 only)	Shark leaves the food ladle during a feeding session.
Foraging behavior after feeding	Frequency of foraging behavior at water surface after feeding.

Monitoring behavior
Swimming behavior was observed three times a day, for one hour, and scored according to a nine-criteria ethogram, detailed in Table 1.

Feeding behavior for each session was assessed and scored according to a seven-criteria ethogram, detailed in Table 2. A summation of the seven feeding scores was recorded as overall "feeding status". The total mass (kg) of food consumed was also recorded.

Case study 1: the impact of construction
An individual *R. typus* was introduced into the Pacific Ocean exhibit of the Osaka Aquarium Kaiyukan on June 15th, 2008. Between July and November 2010, the Aquarium launched a large-scale renovation, which included extensive chipping and scraping. Much of this activity was carried out at night.

During building renovations, behavioral changes were observed in *R. typus*. These behavioral changes intensified in late October, corresponding with an increased frequency of renovation activity. Observed behavioral changes included an increased amount of time spent swimming near the surface (Figure 1a), an increased preference for swimming in a clockwise direction (Figure 1b), and an increased turning frequency (Figure 1c).

Despite the changes in *R. typus* swimming behavior, feeding activity remained unchanged during October and November. Renovations continued as scheduled and the shark became inappetent on December 11th. More invasive aspects of building renovations were cancelled, as stress from the associated noise was deemed to be the most likely cause of the observed behavioral changes and inappetence. Concern for the welfare of the *R. typus* resulted in the shark being transferred to the Osaka Aquarium Kaiyukan Biological Research Institute of Iburi Center (OBIC) on 7 January 2011 to facilitate its recovery. The condition of the shark improved; feeding status normalized within a month and, although direct comparison was not possible due to differences in tank design, the shark resumed what was considered to be normal swimming behavior.

Case study 2: the impact of an earthquake
An individual *R. typus* was introduced into the Pacific Ocean exhibit of the Osaka Aquarium Kaiyukan on 11 September 2007.

The Great East Japan Earthquake (magnitude = 9.0 - 9.1) struck off the coast of Sanriku at 14:46 JST on Friday, 11 March 2011. It was the fourth most powerful earthquake recorded in global history (Shibahara, 2011; Imamura and Anawat, 2012). The primary earthquake and a series of aftershocks

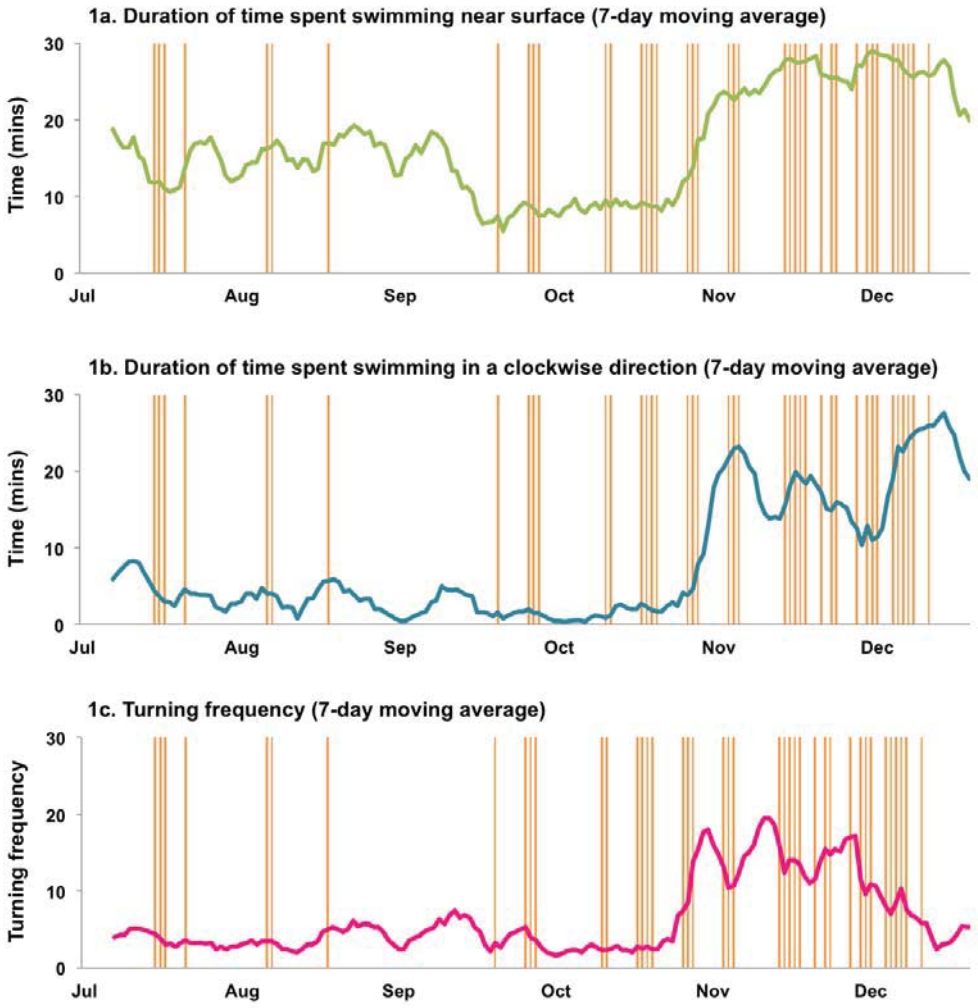

Figure 1. Change in swimming behavior of a whale shark, *Rhincodon typus* (Smith, 1828), during a large-scale renovation of the Osaka Aquarium Kaiyukan (Japan) in 2010, showing: (**a**) duration of time spent swimming near surface; (**b**) duration of time spent swimming in a clockwise direction; and (**c**) turning frequency. Orange bars indicate days when construction activity was undertaken. Note the behavioral changes in late October when construction activity intensified.

swept the Osaka Aquarium Kaiyukan. A seismic magnitude of 3.0 was recorded in Minato Ward, Osaka City (Japan Meteorological Agency, 2012). The earthquake visibly impacted the eight-story Aquarium. Underwater piping and airtight covers were damaged, waves formed in the Pacific Ocean exhibit and water in the exhibit became excessively cloudy, resulting from suspension of particulate material from damage to the building and possibly also from air entrainment through water surface disturbance.

After the initial earthquake a number of aftershocks were recorded across Japan, some measuring a seismic magnitude of 5.0 or greater. No signs of unusual behavior were observed in the *R. typus* immediately following the primary earthquake, but nine days thereafter feeding activity began to change. During the next four months there were several incidences (n = 6) of inappetence, which appeared to be related to the Great East Japan Earthquake and the many subsequent aftershocks with a 5.0 magnitude or greater (Figure 2a). In addition, from late May, the *R. typus* spent a lower proportion of time near the surface of the exhibit (Figure 2b). By late June, the shark started swimming in smaller, tighter circles in the center of the tank, and the time to circumnavigate the exhibit started to decrease (Figure 2c).

The observed changes in shark behavior prompted the husbandry team on July 23rd to transfer the *R.*

typus to the OBIC, a shorter building than the Osaka Aquarium and less susceptible to seismic shaking. Within a day the appetite of the *R. typus* returned and its feeding activity continued to improve over time. Although the swimming behavior of *R. typus* could not be directly compared between the OBIC tank and the Pacific Ocean exhibit, due to differences in tank design, the shark resumed what was considered to be normal swimming behavior.

DISCUSSION

Case study 1: the impact of construction

Elasmobranchs detect sound using a well-developed sense of hearing, which plays a critical role in their lives (Myrberg, 2001; Casper and Mann, 2009; Casper et al., 2012). Elasmobranchs and teleosts detect audible and non-audible vibrations differently. Elasmobranchs lack an internal gas chamber and there are compositional differences between teleost otoliths and elasmobranch otoconia (Casper et al., 2012; Hawkins and Popper, 2012). As a result, the physiological response of bony fishes to sound and vibration may be more profound (Casper et al., 2012; Hawkins and Popper, 2012) than that of elasmobranchs. The inner ear and semicircular canals of *R. typus* are the largest in the animal kingdom, highly responsive to long wavelength, low frequency, sounds (Martin, 2007). It is possible that *R. typus* in the Pacific Ocean exhibit was the most vulnerable species to long wavelength, low frequency, sounds generated by the construction activities associated with Aquarium

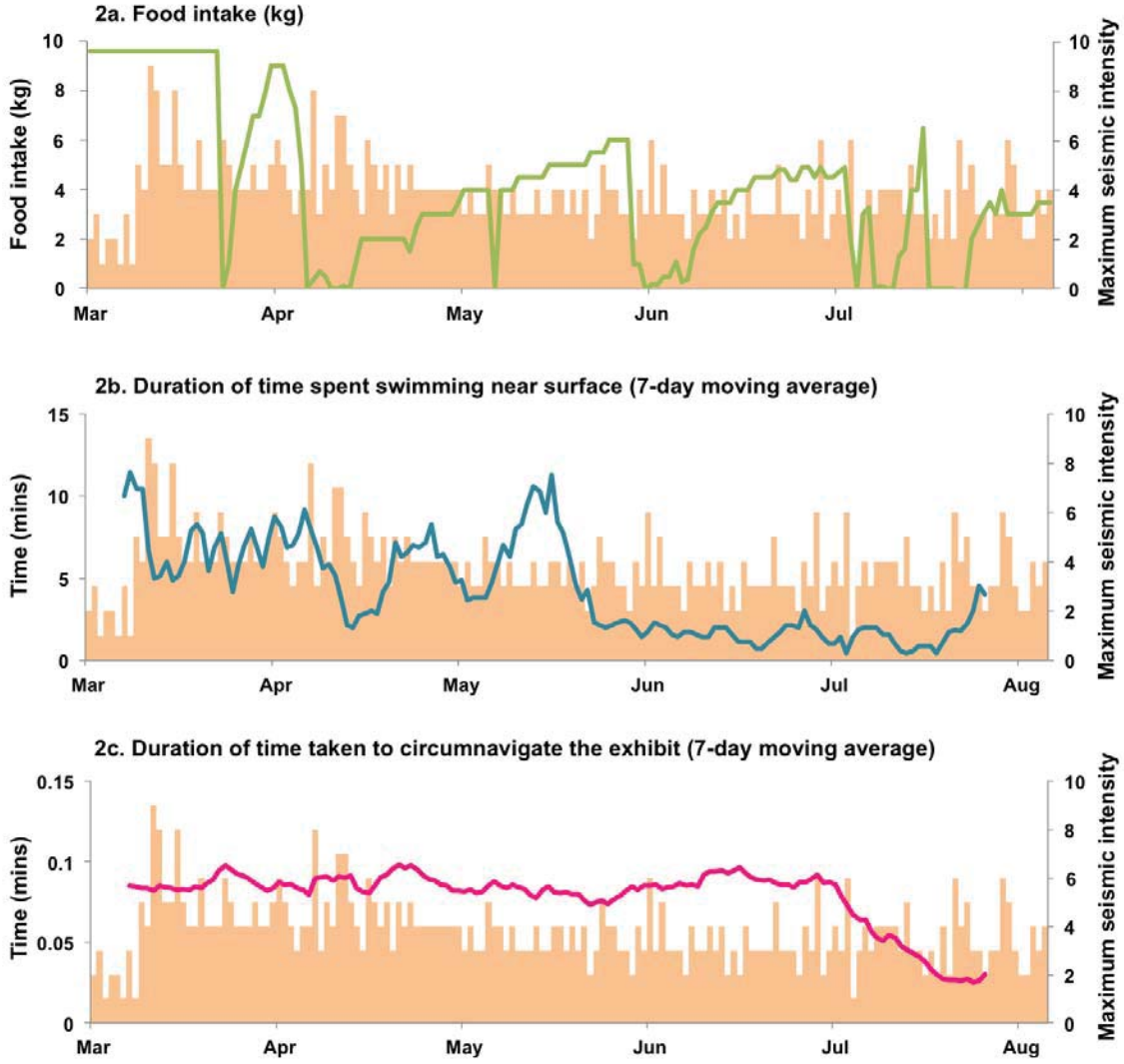

Figure 2. Change in feeding and swimming behavior of a whale shark, *Rhincodon typus* (Smith, 1828), during a period of significant seismic intensity (orange bars) at the Osaka Aquarium Kaiyukan (Japan) in 2011, showing: (a) food intake, (b) duration of time spent swimming near surface, and (c) duration of time taken to circumnavigate the exhibit.

renovation. During building renovation, teleosts and other smaller elasmobranch species showed no noticeable behavioral changes. It should be noted, however, that no species aside from *R. typus* was quantitatively assessed using an ethogram.

Avoidance behavior is the primary defensive mechanism used by fishes in the event of adverse environmental change, predator presence or social conflict (Ursin and Eriksen, 2004; Huntingford et al., 2006). Avoidance behavior is a known response to sound in various teleost species (Malar and Kleerkoper, 1968; Blaxter and Hoss, 1981; Knudsen et al., 1992; Mueller et al., 1998; Buscaino, 2010). Nedwell et al. (2003) described two methods to assess teleost avoidance behavior to noise: startle reaction and fish activity level. The startle reaction was defined as a sudden C-shaped flexure of the body. Fish activity level was defined as a change in movement, commonly expressed as increased swimming speed and random turning. In a study by Popper and Hastings (2009a) on the impact of anthropogenic noise, it was observed that wild fishes responded to unpleasant sounds immediately, by employing avoidance behavior. Whereas, fishes in aquaria were unable to make such substantial movements in response to sound. According to Smith et al. (2004), rapid changes in sound characteristics stimulated alarm behaviors in goldfish, *Carassius auratus* (Linnaeus, 1758), and could elicit a greater stress response than chronic exposure to noise. This finding suggests the possibility of habituation by fishes to continuous exposure to a homogeneous sound. Avoidance behavior in elasmobranchs has been observed in response to the sudden onset of an intense sound (Myrberg, 2001). It is possible that intermittent noise associated with the construction work at the Osaka Aquarium Kaiyukan triggered a startle response in *R. typus* and resulted in the observed increase in activity level of the shark.

Case study 2: the impact of an earthquake

Modified *R. typus* swimming behavior following the Great East Japan Earthquake may have been a learned response. Experiments testing teleost response to aversive stimuli have been used to study cognition and memory (Portavella et al., 2004; Yue et al., 2004; Lee et al., 2010; Xu et al., 2012), as well as other aspects of fish biology, including hearing (Csányi, 1986; Fréon et al., 1993; Soria et al., 1993; Valente et al., 2012). Aversive stimuli studies in elasmobranchs have been employed to better understand their sensory organs (Tester and Kato, 1966; Nelson, 1967) and level of cognition (Schwarze et al., 2013). Studies have shown that teleost fishes are able to retain their memory of aversive stimuli for a period of time (Olla and Davis, 1989; Csányi et al., 1989; Magurran, 1990).

When the Great East Japan Earthquake shook the Pacific Ocean exhibit, in addition to the resulting intense low frequency vibrations, an increase in particulate matter and suspended gas bubbles was observed. Dissolved gas pressures also may have changed, possibly indicative of a near-shore ocean swell, seismic activity or some other change. It is suggested that similar environmental changes, resulting from the aftershocks, may have triggered a learned aversion response in the *R. typus* and caused the abnormal swimming and feeding behavior of the shark observed in the months following the primary earthquake.

PREVENTATIVE MEASURES

Habituation

Teleosts and elasmobranchs have demonstrated habituation (i.e., a diminishing physiological or emotional response to a frequently repeated stimulus) to sound (Knudsen et al., 1992; Mueller et al., 1998; Myrberg, 2001). If the *R. typus* had been exposed to construction noise, with a gradually increasing intensity over time, then habituation to the sound may have developed.

Masking

Masking occurs when the threshold for a particular stimulus is increased through the use of a "masker" stimulus, which interferes with and covers the primary signal by partially or entirely reducing its audibility (Hawkins and Popper, 2012; Landos, 2012). A number of studies have examined the effects of masking on fishes (Smith et al., 2004; Wahlberg and Westerberg, 2005; Wysocki et al., 2006). Hawkins and Popper (2012) concluded that masking, and a temporary threshold shift (TTS) for audio stimuli, at continuous elevated noise levels, was likely to occur in elasmobranchs. With careful verification that the masker stimulus does not become a stressor to *R. typus*, the use of a masker may be employed to mitigate the impacts of construction noise in the future.

Conditioning

Positive reinforcement training, via operant conditioning and environmental enrichment, has been used successfully to reduce stress in fishes (Galhardo and Oliveira, 2009). If food and feeding

were associated with noise resulting from construction, then a shift in the perception of the sound from a negative to a positive stimulus may be possible.

Timing

Hawkins and Popper (2012) suggested that the behavioral response of fishes to noise might vary depending on age, size and activity at the time of exposure. They noted that fishes might be less responsive to sounds while feeding or laying eggs, as compared to how they would respond when resting. If construction activities were timed to commence during *R. typus* feeding sessions, the responses of the shark may have been less pronounced.

Observations of *R. typus* nocturnal swimming behavior (from 18:00 to 6:00), under normal aquarium conditions, indicated homogeneous activity with little variation, suggestive of a resting and recovery phase. This suggestion was corroborated by an occasion when SCUBA divers attempted to raise an *R. typus* from the bottom to the surface of the exhibit, for a medical exam, in the late evening (20:00). The shark remained calm for several minutes but then "roused" and began to swim vigorously, suddenly became more alert. The major renovation work at Osaka Aquarium Kaiyukan was undertaken at night to avoid inconveniencing aquarium visitors. However, if nighttime normally represents an opportunity for *R. typus* to rest and recover, the noise associated with nocturnal construction work may impose an elevated stress on the shark. It is suggested that future construction work should be scheduled during the day, allowing time for *R. typus* to rest during nocturnal hours.

Quantification of stress

One way to better understand the impact of repetitive stressful stimuli (e.g., construction noise, earthquakes and aftershocks, etc.) on elasmobranchs is to quantify biochemical changes in the blood. For example, stress resulting from hyperactivity is reflected in changes to acid-base balance, serum osmolality, metabolites, stress hormones and hematology (Hight et al., 2007; Manire et al., 2007; Hassan-Hassanein, 2010). However, the process of restraining large sharks to obtain blood samples can further stress the animal, confounding and biasing results. Husbandry training is actively conducted at the Osaka Aquarium Kaiyukan to facilitate *R. typus* health management. Husbandry training includes habituation to restraint, palpation, gill exams weighing, biopsies, ultrasound exams, and blood sampling (Ito et al., 2012; Sodeyama et al., 2012). As a result of husbandry training, blood sampling can be accomplished with a minimum of capture and restraint stress, allowing for a more accurate assessment of blood parameters. In the future, the husbandry team at Osaka Aquarium Kaiyukan will more comprehensively assay *R. typus* blood parameters (i.e., hematology, serum chemistry, blood gas and stress hormones) as a mechanism to assess potential stress events.

Difficulty handling animals and the cost of blood analyses has historically hindered research on stress in aquarium fishes. However, the ethical maintenance of animals under human care necessitates that potential stressors be thoroughly investigated and minimized. Aquarium personnel should actively assess stressful stimuli within their aquaria, using ethograms, blood parameters and other stress indicators, and develop solutions to mitigate those stimuli. Results from these studies should be actively disseminated throughout the public aquarium community.

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Chapter 17

Preliminary investigation of electricity in aquaria with elasmobranchs

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Abstract: It has been well known for some time that elasmobranchs detect faint electric fields that aid in navigation and prey location (Hueter et al., 2004). Electrical components within aquaria usually operate with some measure of errant voltage "leaking" into the aquatic system, but how this may affect elasmobranchs has not been thoroughly investigated. A total of 39 aquaria housing elasmobranchs were tested for electrical potential (voltage). All aquatic systems, with the exception of one, had detectable levels of electrical potential. The highest recorded potential was 482 mV, with 29 (74.6%) of the aquatic systems recording over 100 mV. Multiple readings were taken from 32 aquaria, yielding variances between readings of >100 mV in 13 (40.6%) of the systems. There was no observed significant correlation between the magnitude of measured electrical potential in aquaria and the exhibition of idiosyncratic behavior by elasmobranchs.

INTRODUCTION

In an effort to provide a healthy and dynamic environment for elasmobranchs, we surround them with many potential sources of electromagnetic noise. Despite anecdotal cases of elasmobranchs being adversely affected by errant voltage, there is a dearth of information about the possible effects of the associated electromagnetic fields and at what point they may become problematic for sharks, skates and rays. As researchers have discovered more about the sensitivity of the elasmobranch electro-sensory system, it has become clear that aquarists must better understand the impact of errant voltages from life support systems (and other sources) on the animals within their care.

The nature of voltage and current

Voltage is the potential for electrons to move, from a source with more electrons to a destination with fewer. The actual movement of electrons is termed current. Any aquarist who has been accidentally shocked by a broken glass heater or wet power strip is very familiar with current. Voltage is more difficult to comprehend, as humans have no innate way to sense it. Many taxa, however, can detect voltage, including some amphibians, bony and jawless fishes, mammals (monotremes) and elasmobranchs.

All aquatic organisms generate a voltage, relative to the water, via ion transport during natural body processes such as osmoregulation and through muscle action (New and Tricas, 1998). Voltage (and current) that moves in one direction is called

direct current (DC), while voltage (and current) that oscillates back and forth is called alternating current (AC). The passage of ions across mucous membranes produces DC, while muscle action potentials create AC.

Electric fields can be categorized as uniform and non-uniform. Uniform fields are consistent in magnitude and direction. In the context of this article, they are fields generated by a distant source and are considerably larger than the length of the animal—e.g., an electromagnetic field generated by the alternating orientation of magnetic components within the sea floor. Non-uniform fields are smaller, multipole fields, such as those generated by prey species (Peters et al., 2007) (Figure 1).

Electro-sense has evolved, disappeared, and evolved again many times over the eons (Kempster et al., 2012). Elasmobranchs can sense both DC and low-frequency electric fields (Bedore and Kajiura, 2013).

Ampullae of Lorenzini

Elasmobranchs sense sources of voltage via an organ called the ampullae of Lorenzini, described hereafter. Pores on the surface of elasmobranch skin lead to canals that are lined with tightly joined epithelial cells, which are covered by collagenous fibers (Figure 2). The canal walls are highly resistant: 6 million Ohms/cm. The canals terminate at alveoli, clusters of which form the ampulla chamber. The chamber is lined with receptor and supporting cells, which, like the cells of the canal wall, are tightly joined. The high resistance of the canal wall, coupled with the lack of gaps between the cells, maintains the internal potential of the animal, compared to the potential of the external environment. The canals are filled with a highly conductive ionic jelly, called lumen, which conveys external potential through the canals to the ampulla. The receptors produce a steady discharge of neural signals; positive (cathodal) stimuli increase neural activity, while negative (anodal) stimuli decrease neural activity. Multiple ampullae are grouped into clusters and distributed around the head of an elasmobranch and, in the case of skates and rays, also the pectoral fins (Kalmijn, 1974; Bleckmann and Hofmann, 1999; Hueter et al., 2004). Signals from the ampulla are interpreted somatotopically—i.e., neural impulses inform the animal from which direction the stimuli are coming (Kalmijn, 1974; Bleckmann and Hofmann, 1999; Hueter et al., 2004).

As mentioned, aquatic animals produce their own bioelectric fields. Elasmobranchs employ different

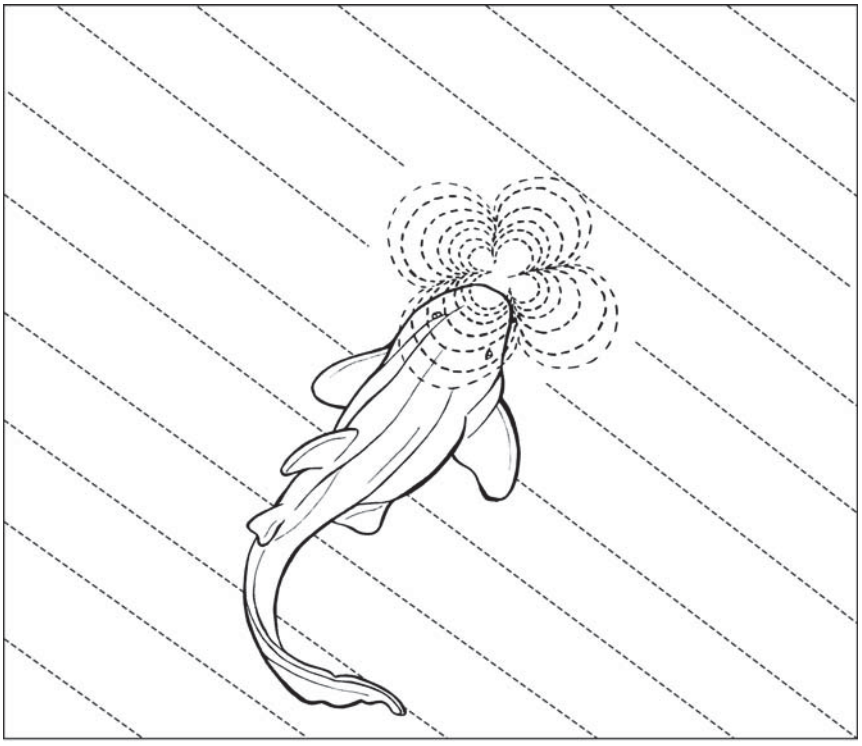

Figure 1. Illustration of a shark swimming through a large uniform field (parallel lines), generated by a distant source (e.g., the magnetic field of the earth), and encountering a small non-uniform field (clover-shaped lines), generated by a prey species.

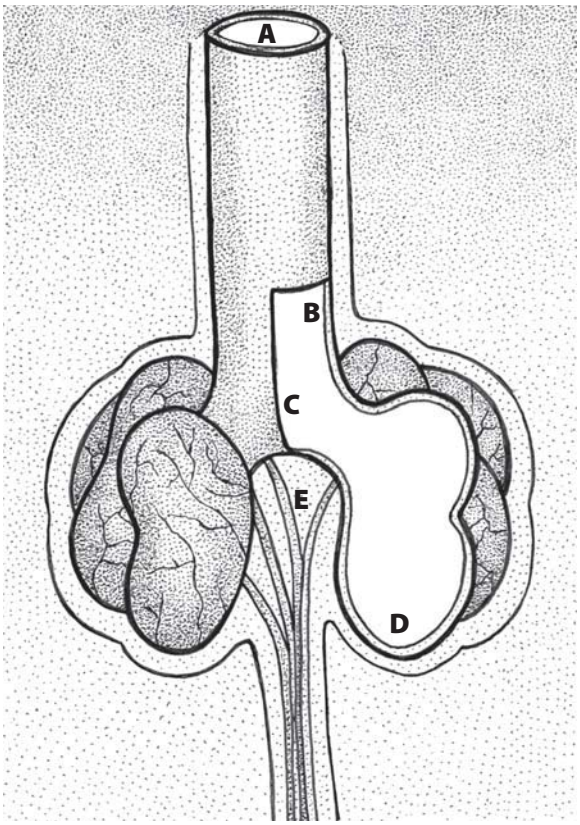

Figure 2. Anatomy of an ampullary organ, showing: (A) pore; (B) resistant canal wall; (C) conductive lumen; (D) alveolus; and (E) afferent nerve.

strategies to nullify the interference of their own bio electric field. Their neurological system has an adaptive filter, a mechanism by which electro-sensory signals resulting from their own movement are eliminated from the brainstem in favor of novel signals (Bodznick et al., 1999). Elasmobranchs may further reduce electro-magnetic interference, while seeking prey, by minimizing body movement, employing a "sit-and-wait" strategy, or by temporarily remaining rigid (Montgomery and Bodznick, 1999).

By the numbers

The electrical potentials encountered by wild elasmobranchs are minute and the ampullae of Lorenzini is correspondingly highly sensitive. Elasmobranchs produce fields below 50 µV and teleosts produce fields near 500 µV. Wounded animals produce fields of a higher magnitude (Kalmijn, 1972; Kalmijn, 1974). Animals in a laboratory setting have exhibited responses to potentials as low as 5 - 10 nV/cm. In laboratory settings, excised ampullae of Lorenzini displayed potential thresholds of 5 nV/cm, and dusky smooth-hound, *Mustelus canis* (Mitchill, 1815), tested in the wild, exhibited behavioral responses to potentials of 5 nV/cm (Bleckmann and Hofmann, 1999). Researchers measured a voltage gradient at the surface of the Atlantic Ocean ranging from 0.05 - 0.5 µV/cm.

The majority of literature on electro-sense in elasmobranchs focuses on minimum potential thresholds necessary to elicit a neurological or behavioral response. Another major focus of work, however, has revolved around human attempts to illicit an avoidance response in elasmobranchs by using electromagnetic fields to repel wild elasmobranchs from divers and swimmers, and to minimize elasmobranch bycatch in commercial fisheries. Unfortunately, little research has been conducted that illuminates what happens between these two electromagnetic extremes, which would be of great interest to aquarists who maintain elasmobranchs in aquaria.

Studies suggest that, like many elasmobranch sensory systems, the electro-sense is highly flexible and can readily compensate for background electromagnetic noise within seconds. Adaptability, while maintaining functionality, has been demonstrated with background potential levels as high as 1 mV, which is 2,000 times the usual potential threshold (Bodznick et al., 1993).

Directly measuring the minimum voltage that elicits a behavioral response is difficult and has only been determined for a few species (New and Tricas, 1998). Scalloped hammerheads, *Sphyrna lewini* (Griffith & Smith, 1834) and sandbar sharks, *Carcharhinus plumbeus* (Nardo, 1827), both of which are held in public aquaria, responded to a median stimulus of 0.025 - 0.030 µV/cm (Kajiura and Holland, 2002).

Ampullae as an indicator of modality

The number and distribution of ampullae do not indicate sensitivity to potential (i.e., the minimum threshold an animal can sense), but they do offer clues to natural history. Ampullary systems have been completely mapped for only a small percentage of species. However, work in this area demonstrates that the morphology of the ampullary system can vary widely from species to species, and that variation reflects evolution to particular ecological niches.

Pore abundance varies widely, from 178 in the horn shark, *Heterodontus francisci* (Girard, 1855), to over 3,000 in species such as the largetooth sawfish, *Pristis microdon* (Latham, 1794). Some Pristiformes, including *P. microdon*, are found in extremely muddy waters, where electro-sense

would confer an advantage. Animals with keener vision tend to have fewer pores (Kempster et al., 2012). In benthic elasmobranchs, such as the yellow stingray, *Urobatis jamaicensis* (Cuvier, 1816), the majority of ampullary pores tend to be located ventrally, especially around the oral region, where they help the ray locate buried prey. Yet *U. jamaicensis* have a greater proportion of dorsal pores than the bentho-pelagic cownose ray, *Rhinoptera bonasus* (Mitchill, 1815). The higher proportion of dorsal ampullae may aid *U. jamaicensis* in predator detection and avoidance, as danger is likely to rain from above, whereas *R. bonasus* spends much of its time in the water column (Kempster et al., 2012; Bedore and Kajiura, 2013).

Dorsal pore versus ventral pore distribution is typically asymmetric, but the left versus right side of both surfaces have a symmetrical distribution of ampullae. Differential stimulation between the two sides may help an animal orient within a large field (Peters et al., 2007). The exact arrangement of the pore array varies from species to species; reflecting ecological advantage gained through the filter of evolution. Camperi et al. (2007) modeled the electrical inputs for a barndoor skate, *Dipturus laevis* (Mitchill, 1818), as it traveled near a potential food source, and compared the inputs to those of a hypothetical skate with evenly-spaced pores and canals of equal length. The magnitude of change in voltage for *D. laevis*, with a heterogenous pore array, was much greater than that of the hypothetical skate, demonstrating a greater capacity for detecting prey (Camperi et al., 2007).

Canal length has implications for the range and sensitivity of the electro-sense. Longer canals sample along a greater distance and are better suited for signals further away (far-field). Shorter canals can discern smaller gradients in near-field electrical potentials. Most elasmobranchs have canals of various lengths to accommodate a wide range of voltage potentials. However, benthic elasmobranchs, which are closer to geomagnetic fields and closer to benthic prey, have a greater proportion of shorter canals (Kempster et al., 2012).

The skin of freshwater fishes is relatively impermeable to ions, to help maintain osmotic balance. Their interior is of homogeneous potential, or isopotential (Kramer, 1996). Small potential gradients are managed by mini- and micro-ampullae, which are small, have very short canals, and are individually located on the head and pectoral fins (Kalmijn, 1974; Hueter et al., 2004). A study comparing the ampullary systems of a euryhaline ray with those of a marine ray concluded that the two morphologies had more in common than those of a euryhaline ray and a freshwater ray.

Employment of electro-sense

Most experiments with live elasmobranchs have focused on smaller species, which are easily acquired and maintained in aquaria. As a result, more is known about the electro-sensory system of smaller species. Lesser spotted dogfish, *Scyliorhinus canicula* (Linnaeus, 1758), presented with a piece of whiting, *Merlangius merlangus* (Linnaeus, 1758), and an electric field generated by two electrodes, simulating live prey, showed much more interest in the two electrodes. The sharks only ate the food item if they physically bumped into it. This finding suggested that electrical stimulation was more attractive to *S. canicula* than chemical stimulation (i.e., the smell of the whiting) (Kalmijn, 1971), a suggestion corroborated in the field in both *M. canis* and blue sharks, *Prionace glauca* (Linnaeus, 1758) (Hueter et al., 2004).

A possible mechanism for how elasmobranchs use a non-uniform field to locate the source is illustrated in Figure 3. Haller's round ray, *Urolophus halleri* (Cooper, 1863), detects conspecifics during mating season by sensing the bioelectric fields generated by the movement of the spiracles, mouth and gill slits during ventilation. Male *U. halleri* use the fields to detect females buried in the substrate, while females use the fields to seek out other females. Male Atlantic stingrays, *Dasyatis sabina* (Lesueur, 1824), become more sensitive to the frequencies produced by females during mating season. Researchers have been able to induce this seasonal change in electro-sense acuity by implanting male *D. sabina* with androgen steroids (Montgomery and Bodznick, 1999; Tricas and Sisneros, 2004).

Embryos of the clearnose skate, *Raja eglanteria* (Bosc, 1800), circulate water through their egg case by moving their tails. Although this movement is necessary, to refresh the water in the egg case, it sends a trail of chemical cues to potential predators, which may also sense the electrical field generated by the movement of the tail. When *R. eglanteria* egg cases were exposed to electromagnetic fields, simulating a predator, it elicited a "freeze response" in the embryo, ameliorating the chemical and electrical cues given off by the unborn pup. A "freeze response"

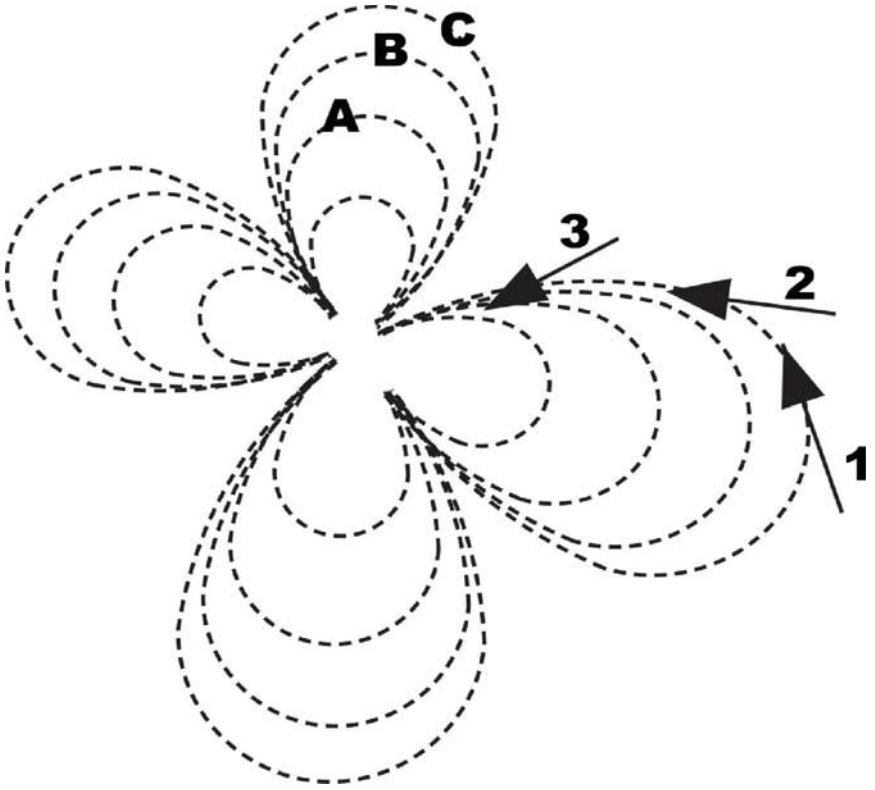

Figure 3. Diagram of a multipole electrical field, showing a hypothetical method of navigation that may be employed by an elasmobranch. Isopotential lines are indicated by A, B, and C, akin to a contour map. Each point along line A is of the same potential and each point along line B is of the same potential, but of lower magnitude than line A, and so on for lines B and C. The arrows represent an approaching elasmobranch encountering the field at point 1. If it maintains its angle of approach relative to the field as it progresses through points 2 and 3, it will ultimately reach the source of the field.

has also been demonstrated in newborn *S. canicula* (Tricas and Sisneros, 2004)

It has been observed that elasmobranchs use the geomagnetic field of the earth, as well as the electromagnetic fields generated by their own movement through the water, to aid navigation (Kalmijn, 1974). *U. jamaicensis* was shown to differentiate between an anode and cathode in a dipole field (Siciliano et al., 2013). Both *S. canicula* and *U. halleri* have been demonstrated to use electromagnetic fields for orientation (Tricas and Sisneros, 2004).

ASSESSING VOLTAGE IN AQUARIA

The most common method to check for errant voltage in an aquarium is to use a voltmeter or multi-meter. One of the probes of the multi-meter is connected to a ground and the other is placed in the water. The multi-meter is then monitored as electrical components, in and around the exhibit, are individually unplugged. A significant drop in the magnitude of the voltage implicates the equipment responsible. This method was successfully used by the authors to trace the source of an electrical leakage in a stingray touch pool at the Virginia Marine Aquarium and Science Center (Virginia Beach, Virginia, USA). The problem was first denoted by the appearance of abrasions on the rostrums of a large number of *R. bonasus* in the touch pool. Following the elimination of more obvious environmental challenges, the possibility of an electrical leakage was suspected and confirmed. The source of the leakage was a malfunctioning heater. Once the heater was removed, the lesions started to heal and were gone within two weeks.

METHODS

A survey was conducted during 2013 - 2014 to assess the impact of electricity in public aquaria maintaining elasmobranchs. The intent of the survey was to illuminate possible relationships between observed voltages, aquatic system design

and/or observed idiosyncratic or unusual behavior exhibited by elasmobranchs.

Institutions participating in the survey provided detail about their aquatic systems, specifically: tank construction materials; location of electrical life support system components; presence or absence of system-specific grounding probes, and if they were regularly inspected; species maintained within the exhibit(s); and any history of upgrades to life support systems, structural components and/or habitat décor. Survey participants were also asked to report on any idiosyncratic behavior noted within their elasmobranch collections, both at the time of the survey and historically, especially if electricity had been implicated.

Survey participants were also asked to test their aquatic systems for electrical potential (measured in mV), by connecting the water to a "ground" via a multi-meter. Respondents were asked to ensure that their equipment was calibrated and functioning correctly, and to take multiple readings throughout the day if at all possible.

RESULTS AND DISCUSSION

In total, 41 systems from 19 institutions were tested for electrical potential using a multi-meter. Data from two systems was excluded, as there were questions about the veracity of the technique and the robustness of the data sets. All of the remaining aquatic systems (n = 39), with the exception of one, had detectable electrical potential. The highest recorded potential was 482 mV and 74.6% of the aquatic systems had readings over 100 mV. Many survey respondents took multiple readings from individual aquaria (n = 32) and observed variances between readings of over 100 mV in 40.6% of systems.

Six respondents reported unstable electrical potential readings that bounced between two numbers, or dropped over time until it stabilized. It is possible that "bouncing" potential may have been the result of water movement at the testing site, as testing in the calmest area of an aquarium typically produces the most stable readings. A slowly decreasing voltage can indicate the presence of an accumulated charge that is "bleeding out" via the multi-meter, akin to a battery running down. It is possible that this situation arises when an electrical component responsible for charge buildup is turned off at the time of testing, which raises concern over the effectiveness of the grounding of the system. In almost every case, husbandry teams had never inspected the grounding mechanism for their building or their exhibits. No significant difference in electrical potential was observed between systems with dedicated grounding probes versus those without.

Elasmobranchs in 40.6% of systems displayed idiosyncratic behaviors, including repetitive pacing, rubbing on tank walls, darting across the tank, breaching inclined swimming, mouth agape, slack jaw, and inappetence. Some animals had incurred damage to the rostrum or nodules on the leading edges of their pectoral fins, suggesting repetitive contact with tank walls and/or décor. There was no significant correlation between the magnitude of measured electrical potential and the presence of idiosyncratic elasmobranch behavior. However, in two specific cases, husbandry teams were able to trace the cause of unusual behavior and/or physical damage to the chronic disturbance of elasmobranchs resulting from an electrical source, in both cases faulty heaters.

One survey respondent described a wave generator that added 20 mV of measurable voltage to a system when it was in operation. Aquarists from another facility reported health challenges associated with strong magnetic fields emanating from pumps adjacent to the aquarium. Half of their elasmobranch collection had contracted a fungal infection and the sharks periodically presented with a slack jaw or mouth agape. Once the suspect pumps had been removed, the slack jaw/mouth agape behavior and the fungal infection resolved.

Shark mortalities were observed at another facility where, ultimately, it was determined that electricity was bleeding into their elasmobranch exhibit. Prior to observed mortalities, the sharks exhibited darting behavior and inappetence, and had sustained extensive physical damage to their rostrums. The sharks actively avoided a portion of the exhibit. In this case, teleost fishes were also notably affected by the presence of the electricity.

CONCLUSIONS

Standard multi-meters and voltmeters are not capable of detecting the minute electromagnetic fields found in nature, nor are they adequate for determining the size or shape of a field. The commonly accepted technique for testing errant voltage, described above, tells us more about the

conditions within the aquarium than it does about how electrical potentials may be affecting elasmobranchs. The method described may be useful for locating a faulty piece of equipment, but no correlation was observed between the data provided by the multi-meters and the behavior of the animals. Given these restrictions, animal behavior remains the best indicator of an environmental challenge, which, only in some specific cases, may be the result of errant electrical potential.

Elasmobranchs are surrounded by electrical noise. The action of water moving through the magnetic field of the earth is sufficient to induce an electric field, so a marine aquarium will always have electrical noise (Kajiura, personal communication). Although elasmobranch electro-sensory systems are very adaptable to a constant level of input from background sources, these sensory systems did not evolve in an environment of myriad electrical components turning on and off and/or leaking electricity into the water. An ever-variable background level of electromagnetic input, sometimes of a magnitude well in excess of what would be encountered in the wild, makes adaptation of elasmobranchs to background electrical noise in aquaria almost impossible (Bodznick et al., 1993; Kajiura, personal com-munication). In addition, the highly conductive nature of seawater can exacerbate these challenges. Even if no obvious path exists between an electrical source and system water, there are many insidious routes for electricity to travel into or through an exhibit—e.g., a trail of salt creep, a small crack in concrete allowing water to contact ferrous rebar, microfractures in the glass casing of a heater, to name just a few (Stevens, 1994). Grounding systems can help dampen electrical noise, but they, like other life support components, must be regularly inspected and maintained to assure their functionality. Any corrosion or missing connections can render a ground useless or, worse, create a shock hazard.

Investigating this issue was fraught with challenges. Data and testimonials from survey respondents were incomplete and, at times, inaccurate. In some cases, this situation was interpreted to be the result of sensitivities surrounding animal losses and/or a culture of corporate secrecy. Written information provided by one survey recipient was wholly inconsistent with accounts relayed in person. At least one institution, known to have significant issues with voltage leaks in their elasmobranch system, did not respond to the survey at all. If we are to advance the husbandry of animals in our care, it is critical for aquarium colleagues to share their experiences, both good and bad. The authors found colleagues in the academic community to be particularly willing to help and will cultivate such connections in the future.

Great care should be exercised when interpreting the results of this preliminary study. Although aquarists at each facility were asked to calibrate their equipment, different makes/models of multi-meter were used in almost every case. Each system tested was unique, and the time of day, temperature, humidity and other environmental conditions were not standardized. As a result, the findings should be carefully judged on their empirical merits only and cautious conclusions should be drawn. Nevertheless, the reader is directed to the value of the general approach outlined in this chapter re assessing their systems for electrical potential. Benchmarking aquatic systems and then periodically checking the same systems under the same conditions can be a valuable tool for the detection of changing electrical conditions in and around elasmobranch exhibits.

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Chapter 18

Elasmobranch touch pools

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Abstract: Advances in aquarium science technology and animal husbandry have provided an excellent foundation for creative visitor interactive programs involving all types of elasmobranchs. The demand for more intimate experiences is driving the industry to the limits of technology, animal welfare and visitor engagement. Recognizing and applying best practices in the design, construction and management of elasmobranch touch pools is crucial to the long-term success of these experiences. Human safety, animal welfare and health policies, related to collection management, are necessary to ensure the wellbeing of the elasmobranchs, as well as the quality of the experience for the participating public.

INTRODUCTION

In the 1980's, a transition occurred in public aquarium and zoo education. The use of dried and preserved animal artifacts, to supplement educational experiences, became overused and less effectual. The "take home" message was frequently uninspiring and lackluster. A revolution was occurring across the industry as the public wanted to get more in touch with the "wild side" of the institutions they were visiting. Providing personal, intimate experiences with animals became a conduit for education and the visitor experience changed forever. Today, hundreds of institutions worldwide incorporate live animals into their visitor education experiences. Among the varied animal interactions provided by modern institutions, aquatic touch pools are particularly popular and are used to bring visitor 'finger-to-fin' with the aquatic world.

The concept of a touch pool or touch tank is relatively simple. An aquarium open at the top, or a water-filled pool, holding aquatic organisms, is placed within easy reach of visitors. Mediated by staff or trained volunteers, visitors are encouraged to explore the contents of the pool with their hands. Successful touch pool experiences incorporate a variety of different aquatic organisms. Historically, and still most commonly, aquatic invertebrates are used in touch pools. But there is an impetus to provide 'bigger' and more personal experiences, with an increasing variety of animals. Elasmobranch touch pools are a logical progression of this trend. Elasmobranch touch pools tend to be larger, technologically more sophisticated and require more staff time for husbandry and maintenance than traditional touch pools. Facilities that rush to develop elasmobranch touch opportunities, without appropriate research, experience or clear goals, struggle to meet the demands of their visitors and the animals in their care. Visitor satisfaction, human safety and animal health can all suffer. Visitor safety and high impact experiences, coupled with high animal welfare standards, are critical to the success of touch pool programs. Thoughtful exhibit design and comprehensive management practices yield long-term success and the intended positive outcomes for visitors and animals.

This chapter outlines some core principals that should be considered when developing a new elasmobranch touch pool experience or reviewing an existing program. Throughout this chapter reference will be made to a survey that was conducted in 2013 (hereafter "2013 Survey"), which reviewed many aspects of existing

elasmobranchs touch pool experiences, drawn from 40 aquaria worldwide.

TOUCH POOL MANAGEMENT

Making the commitment

Before any discussions regarding exhibit design or management take place, aquarium personnel should take the time to establish clear goals for the elasmobranch touch pool at their institution. Elasmobranch touch pools require substantial resources to start-up and maintain, as well as ongoing management far beyond any similar visitor experience. Unlike traditional exhibits, touch pools are more subject to variables connected with visitor impact. Touch pools cannot operate autonomously and require constant monitoring. In addition, elasmobranch touch pools bring with them increased risks and liabilities. The ramifications of investing in such a visitor experience can be far-reaching and may materially impact the operation of the aquarium. Start-up capital for an elasmobranch touch pool is only a small fraction of the total investment needed over time. An elasmobranch touch pool is a commitment that must be well considered and stakeholders from husbandry, to operations, to finance, should all be represented when considering whether a touch pool experience is a sustainable undertaking for the institution. Animal welfare, visitor satisfaction and operational viability should be the ultimate goals of an elasmobranch touch pool.

Risk management and liability

Risk management and liability mitigation are important aspects of any touch pool operation, and must be thoroughly addressed. The risk and liability associated with a visitor physically engaging elasmobranchs is real. Teeth are only one of the defensive measures available to elasmobranchs. However, the magnitude of risk and liability can be inflated, resulting from populist sensationalism perpetuated by tabloid media.

Aquarium personnel must speak openly and honestly with risk management and liability experts to ensure informed decisions are made regarding the touch pool experience. A strategy outlining how to respond to negative publicity should be held early in the process to help inform operational planning and exhibit design. Publications detailing the risks associated with aquatic animals in public spaces should be consulted (refer: Nemetz and Emmet, 1993; Lehane and Rawlin, 2000; Lewis et al., 2003; and Smith and Cradon, 2003) In 2013, the National Association of State Public Health Veterinarians published the Animal Contact Compendium of Measures to Prevent Disease Associated with Animals in Public Settings (NASPHV, 2013). While the document does not specifically address touch pools, it does provide a foundation for developing measured approaches to mitigating risk, including ways to communicate risk to visitors in a positive and empowering way. Local, State and Federal liability laws may be invoked under certain circumstances. A facility with a touch pool must be fully prepared to justify the suitability of their program, establishing appropriate management and response protocols that demonstrate due diligence, best practices and transparency. Appropriate insurance policies and safety signage must also be in place.

Supervision

Above all other considerations, safety of visitors, the staff and the living elasmobranch collection must be the prime objective of any interactive animal experience. Firm, friendly supervision of the experience ensures consistent, positive interactions for touch pool participants. Passive supervision can be provided through the use of cameras, but active supervision using trained staff or volunteers is more effective and highly recommended. Active supervision provides for immediate response to animal welfare concerns and challenges surrounding the visitor experience. Employing staff or volunteers as touch pool monitors also provides an ideal opportunity to communicate conservation education messaging. Appropriate supervision of a touch pool can make all the difference between a glowing report from visitors and a complaint. It should be acknowledged that trained active supervisory staff represents one of the core ongoing costs of ownership for an elasmobranch touch pool.

Supervision should be employed whenever visitors have access to the pool. Dedicated barriers or covers should be employed to prevent non-supervised visitor interactions when the exhibit is closed. In many cases, visitor access to touch pools is not only provided during regular operating hours, but also during special events and facility rentals outside of normal operating hours. The 2013 Survey revealed that every institution (n = 40), with the exception of one, used staff and/or volunteers to monitor touch pool activities. Of those aquaria, 50% had dedicated training programs for touch pool interpreters.

Training
The following topics are recommended for the training of staff and volunteers who will supervise and interpret elasmobranch touch pools:

- Techniques for touching the live collection, and, where appropriate, feeding methods;
- Typical and atypical behavior of the live collection, and possible triggers for stress;
- Protocols to respond to real or perceived injuries sustained while visitors are participating in touch pool activities;
- Water safety and emergency response training, including the use of emergency response tools (e.g., life rings or body hooks);
- Techniques to encourage positive interactions between the live collection and visitors, particularly techniques to alleviate visitor apprehension and encourage the timid to participate;
- Information about the natural history and biology of the live collection, including general information about elasmobranchs;
- Key educational talking points that convey core messaging in a positive manner; and
- Techniques to encourage turn over of visitors at the exhibit during periods of heavy visitation.

Communication
It is crucial that touch pool supervisors and husbandry staff remain in constant communication. Touch pool supervisors are frequently the first to recognize early signs of potential animal health concerns. Daily reporting on the behavior and feeding pattern of the living collection can aid the process of communication, and can lead to early responses to issues of concern that have animal health or visitor experience implications.

SPECIES SELECTION

More than 500 species of rays and skates, two species of chimaera, and 400 species of sharks are currently described. Not all species are suitable for touch pool applications. The selection of species for a touch pool may seem logical, but is not always the case. Species with a proven track record in touch pools should be the starting point for formulating a stocking list. Adopting a trial-and-error approach to species selection is discouraged and can prove economically and politically costly. Consulting with experienced colleagues is highly recommended when developing a species list for a touch pool.

Species selection tool
To aid in the selection of touch pool species a tool has been developed (Figure 1). Used in conjunction with life support system and exhibit design, this tool will aid in determining appropriate elasmobranch species for a touch pool.

Common touch pool species
The 2013 Survey showed that the most common elasmobranch touch pool species were: cownose rays, *Rhinoptera bonasus* (Mitchill, 1815), southern stingrays, *Dasyatis americana* (Hildebrand & Schroeder, 1928), Atlantic rays, *Dasyatis sabina* (Lesueur, 1824), whitespotted bamboosharks, *Chiloscyllium plagiosum* (Anonymous [Bennett], 1830), brownbanded bamboosharks, *Chiloscyllium punctatum* (Müller & Henle, 1838), and epaulette sharks, *Hemiscyllium ocellatum* (Bonnaterre, 1788).

Nurse sharks
Nurse sharks, *Ginglymostoma cirratum* (Bonnaterre, 1788), were a very popular species for touch pools when interaction programs were in their infancy. Their hardy and compliant nature, ease of care and low cost of acquisition, made *G. cirratum* appear to be an ideal candidate species, so high numbers of smaller specimens were collected for touch pools. The small sharks thrived and quickly outgrew their exhibits. As a result, many aquaria were faced with a growing population of larger *G. cirratum* and no repositories for the surplus animals. Some sharks were moved to larger, non-touch exhibits, but others were released back into the wild or euthanized. *G. cirratum* are a good example of a species that seem to be well-suited to a touch pool, but when long-term management of the species is considered, turn out to be unsuitable. This species epitomizes the need for thoughtful exhibit goal setting, collection planning and exhibit design. Nurse sharks are not recommended for most touch pool situations.

Freshwater stingrays
Freshwater stingrays in the genus *Potamotrygon* (Garman, 1877) are rarely used in touch pools, but with some simple precautions can be successfully incorporated into a program (Atteberry, personal communication). Touch pool experiences involving freshwater stingrays provide excellent opportunities to teach visitors about elasmobranch diversity and about freshwater conservation issues. The use of freshwater stingrays in touch pool programs can also generate holding and breeding space for this conservation-dependent group. However, before incorporation of these beautiful and exotic sting-

A

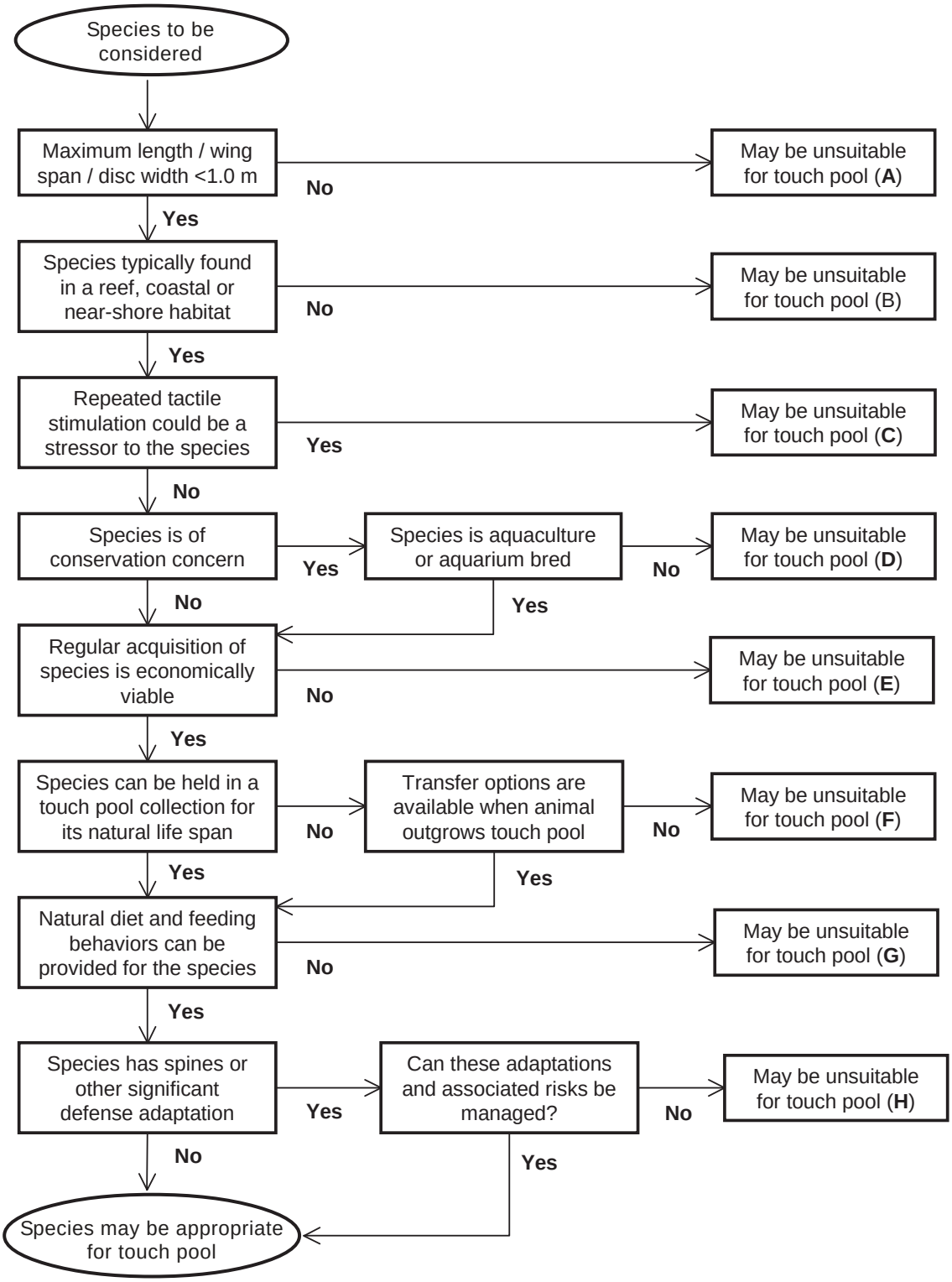

Figure 1. Touch pool species selection tool, including (**A**) a decision tree (above) and (**B**) explanatory notes (right page).

B

(A) *Size:* Most touch pool designs and management budgets cannot provide for a collection where individual specimens exceed 1.0 meter in size; total length (TL), disc width (DW) or wing span (WS). If larger species are desired, life support systems must be suitably designed to manage the nutrient load. The exhibit must provide ample space for: the natural swimming behavior of the larger animals, zones of easy accessibility to visitors and zones where touching is not possible, allowing the elasmobranchs a period of respite. Some aquaria may consider temporarily holding smaller, younger individuals of a larger species in their touch pool. In this case, rigorous transfer plans for the species should be established prior to acquisition, which should not include release into the wild or euthanasia.

(B) *Habitat:* Open water species may not be suitable for the complex environment of a touch pool, as tactile stimulus can induce a flight response resulting in physical injury. Pelagic or open-water species do not typically encounter vertical obstacles and will not respond well to the close quarters of a touch pool. If a species naturally encounters obstacles and objects that bump or brush the body (e.g., mangrove roots), is typically exposed to unexpected changes in the environment (e.g., reef drop-off versus a lagoon), or encounters acute visual disturbances (e.g., splashing or crashing of waves), then it may more readily habituate to the repeated tactile stimulation and environmental changes associated with a touch pool.

(C) *Tactility:* The premise of a touch pool is the tactile interaction between visitor and animal. It is possible to habituate, or desensitize, some elasmobranch species to touch, which must be completed before the animal is used for interaction with visitors. Some elasmobranch species do not habituate to tactile stimulation and do not make good candidates for a touch pool. In addition, to provide sufficient touching opportunities for visitors, touch pools are densely stocked to provide ease of interaction. Species that do not thrive in inter-specific or intra-specific groups, or that tend to be highly competitive for space or food, may be contraindicated for a touch pool situation.

(D) *Conservation status:* Species of conservation concern may not be available without costly permitting and a time-consuming acquisition processes. Additionally, such species may not be available in the numbers, or frequency required, for most touch pool scenarios. In general, careful thought should be given to the ethical implications and suitability of an endangered species for a touch pool.

(E) *Economics:* Whether obtained through wild collection, aquaculture or an aquarium breeding program, species sourcing logistics and economics must be carefully examined and determined to be sustainable. Touch pools require a large number of specimens and a routine addition of specimens over time. If specimen acquisition and resource costs are unviable over the lifetime of the touch pool, then the species should not be considered a candidate for display.

(F) *Life history:* Some species may be excellent candidates for a touch pool before they become sexually-mature and then become too large or present a risk to visitors once they mature. If a species cannot be maintained in a touch pool for the entirety of its life—e.g., it becomes too large, or its behavior changes when it reaches sexual maturity—then a coherent transition plan must be in place. Common species may be a challenge to transition if there is typically a surplus throughout the aquarium community. If transitioning a species is a unviable, then it may not be a suitable candidate for a touch pool.

(G) *Food and feeding:* Reducing specimen stress must be a core consideration when planning and designing a touch pool. Allowing an animal to exhibit natural feeding behavior promotes health and reduces stress. The provision of an appropriate ration of nutritious food (including supplements), which closely replicates wild diets, is essential to maintaining animal health, reducing stress and supporting a strong immune response. If exhibit design, husbandry constraints or limited resources do not allow for a high-quality diet or accommodate natural feeding behavior, the species may not be suitable for a touch pool.

(H) *Defense:* Many elasmobranch species have physical and behavioral adaptations that pose a risk to humans. Many stingrays have spines or barbs that can cause injury during human interaction. Spines can be humanely clipped; a procedure that may be required every 3 - 4 months. For some species, the use of sedation or anesthesia may be required to appropriately conduct the clipping procedure. Both sharks and rays have the potential to bite a human during an interaction. The use of management techniques—i.e., dedicated feeding stations, isolation during feeding, careful instruction to visitors about how to touch an animal, etc.—can substantively minimize the risk of these negative interactions. Some species may not be suitable for a touch pool because their defensive responses are too aggressive or potentially injurious to humans.

rays into a touch pool program, various issues must be considered. Envenomation by freshwater stingrays can result in a severe injury, typically considered to exceed that of marine stingrays (Reynolds et al., this volume). The small barbs or hooks on the dorsal ridge and tail are also mildly venomous. Spines should be carefully clipped on touch pool specimens (refer to Reynolds et al., this volume). The freshwater environment necessary for potamotrygonids introduces an enhanced risk of zoonotic disease (see below), as microorganisms found in freshwater may be more easily transferred to humans than those living in saltwater. Hand washing stations or hand sanitizers near the touch pool will assist visitor hygiene (Holbrook, personal communication).

LIFE SUPPORT SYSTEMS

Well-designed and appropriately sized life support systems are an essential element of a successful touch pool. From a visitor perspective, a touch pool is not inviting if the water is colored, turbid, high in suspended material (particulates) or covered in floating material (surfactants). The appearance of the water may directly affect how visitors approach a touch pool, the duration that visitors interact with the exhibit and the emotions they take away from the experience. An appropriate life support system provides good water aesthetics for the visitors, as well as optimum water quality for the animals, which is essential for a healthy, immunologically robust collection.

Touch pools have water quality challenges that are uncommon to many aquarium systems. Nutrients and oils from human hands are combined with nutrients from animal food and waste, creating a complex chemical soup that needs to be remediated by the life support system. Many early touch pool life support systems were underdesigned and undersized, not fully accounting for the impact of human interactions and the dense population of animals. The resulting water quality was poor, animal health was unstable and the visitor experience suffered. When designing a life support system for a new touch pool, or evaluating the efficiency of an existing aquarium, it is important to consider the total nutrient load, or bio-load. Although it is difficult to accurately calculate the bio-load, approximations can be made. The principals of adequate system water volume, appropriate turnover rate, and robust biological, mechanical and chemical filtration all apply, as for any elasmobranch aquarium (Mohan and Aiken, 2004). Reference to water treatment systems on successful touch pools at other aquaria can provide an excellent starting point for preliminary calculations, which should be considered within the context of the proposed animal collection, the number of visitors expected per day and the average visitor dwell time.

System volume calculation

A touch pool must be of sufficient volume to maintain good water quality even at peak times, when animal stocking density is high and visitors are greatest in number. Appropriate system volume helps dilute and hydrolyze waste products, and allows the life support system time to process dissolved and particulate material. To aid in the calculation of an appropriate volume for a touch pool, a model formula was developed and tested. This formula accounts for both contributions from the animal collection and also the visiting public who interact with the exhibit.

Volume for the collection

Determining the volume of water required to adequately maintain elasmobranchs can be approximated using the anticipated number and size of the animals in the collection, or the collection to volume ratio (CVR). A good starting 'rule of thumb', which has been used successfully for many years, is 15 L of water for every 1.0 cm of fish (i.e., 1" = 10 US gal). Using data from the 2013 Survey yielded an average of 38 specimens from each touch pool, with an average size (TL for sharks and DW for rays and skates) of 61 cm. Using the CVR tool we obtain a theoretical volume for the average collection (TVAC) of 34.77 m^3 (38 specimens x 61 cm x 15 L). This figure can be used as a starting point for calculating the size of a new touch pool, but does not account for the impact of human byproducts, which must also be considered when determining an appropriate exhibit volume.

Volume for the visitors

Each visitor to a touch pool implies hands coated with debris, lotion and other organic and inorganic material. When considering visitor impact on a life support system, it is necessary to estimate the maximum number of people who will interact with the exhibit during one day. This figure can be calculated by factoring maximum exhibit visitor capacity by the number of times visitors turn over in a day. The 2013 Survey yielded an average maximum exhibit capacity of 43 people. Average

visitor residency time was 10 min, equating to a visitor turnover of 48 times/day for an aquarium open for 8 h. These figures yield a result of 2,064 visitors per day. By arbitrarily assigning one visitor to 15 L of water we equate visitor to volume ratio (VVR) to CVR. However, it is unlikely that one visitor would add as much nutrient load to the water as one centimeter of fish, so a correction factor (CF) of 50% has been arbitrarily assigned to the calculation. Using the VVR tool we obtain a theoretical volume for the average maximum visitor number (TVAV) of 15.48 m^3 (2,064 visitors x 50% x 15 L).

The final calculation

By adding TVAC to TVAV we obtain an estimate of total touch pool volume required to support the nutrient impact anticipated for both the elasmobranch population and visitor interactions. Thus, the theoretical touch pool volume = TVAC + TVAV = 50.25 m^3. Results from the 2013 Survey yielded an average touch pool volume of 52.96 m^3, only 5.4% more than the calculated theoretical volume. The survey reported few challenges to water quality in existing touch pools, even on busy days, suggesting that the size of the exhibits was adequate. Given the close agreement between the calculated theoretical volume and the actual volume of the average touch pool, it is proposed that these equations can be used as an estimator of required exhibit volume. To summarize:

Touch pool volume = TVAC + TVAV

TVAC = specimen number x average specimen size (total length or disc width in cm) x 15 L

TVAV = total number of visitors per day x 50% x 15 L

System turnover time

The turnover time, or the time it takes for one volume of the touch pool to pass through the life support system, affects the health, appearance and success of the exhibit. Turnover time should be based on the exhibit volume, collection composition and number of visitors interacting with exhibit, as well as the life support system components and the design philosophy of the life support system engineer. The CVR and VVR calculations can help inform the anticipated touch pool turnover time. The average turnover time for elasmobranch touch pools, reported in the 2013 Survey, was 45 min for exhibits in the range of 1.9 - 302.8 m^3 in volume.

Water movement

Movement of water within a touch pool assists with effective water quality management. Touch pools should be designed to have integrated and effective water movement mechanisms—i.e., well located influent and effluent lines, and strategically placed water jets directed to encourage rapid mixing of pool water with polished water returning from the life support system. Surface skimming should be optimized to quickly remove positively buoyant particulates and surface-active compounds (oils and other surface films), which frequently result from human interaction with the water.

Water movement is also a key management tool for the animal collection. Many touch pool species will respond to areas of higher current by constantly moving in and out of the area. As a result, areas of increased water movement are best placed in close proximity to visitors. Animals will be encouraged to move in and out of the area, allowing for more frequent animal-visitor interactions, and waste products will be quickly transported away from the area. Areas of reduced water movement should also be provided so that animals can retreat to calmer waters for a rest period should they desire. Typically, these areas should be located in zones away from visitors and may also be appropriate for the establishment of feeding stations. Water currents can also be adjusted to steer elasmobranchs away from the walls or décor, minimizing the risk of contact abrasions.

Biological filtration

Adequate biological filtration is crucial to the stability and health of any aquarium system. A mature, robust bacteria culture must be established prior to animal introduction, and maintained throughout the lifetime of the exhibit. New touch pools must be biologically cycled before animals are added to the exhibit to avoid system collapse, reducing the risks of animal mortality and exhibit down time.

Marine or freshwater aquaria maintained at 23°C require at least 30 - 40 days, and sometimes as long as 100 - 120 days, to establish the beneficial bacteria necessary to support aquatic life. Bacterial growth can be accelerated by increasing the water temperature to 30°C, but the temperature will then have to be slowly decreased (e.g., 0.5°C/day), to preserve the bacteria, before the live elasmobranch collection can be added to the aquarium. A touch pool is more densely populated than the average aquarium, so nutrients added to the system

during the maturation process must be similarly elevated to accommodate the large animal collection as well as the hands of visitors. Newly introduced animals produce heavy nutrient loads and can quickly overload biological systems. When an exhibit first opens, the corresponding onslaught of visitors will exacerbate the situation. Growing bacterial populations to densities beyond what would be required during normal operation is a good strategy to cater to the challenges of the first few weeks of operation.

To successfully pre-populate a biological filter, a dense 'primordial soup' of nutrients and oxygen, as well as elevated temperatures and alkalinity, must be provided. Feeding the system with nutrients at a concentration of 3 - 6 mg/L N-NH_3 throughout the maturation process is recommended. It is important to remember that nitrifying bacteria are dependent on, and consume, oxygen, carbonates and bicarbonates. Therefore, aggressive cycling of the biological filter will require good gas exchange and the addition of buffering compounds to maintain alkalinity, ensuring optimal performance of the bio-filter and maximum bacterial growth during cycling (Christie, personal communication). Once the touch pool biological system is mature, bacteria should be able to consume all added nutrients within a day. Water chemistry results should be consistent and remain at or below accepted industry standards, regardless of the collection density and visitor numbers.

Mechanical Filtration

Mechanical filtration, or the removal of particulate matter from the water column, is important to the long-term health of the touch pool system and to the visitor experience. Suspended organic material can affect water clarity and color, and large particulates, such as feces and uneaten food, can make the pool uninviting to visitors and pose a health challenge for the collection. In addition, excess organic material will be consumed by heterotrophic bacteria, which will depress oxygen, pH and ORP. Robust particulate removal technologies, such as pressurized sand filters or drum filters, should be considered when developing touch pool life support systems. The 2013 Survey revealed that 86% of elasmobranch touch pools had either pressurized sand filters or canisters, with pleated cartridge filters. A mechanical filter is most efficient when it has access to the particulate load. Good water circulation and strategic water currents within the touch pool will keep heavy particulates suspended. A high turnover rate through the life support system (e.g., a volumetric turnover time of 30 mins) will then allow mechanical filters access to the suspended material.

Chemical Filtration

Visitors can interpret clarity and color as a measure of water health. Appropriately applied chemical filtration strategies, such as ozone, ultraviolet light (UV) and activated carbon, not only provide for optimal water appearance, appreciated by quests, but also aid in disinfection.

Ozone is created from oxygen in the presence of UV light and/or an electrical discharge. Ozone is a strong oxidizer and will react with any organic substance. Appropriately applied in freshwater or marine elasmobranch touch pool systems, ozone can improve water clarity and reduce color. Ozone also helps control microorganisms that may become pathogenic to elasmobranchs and reduces the risk of zoonosis. In freshwater systems ozone is typically applied via a pressurized contact chamber. In marine systems ozone is most frequently introduced via a foam fractionator or protein skimmer. Ozone-fed foam fractionators are an excellent choice for touch pools as they process both dissolved and particulate organics and export them out of the system, where they can no longer impact water quality. In this way, foam fractionators provide both mechanical and chemical filtration. The 2013 Survey showed that 83% of institutions had foam fractionators on their elasmobranch touch pool systems and 84% of those also employed ozone. Ozone dosing at levels appropriate for micro-flocculation (typically 0.01 - 0.05 mg/L) will also improve system ORP and reduce organic dyes that color the water.

UV is another mechanism to disinfect water in a touch pool system. The efficacy of UV disinfection is affected by water clarity and contact time, so UV reactor location within the life support system, and the associated proportion of system water flow it receives, must be carefully considered. Like ozone, UV disinfection improves water clarity and reduces color. Under half (40%) of the 2013 Survey respondents reported the use of UV disinfection on their touch pool and only 20% used a combination of both UV and ozone.

Activated carbon is another common chemical filtration media used for touch pool systems. The chemical structure of carbon attracts dissolved

organics and its high surface area provides ample space for their attachment or adsorption. Activated carbon is typically incorporated within a woven or pleated cartridge filter and it is an excellent tool for augmenting mechanical and chemical filtration for elasmobranch touch pools. Should water quality start to decline in a touch pool system, the addition of activated carbon can be a useful way to boost the efficacy of the life support system.

DÉCOR, SUBSTRATE AND LIGHTING

A successful touch pool exhibit encourages intimate, one-on-one experiences between visitors and live animals. The replication of a natural habitat and animal swimming behavior must be balanced by the ability for visitors to have frequent encounters, both tactually and visually.

Exhibit décor

Exhibits should be designed so that animals will move close to the visitor and not hide in, or go behind, the décor. An understanding of the natural behaviors of the animal collection should guide the design of the décor and the internal features within the touch pool. In addition, flexibility should be incorporated into the design of the exhibits and the décor to allow for changes in the composition of the animal collection, as well as changes to accommodate the needs of the husbandry team, education staff and the visitors.

Lighting

Good illumination enhances the visitor experience, but touch pool lighting design must also consider the needs of the live collection. A bright wash of light over the entire surface of the pool may cause excessive reflection for the visitor and limit visibility of the animals within the water. In addition, uniformly bright lighting may not be suitable for animal health. However, a touch pool that is dimly illuminated may promote visitor anxiety, as they are not able to clearly see an approaching animal while they position their hand. Creative use of lighting techniques and intensities provides a welcoming exhibit and promotes visitor engagement. When visitors can clearly see what they are touching, they are more likely to engage freely, with comfort, and for longer periods of time. Recent work on sandbar sharks, *Carcharhinus plumbeus* (Nardo, 1827), suggests that environmental lighting plays an important role in their physiological welfare (Cusack, 2011). Animals should be provided the choice to be in brightly or dimly lit areas. Providing an appropriate photoperiod for the animals, with changing lighting conditions to simulate dawn and dusk, is highly recommended.

Substrate

Collection animals within a touch pool can develop contact abrasions from constantly encountering exhibit décor, or rubbing the walls and floor of the exhibit. Well-directed water currents and appropriate substrate can materially improve the environment for the animals and minimize the risk of contact abrasions. Some examples of appropriate substrate for touch pool elasmobranchs include:

- Freshwater stingrays (*Potamotrygonid* spp.): mud or fine sand.
- Eagle rays (Myliobatidae): sand or aragonite.
- Stingrays (Dasyatidae): fine to coarse sand or smooth rock.
- Smaller temperate ram-ventilating sharks: sand to small, smooth gravel or rock.
- Smaller tropical ram-ventilating sharks: fine sand to aragonite.
- Benthic, non-ram ventilating sharks: sand, aragonite or smooth rock.

EXPERIENCE DESIGN

Elasmobranch touch pool design must be thoughtfully and carefully conceived, and continuously evaluated throughout its operation. The physical design of a touch pool should effectively support the needs of the animals, the visitors, and husbandry and education personnel.

All stakeholders should be included in the design process from the beginning, to ensure every aspect of the project has been addressed and that the chances of a successful exhibit are maximized. Input from husbandry staff and life support technicians during this process is crucial. Educators who will be interacting with visitors and interpreting the exhibit must also take part in the exhibit development process. Designers are encouraged to also engage the following departments: visitor services, who will inform visitors of the experience when they enter the institution; finance, who will manage the finances for the operation of the touch pool; marketing, who will sell the experience; and human resources and risk management, who will manage legal support for the exhibit, both internally and externally.

Key considerations for the live collection

The design of an elasmobranch touch pool should take into account the needs of the species to be displayed and be sufficiently flexible to accommodate changes to those needs. In turn, collection planning should take into account the design of the touch pool and associated life support system capabilities.

The most popular geometric footprints for an elasmobranch touch pool were a circle, oval or ellipse (2013 Survey). It was reported that these shapes accommodated the biomechanical needs of sharks and rays, and provided space for the 'swim-and-glide' behavior required by most species.

In general, rounded corners and gentle slopes, between the sides and bottom, encourage animals to turn comfortably and easily maneuver within the pool. Narrow spaces and acute corners should be avoided, as should an excess of obstacles within the swimming path of the animals, which can lead to abrasions from repeated contact.

Any life support equipment within a touch pool should be placed in a manner that does not create a risk of entrapment. In addition, life support or maintenance equipment should have rounded edges and smooth surfaces to reduce the risk of lacerations or contact abrasions. If space and budget allow, touch pools should include adjoining holding pools to facilitate animal isolation for medical examinations and procedures. Isolation pools can also be used to separate animals to aid feeding strategies and to manage competition. At a minimum, an off-exhibit holding tank should be included as part of the scope for an elasmobranch touch pool project.

When designing a touch pool the development team should consider a number of key questions related to the living collection that will help inform the design. These questions have been summarized in Table 1.

Key considerations for visitors

A touch pool experience should be welcoming, comfortable and accessible to all visitors. The challenge for the development team is to ensure that all these goals are carried from the design through to touch pool operation. The purpose of a touch pool is access. It is critical that the touch pool design addresses the needs of all audiences, giving accessibility to all, regardless of capability. Key elasmobranch touch pool design criteria are outlined below.

Water depth

Water depth is important for the comfort of both the live collection and the visitors. If animals feel trapped or constrained in shallow areas, they may be less likely to approach an area where interactions are planned to occur. However, visitors may feel less comfortable extending their arms too deep into an exhibit, or having to reach too far to make contact with an animal. The 2013 Survey indicated that although touch pools varied greatly, they had an average depth, in the touching zone, of 48 cm.

Underwater viewing

Underwater viewing provides an alternative way of experiencing the live collection and can be facilitated by underwater cameras or by acrylic viewing panels in the walls of the pool. Some timid visitors may choose to view the elasmobranchs for a while and only then take the bold step of reaching out and touching an animal. Underwater viewing can also help ease visitor pressure on days of high occupancy, when access to the live collection may be delayed.

Pool edge

The edge of the touch pool must be strong and comfortable for visitors to lean on while they engage with the live collection. With an average visitor stay time of 10 min, a comfortable body position is crucial for an agreeable experience.

Ancillary seating

Not all visitors will enjoy the dynamic environment at the edge of the touch pool. Ancillary seating should be provided for those people who wish to take a more passive approach to the experience, or for those waiting their turn to access the edge of the pool.

Special needs

Whenever possible, an elasmobranch touch pool should incorporate opportunities for visitors with special needs to participate comfortably and to the best of their abilities. Wheel chair access at the edge of the touch pool should be incorporated into the exhibit design. Braille text and a highly tactile relief on labels, to promote access for the sight challenged, is encouraged.

When designing a touch pool the development team should consider a number of key additional questions, related to the visitor experience, which will inform the design. These questions have been summarized in Table 2.

Table 1. Key questions for the design of an elasmobranch touch pool: the living collection.

Species

a. What species are intended to be maintained or are in the exhibit now?
b. Is there potential for the species composition to change in the future?
c. For existing touch pool exhibits, what species did not thrive and why?

Behavior

a. What are the behavioral requirements of the live collection and will species requirements change in the future?
b. Do animals swim continuously, or require space on the bottom for rest?
c. How do the animals use the water column, surface area and exhibit perimeter?
d. How do the animals in the exhibit use and/or interact with the walls?
e. What is the preferred shape of the exhibit for the intended species?
f. Is there potential for exhibit inhabitants to jump?
g. Is stereotypic behavior common in the species to be maintained in the pool?

Food and Feeding

a. What are the feeding requirements of the collection?
b. Are there special feeding techniques required of aquarists, and does the pool design facilitate those techniques?
c. How will food competition be managed?
d. How will inappetence in the collection be managed?
e. What is the maximum amount of food that will be offered to the collection?
f. Will aquarists enter the exhibit to perform feedings, or conduct feedings from the side?
g. Will visitors feed the collection or will this be done by staff only?
h. If visitors feed the collection, how will daily food ration be determined and monitored?

Maintenance

a. How will entering and exiting the pool be facilitated safely and effectively?
b. What are the maintenance requirements of the exhibit?
c. Will special equipment be required for maintenance?
d. Will maintenance duties be performed during, or outside of, regular opening hours?
e. Is pool décor easily accessed for maintenance?
f. Will husbandry staff be required to enter the water for maintenance?

Design

a. What are the spatial requirements of the collection for movement within the exhibit?
b. Does the animal collection have the option to avoid visitor interaction?
c. Can exhibit décor be changed to meet the needs of the animals, current and future?
d. How will animal health management be facilitated by the pool design?
e. Does pool design facilitate the removal of animals regardless of their eventual size?

Infrastructure

a. Is offsite animal holding available?
b. Are routine physicals, training and reproductive activities supported?

HYGIENE AND ZOONOSIS

Physical contact with animals and the act of placing hands in an aquarium are essential to the success of the touch pool experience. The risk of zoonosis—i.e., the transmission of an infectious disease from an animal to a human—and general visitor hygiene must be a part of any touch pool management plan. Some regional zoo and aquarium accreditation associations require institutions to provide easy access to hand wash basins or hand sanitizer dispensers near touch pool

Table 2. Key questions for the design of an elasmobranch touch pool: the visitor experience.

Audience

a. Is the pool intended for use by the general public or school groups in a classroom?
b. Does the pool design consider visitors with special needs and how they may engage in the experience?
c. What ADA requirements must be accommodated?
d. Is the exhibit wheelchair accessible?
e. Have sight- and hearing-challenged visitors been considered when designing exhibit features?
f. Are visitors of all ages and heights able to engage in touch pool activity comfortably and easily?

Space

a. What space is available for visitors near the pool?
b. How many visitors are expected to engage in the pool experience at any given time?
c. Is overall visitor flow through the touch pool area impacted by the level of activity at the touch pool?
d. Can visitors view the pool from the edge only?

Education

a. Are educational programs planned?
b. What are the programming or teaching needs of the docents or educators?
c. Will educators and docents need a sound system to engage visitors?
d. How is information about the touch pool experience to be communicated?
e. Where labels are to be placed around the pool?
f. What conservation messages will be shared through signage, labels or interactives?

Experience

a. Are visitors able to actively and passively engage with the exhibit?
b. Are there other means by which the visitor can engage in the experience besides touching?
c. Are visitors able to view the collection only from the water surface, or is below-water viewing possible?
d. What technologies, like underwater cameras, can be incorporated into the touch pool exhibit that can enhance and extend visitor engagement?
e. Is space available for those not wishing to actively participate, but who wish to watch (e.g. seating, stroller docks)?
f. Is there a desire to provide in-water experiences for visitors to the touch pool?
g. What logistics are needed to facilitate in-water experiences?

Safety and Hygiene

a. What accommodations are available for visitors to sanitize their hands following their experience?
b. What safety and hygiene information should be communicated?

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exhibits. However, there is no legislative requirement for the testing of bacterial concentrations in touch pool water. Regardless, a zoonosis policy should be a key part of touch pool management, as should the monitoring and control of bacterial loads. Engaging with local and regional public health agencies will provide information on regulations and testing protocols for visitor experiences, and help provide a strong foundation for management protocols.

Historically, coliform concentrations have been the measure agencies have used to determine the health of water systems. It should be recognized that in addition to traditional “indicator” bacteria species, other potential pathogens, such *Vibrio* spp., *Mycobacteria* spp., and *Aeromonas* spp., are routinely found in aquarium systems, and can pose a health risk to humans. Understanding what organisms to track, how to properly collect and test samples, and how to interpret results in a

timely fashion, is crucial to properly managing a touch pool and to alleviating real and perceived risks related to human contact with pool inhabitants (Lowry and Smith, 2007; Boylan, 2011; Gauthier 2015; Murray, personal communication). There has been a marked increase in the number of elasmobranch touch pool experiences in recent years. It should be noted that despite this change, there has been no material increase in the incidence of human "infection" related to these interactive experiences.

Appropriate application of hygiene protocols, immediately following the touch experience substantively reduces any risk to visitors, so wash basins or restrooms should be made available in close proximity to a touch pool. However, careful thought should be given to the location of these facilities. Washbasins and sanitizer dispensers located too close to touch pools can result, deliberately or accidentally, in the addition of soaps and disinfectants into the exhibit. Concentrated hand soap, laden with surfactants, and alcohol-based hand sanitizers are not recommended for use near touch pools. Paper or cloth towels used to dry hands may be dropped into the touch pool, representing a risk to animals and life support equipment, so their use is discouraged. Warm air dryers represent a good alternative and provide a more sustainable and economical solution.

Regardless of the precautions outlined above, it is almost inevitable that, in time, a visitor will claim they have a health complaint arising from their interaction with the touch pool, not to mention the rare, but real, possibility of a minor injury from a stingray barb or a shark tooth. Facilities with a touch pool must engage their visitor services and marketing departments before they open to the public, and be fully prepared to respond to a complaint with a pre-planned response and coherent communication protocol. Having high fidelity water quality records available, to verify the health of the system, can be a very useful mechanism to assuage visitor concerns.

CONCLUSIONS

Elasmobranch touch pools represent a positive trend in conservation education. Touch pools provide a personal and inspiring experience that visitors remember long after their visit to the aquarium. When considering the construction of a new touch pool, or evaluating an existing touch pool, several serious issues must be reviewed that affect the overall long-term success of the experience and the ongoing health of the animal collection. These issues include:

1. Aquarium commitment to providing the funding and resources needed to appropriately manage the touch pool for the life of the exhibit;
2. The capacity of the Aquarium to govern and manage risks associated with the touch pool;
3. Thoughtful and ethical selection and management of the live collection;
4. Robust life support system design and ongoing water quality management; and
5. Touch pool design to facilitate animal health, visitor engagement, and accessibility for both visitors and exhibit management staff.

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Chapter 19

Elasmobranchs and guest-immersive programs

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Abstract: Recognizing and applying best practice when developing and managing an elasmobranch guest-immersion, or 'swim with', program is crucial to its success. Immersion programs can present significant and expensive challenges to an institution, but comprehensive research and planning can facilitate the development of new exhibits that readily accommodate safe and sustainable experiences. In many cases, with careful analysis and planning, it is possible to retrofit existing exhibits for guest-immersion programs. Safety, for visitors, aquarium personnel and the live animal collection, must remain the highest of priorities. Maintaining a stable environment for the animals, as a means to maintain a healthy collection, should be a central tenet of a guest immersion program. Best practices in animal welfare and animal health management must parallel efforts to maximize the experience for visitor divers.

INTRODUCTION

Guest-immersion programs involving elasmobranchs (hereafter GIPEs or immersion programs) are very popular with visitors. Immersion programs are typically developed to enhance the visitor experience and to create additional revenue streams for the host facility. Program offerings are informed by these goals, as well as the composition of the elasmobranch collection, the design of the exhibit, the skills of the husbandry and interpretive staff, the available resources and infrastructure, and the governance and risk management prowess of the aquarium team.

Also referred to as 'swim with' programs, GIPEs provide a desirable experience for visitors and can generate significant revenue. However, it is not clear how these programs impact elasmobranch collections. No studies have yet been conducted to exam the impact of GIPEs on animal physiology, behavior or health. Additionally, diving industry bodies, and regional zoo and aquarium accreditation associations, do not set or regulate policies for immersion programs. In an effort to better understand global practices for guest immersion programs in elasmobranch exhibits, a survey (hereafter 2013 GIPE Survey) was sent to national and international industry professionals operating successful GIPEs. A total of 17 institutions responded to the survey and the results have been summarized in Table 1. A core focus of the survey was to better understand possible GIPE impact on the wellbeing of elasmobranch collections.

ENVIRONMENTAL STABILITY

The ultimate goal of any husbandry management plan for an exhibit where GIPEs are conducted should be to ensure that there is minimal impact on the elasmobranchs. Changes to the environment should be kept to an absolute minimum. Environmental stability and consistent husbandry practices will help support both animal health and human safety.

Lighting

Elasmobranchs have excellent vision (Gruber, 1977) and even subtle lighting changes are easily detected (Gilbert, 1963; Gruber and Cohen, 1978). The eyes of some elasmobranch species can be damaged by constant, non-variable bright light (Cusack, 2011). Lighting schemes for exhibits

Table 1. A summary of current trends from a survey of 17 institutions operating guest-immersion programs involving elasmobranchs (GIPEs).

Current trends in guest-immersive programs in elasmobranch exhibits (GIPEs)

Development

- Annual visitation, institutional budget, exhibit shape, and collection size / composition had little influence on the ability for an institution to successfully support a GIPE.
- The goals of GIPEs developed after 2000 typically focused on three areas: the generation of additional revenue; meeting the conservation education mission of the sponsoring institution; and providing new and engaging experiences for visitors.
- Most GIPEs were operated within aquaria of 350 m^3 volume or greater.

Management

- Most GIPEs were managed either in part, or solely, by the husbandry department.
- When co-management was employed, other departments typically included the marketing and/or dive operations departments.
- GIPEs involving diving required visitors to have at least an Open Water SCUBA certification.
- 88% of GIPEs requiring SCUBA were led by a staff member certified to at least the level of Master Diver.
- Emergency response drills were conducted on a regular basis in almost all facilities that operated GIPEs and were typically conducted more than four times a year.
- Emergency Medical Services response time to a call from a GIPE program was typically less than 10 min.

Scheduling and programming

- Institutions operated 10 - 20 GIPEs/week, and hosted an average of 4 - 5 visitors/session.
- 88% of GIPEs engaged visitors for an hour or more.
- 100% of aquaria included conservation messaging during their GIPE.
- GIPE participants typically entered the water by ramp, ladder or stairs.
- 55% of GIPEs provided an alternative entry for divers with special needs.
- 33% of GIPEs allowed participants to wade in an exhibit with elasmobranchs.

Economics

- The cost to participate in a GIPE was US$100 - $150.
- The annual gross revenue generated by most GIPEs was between US$20,000 - US$40,000.
- The highest grossing GIPE generated over US$1 million per annum.

that support GIPEs should be carefully designed. In traditional elasmobranch exhibits, lighting schemes address the needs of the collection and the needs of visitors observing from the exterior. In exhibits that support GIPEs, these lighting requirements still need to be addressed. However, lighting should also address the needs of divers entering and exiting the water, as well as provide illumination for divers to clearly see the animals from within the exhibit. With the exception of dawn and dusk, illumination should remain consistent throughout the day. Changes in lighting patterns and intensity, every time a program starts or ends, may affect the behavior of the live collection and should be avoided.

Animal collection

When changes to the collection occur, through acquisition or transfer of animals, new territories are defined, new chemical cues may be encountered, swimming patterns may be reconfigured, and individual space relationships

between specimens may change. These changes in animal interaction and behavior can impact the safety of divers, and the animals themselves, during immersion programs. The timing of animal additions and transfers should be scheduled to allow adequate adjustment and recovery time for the entire collection before immersion programs are re-commenced. Husbandry and animal health staff should be consulted when determining appropriate times to suspend and resume GIPEs.

Exhibit structure

As with changes to the composition of a collection, physical changes to an exhibit can affect the behavior of the animals. The addition or removal of décor, or increased or decreased access to areas of an exhibit, may modify the way animals react to additional novel stimuli such as divers. Any changes to the exhibit should be scheduled to allow the animal collection plenty of time to become familiar with their surroundings before resuming immersion programs. Animal behavior prior to, and following, changes to the environment should be carefully tracked and analyzed to determine if dive plans or diver activities should be modified before immersion programs can be resumed. Husbandry and animal health staff should be consulted throughout this process.

Food and feeding

Many animals become conditioned to a regular feeding routine, including time of day and manner in which food is presented, and typically respond to learned environmental cues to initiate foraging (Clark, 1959; Takeda, 1961; Ramirez, 1999; Lechner et al., 2000). Changes to the food ration or randomly-changing feeding times can lead to frustration in the animal collection, which can disrupt food intake with potential health implications, and also lead to increased risk to GIPE participants as the animals may mistake ambiguous stimuli as a cue to feed. Regular feeding times afford the husbandry team an important opportunity to observe individual animal behavior, providing valuable insight into the health and wellbeing of the collection. Disrupting the regular feeding routine and food ration is not recommended.

According to the 2013 GIPE Survey, 83% of institutions fed their animal collection on days when GIPEs were scheduled. Of those institutions, 77% conducted immersion programs within three hours of feeding their collection. No evidence to date indicates that feeding prior to immersion programs poses a safety risk to visitor participants. No survey respondent reported negative interactions between the live collection and program divers following a feeding session. In general, it is recommended that feeding routines (i.e., ration, timing, duration, and technique) remain constant, regardless of whether an immersion program is scheduled or not.

Conditioning

Desensitization, or conditioning, to changes in the environment is known to reduce stress and has been incorporated into many animal management plans (Whittaker and Laule, 1998; Laule et al., 2003; Weiss and Wilson, 2003; Laule, 2010; Hellmuth et al., 2012). Twelve institutions (70.6%) used strategies to condition the live collection to GIPE activities (2013 GIPE Survey). These strategies ranged from mock dive sessions using staff divers only, to placing GIPE equipment in the exhibit to desensitize animals to its presence. One institution reported that regular dive operations were sufficient to prepare animals for immersion programs with visitors. Conditioning the animal collection prior to the commencement of an immersion program is highly recommended.

DIVING

One of the biggest differences between a GIPE and non-GIPE exhibit is the frequency with which divers enter the water and their activity once immersed. If program scheduling and the increased activity of divers in the water are not carefully considered, GIPEs can have a material impact on the capacity of the husbandry staff to do their job and they can disrupt the normal daily rhythms of elasmobranchs within the exhibit.

Public aquaria routinely require divers to enter exhibits to perform maintenance activities. Removing algae from décor, cleaning substrate, cleaning and polishing acrylic surfaces, and other general ‘housekeeping’ activities, all require hours of time underwater. The duration of these dives is typically consistent, and the activities involved, including the movement of divers, are routine. Dive plans dictated by these husbandry activities should be carried out without interruption, regardless of GIPE scheduling.

GIPE participants should be clearly instructed about how to slowly enter and exit the water, and how to move once inside the exhibit. Whenever possible, GIPE diving pathways should follow those of non-GIPE dive operations. This precaution means that the animals will have little to differentiate between program and non-program

divers. Similarly, using the same general locations for diving activity and for transiting is encouraged for both GIPE and non-GIPE divers.

Where possible, the same methods of locomotion and movement within the exhibit should be employed. Slow, gentle and deliberate movements should be encouraged and flailing limbs discouraged. Blocking the swimming path of elasmobranchs should be discouraged, as should explosive blasts of exhaled air. If non-GIPE divers remain negatively buoyant and walk on the bottom during dive operations, GIPE divers should adopt the same pattern of movement. An elasmobranch habituated to divers on the bottom may change its behavior, if divers float mid-water or overhead, potentially compromising both diver and animal safety (Ritter and Amin, 2012). The animal could also modify its swimming pattern to avoid divers altogether, lessening the impact of a close encounter with a shark or ray.

Choosing observation areas where divers remain stationary and elasmobranchs are allowed to swim close by, without feeling threatened, is critical to the success of an immersion program. When planning a GIPE dive plan, animal movement patterns during non-GIPE diving operations should be carefully observed. These observations will allow operators to anticipate normal and expected behaviors of the live collection, increasing diver safety, enhancing visitor impact and reducing stress on the elasmobranch collection.

The movement and positioning of divers during a GIPE should be determined by where and how both divers and elasmobranch feel safe and at ease. Placing divers in locations where they cannot see oncoming elasmobranchs increases diver anxiety and tension. This situation is shared by elasmobranchs, who should not be surprised by the sudden appearance of a diver (Barker et al., 2011). The activity or movement of elasmobranchs should never be constrained for the sake of getting GIPE divers close to the animal. Both divers and animals should have a clear open space to retreat. Inexperienced divers don't know what distance guarantees comfort for an elasmobranch, so the tour leader must guide GIPE participants to ensure that the sharks and/or rays have plenty of space.

In general, husbandry teams should be augmented so that expanded diving activities can be accommodated without compromising human health, through fatigue and repeated diving. Ultimately, should the frequency of dives start to impact the elasmobranch collection or the husbandry team, the number of dives may need to be curtailed until a good balance of diving activity in the exhibit, and animal and human health have been reached.

HEALTH MANAGEMENT

Elasmobranch health, as for any animal, is dynamic. Unhealthy animals may exhibit atypical and unpredictable behavior. In addition, when a malady is acute, health management can require the undiluted resources of the entire husbandry team to focus on the animal collection. For this reason, it is recommended that immersion programs be suspended when the health status of a collection changes in a manner that may pose a risk to the collection, staff or GIPE participants. Similarly, elasmobranch hormone levels, like health challenges, can change without obvious warning, and can dramatically affect the predictability and manageability of elasmobranchs. Over 75% of survey respondents (2013 GIPE Survey) indicated that animal health management considerations took precedence over GIPE scheduling at their respective institutions, in some cases resulting in program cancellation for several days.

When elasmobranchs become sexually receptive and mating activity is expected, GIPE schedules may need to be altered or the program suspended. Elasmobranch courtship behavior can be very physical (Brockman, 1975; Klimley, 1980; Tricas, 1980; Gilbert, 1981; Tricas and LeFeuvre, 1985; Whitney et al., 2004), require a lot of space, and, at times, present a material risk to divers in the exhibit. Most survey respondents (2013 GIPE Survey) confirmed that GIPE schedules were modified when elasmobranch mating activity was observed. Dives were typically aborted and the divers exited the exhibit immediately.

THE VALUE OF OBSERVATION

Prior to starting a GIPE, it is helpful to establish baseline elasmobranch behavior patterns and any specific idiosyncrasies for particular elasmobranch species and/or individuals specimens. This information will provide a valuable basis for judging the impact of the GIPE on the animal collection. Nearly all survey respondents (2013 GIPE Survey) reported the use of careful observation periods prior to, and following, the implementation of immersion programs.

Some GIPE teams reported behavioral changes in their animal collection after the start of their program (2013 GIPE Survey). A good indicator of how a GIPE may be affecting an animal collection, or individual specimen, is their behavior during feeding sessions, especially elasmobranchs conditioned to eat at a dedicated station. An alteration to feeding behavior, following the initiation of a GIPE, may be a sign that an elasmobranch is not adjusting to the immersion program, and that some change(s) may be required for the long-term welfare of the animal. Survey recipients reported, in general, that behavioral changes following the commencement of a GIPE were easily managed and corrected, where necessary.

In general, careful, quantitative observation of animal behavior, supported by objective decision-making, will help ensure the safety and welfare of GIPE participants, husbandry staff and the animal collection.

COMMUNICATION AND TRANSPARENCY

In the public aquarium and zoo industry, husbandry professionals are often asked to 'push boundaries' when it comes to balancing the needs of the animals under their care and the maximization of the visitor experience. This situation is rarely more expressed than during the preparation and execution of an immersion program. During the development phase of a GIPE, well in advance of the first dive, discussions need to take place regarding the ultimate goal of the program, the welfare of the animals involved and the continued safety of the divers (visitors and staff) participating in the program. These preliminary discussions should result in clear decision-making protocols and a chain of command that consistently supports each of these priorities. Existing GIPE operators highly recommended that program administrators, dive coordinators, and husbandry and animal health staff all be included in immersion program development. Program administrators bear the burden of risk management and loss in revenue when program schedules are modified. Dive coordinators are skilled at managing the uncertainties of humans in the water with elasmobranchs. Husbandry and animal health staff know the biological needs of the animals and can interpret idiosyncratic animal behavior. All stakeholders should have a voice in developing response strategies and program management pathways for maximum benefit to all.

The goal of a GIPE, whether to generate revenue, to further the conservation education mission, to provide new experiences for visitors or all of the above, must be transparent throughout program development discussions. Whatever the primary goal of a GIPE, the welfare of the animal collection and the safety of visitors and staff must always be the highest of priorities.

CONCLUSIONS

A guest immersion program, or GIPE, provides visitors a unique and thrilling vantage point to view and get closer to aquatic life. While relatively new to the zoo and aquarium industry, immersion programs involving elasmobranchs are the ultimate in 'adrenaline programming', are becoming very popular with visitors and provide an excellent opportunity for conservation education. A review of existing successful GIPEs has yielded some key recommendations for institutions considering the development of a new immersion program, or for those coordinators who would like to evaluate their existing program. Safety for the visitors, the staff and the animal collection is paramount. Training of personnel and clear operating protocols, supported by a clear chain of command, are essential. Keeping the environment and husbandry practices stable for the animals will help ensure that the collection remains healthy. Lighting conditions, exhibit structure, diving activities, composition of the animal collection, and food and feeding should all be kept as constant as possible. Regular and careful observation of animals (esp., behavior, color, feeding, swimming speed, etc.) should be used as a guide to their overall health status and appropriate action taken when a problem has been identified, which may include the temporary suspension of an immersion program. When well-planned and managed, an immersion program can add a safe and valuable dimension to the operation of a modern aquarium.

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Chapter 20

Diving with and handling Elasmobranchs

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Abstract: Effective husbandry and veterinary protocols for elasmobranchs often require staff to enter the water with the specimen(s) to be treated or handled. A thorough understanding of the dangers associated with handling elasmobranchs, as well as the use of equipment and techniques to minimize risk of injury, should be an essential part of training for staff working with these animals. Clear animal handling and diving protocols must be developed, trained and rigorously enforced. People interacting with elasmobranchs should wear protective clothing and personal protection equipment (PPE) supplied by the aquarium. Emergency response procedures must be established so that any injuries to personnel are treated promptly and effectively. With proper procedures and safety protocols in place, it is possible to care for elasmobranchs while maintaining the safety of both the animals and husbandry personnel.

INTRODUCTION

Humans are not the natural prey of elasmobranchs. In general, unless threatened or cornered, elasmobranchs will avoid interactions with humans. However, elasmobranchs, like other animals, can be unpredictable and may cause harm while defending themselves, or accidentally cause injury when in close proximity to a human (e.g., biting a diver while attempting to 'strike' at a nearby food item). In the confined environment of an aquarium, it is the responsibility of the aquarist to recognize the potential dangers associated with managing elasmobranchs and to understand how to work safely around these animals

UNDERSTANDING THE RISKS

Husbandry personnel should be fully aware of the dangers presented by interacting with an elasmobranch collection. All elasmobranchs have the capacity to cause harm, but the severity of the injury can vary depending on the species responsible.

Teeth

Sharks in the family Carcharhinidae can inflict deep, clean lacerations with their serrated teeth, potentially resulting in substantial blood loss. Sand tiger sharks, *Carcharias taurus* (Rafinesque, 1810), have dentition evolved to grasp and hold

prey, so bite wounds can be much less clean, resulting from puncturing and tearing. Many elasmobranch species have smaller teeth or teeth fused into grinding plates. Bites from these animals can cause crushing injuries, in some cases severe enough to break bones.

Spines
Many rays (Myliobatiformes) have venomous spines at the base of their flexible tail, which can be used to pierce or slash at potential predators. Stingray spines have a pointed tip and backward-angled barbs running down the sides. The barbs may anchor the venomous spine so that fragments break off inside the puncture wound. Rays are deceptively flexible and can rapidly and accurately whip their tail in any direction. Rays may have smaller spines on their dorsal surface, which can also have venom associated with them. Many ghost fishes (Chimaeriformes) and some sharks—e.g., the piked dogfish, *Squalus acanthias* (Linnaeus, 1758)—have a venomous spine at the leading edge of their first dorsal fin.

Abrasive skin
Direct contact with some elasmobranchs can result in abrasions from their dermal denticles. While most sharks have fine-scale denticles, some species, such as the zebra shark, *Stegostoma fasciatum* (Hermann 1783), have large angular denticles that can abrade unprotected human skin to the point of drawing blood.

Electric shock
Electric rays (Torpediniformes) have an organ capable of producing an electrical discharge, which is used to stun prey or for self-defense. Some species of electric ray can produce a shock of sufficient magnitude that it can be harmful to humans.

Saw blades
Sawfishes (Pristiformes) can inadvertently harm a human when they scythe their toothed rostrum from side to side, seeking prey or struggling during restraint for a medical procedure.

It is rare for any of these animals to harm a human. However, in an aquarium setting, when an animal is feeding, has been threatened or is cornered, or has been restrained for a medical procedure or transport, their teeth, spines or other defensive mechanisms may injure personnel. Knowing and minimizing the risks is paramount.

UNDERSTANDING THE ANIMALS

Careful observation is critical to understanding and anticipating the reaction of sharks and/or rays to a given situation, with the caveat that one should always be prepared for an animal to react in an unpredictable manner. Careful observation will reveal patterns to the behavior of specific species and individual specimens. Familiarity with these patterns will aid aquarists in making informed husbandry management decisions. For example, some elasmobranchs will have preferred swimming paths, speeds and depths. Interactions between an elasmobranch and other tank-mates may follow a stereotypical pattern. Benthic species may rest in a preferred area of an exhibit. Over time, elasmobranchs may habituate to feeding techniques and adopt predictable foraging patterns.

Ideally, observed behavior patterns will be recorded by husbandry personnel in some form of ethogram and used as a baseline description of normal behavior. Recorded characteristics may include: preferred swimming path, swimming speed, swimming depth, turning frequency, normal body position, ventilation rate, preference for an area of an exhibit and/or tank-mates typically avoided, among others. Deviations from normal behavior patterns can indicate a variety of things—e.g., a change in health status, a change in reproductive status, or that an animal is disturbed, alarmed or otherwise distressed. Although not a behavioral change, a modified coloration may indicate some form of physiological disturbance (Smith, 1992).

ACCIDENT PREVENTION

Planning
Any procedure with an elasmobranch must be well planned, with all participants fully trained and briefed. Regularly performed tasks should be detailed and formalized as standard operating procedures (see below). Irregular or novel tasks (e.g., unscheduled restraint of a shark for an emergency medical procedure) should begin with a detailed plan developed by senior husbandry personnel, in conjunction with other relevant support department personnel. The plan should clearly outline the goal to be achieved, the tasks to be performed, the designated personnel for each task, the relative timing of events, an agreement about when and how to abort the operation, and a clear mechanism for communication throughout the operation. Risks

should be identified and countermeasures prepared.

Where possible there should be two operations commanders: one commander overseeing human health and safety, and the other commander monitoring the overall procedure, as well as animal health and wellbeing. Operations commanders must be given the authority to pause and restart, or even abort, a procedure, as conditions demand (Coy, personal communication). Throughout an operation, human life safety must override all other considerations.

Once a plan has been established all staff involved must be briefed in detail, ensuring that each team member understands their role. A 'table-top' briefing provides an opportunity to ask questions and refine details to ensure a smooth operation. Once an operation begins, extraneous conversation should cease. Team members should be encouraged to speak up should they notice a problem, but otherwise remain quiet and follow the directions of the operation commander(s). Communication should be calm, clear and concise.

Standard operating procedures

A universal understanding of the techniques to work safely around elasmobranchs is essential for all husbandry staff. Standard operating procedures (SOPs) should be developed by experienced personnel and thoroughly reviewed. SOPs should then be circulated throughout the husbandry team and relevant techniques trained. SOPs should be regularly reviewed and updated. Many public aquaria have established SOPs and are generally willing to share this information with staff at new institutions. While the conditions at each public aquarium are different, SOPs from other institutions can be adapted to suit local conditions. SOPs should include the following:

A list of elasmobranch species in the collection, the aquariums in which they are being maintained, detailing associated special conditions, as well as any specific risks associated with individual specimens or a species in general. Distinguishing characteristics of individual animals for identification purposes should also be included.

Standard procedures (e.g., stingray spine clips, shark blood draws) described in detail, noting each step and areas of risk to the animal and human handler(s). Responsibilities for each member of the team conducting the procedure should be included.

Appropriate personal protection equipment (PPE) to be used for each procedure.

Emergency protocols to be followed in the event of an injury, including first responder first aid, emergency communications protocols and post incident reporting requirements.

Safety equipment

People interacting with elasmobranchs should wear protective clothing and personal protection equipment (PPE) supplied by the aquarium. Wetsuits and gloves should be worn when handling sharks. Some aquariums stipulate that staff wear chain mail diving suits (e.g., SharkArmor Tech LLC., San Diego, California, USA), offering partial or full-body protection, in particular when feeding larger predatory elasmobranchs such as bull sharks, *Carcharhinus leucas* (Müller & Henle, 1839), and tiger sharks, *Galeocerdo cuvier* (Péron & Lesueur, 1822). While chain mail suits can be cumbersome, they afford a superior level of protection compared to a neoprene wetsuit.

Kevlar® or chain mail boning gloves are recommended for handling stingrays, as their spines can readily penetrate leather and neoprene. Safety glasses should be worn when clipping stingray spines to protect the eyes from flying fragments.

Safety training

Formal safety training sessions should be conducted on a regular basis to ensure that all husbandry personnel are familiar with safe elasmobranch handling practices. All aspects of elasmobranch handling and relevant husbandry, as well as institutional SOPs should be covered in detail and staff quizzed to ensure they understand procedures and their rationale (Terrell, personal communication). For sophisticated and potentially dangerous operations, it is prudent to have inexperienced staff observe or take on minor roles only. Once newer team members gain competence and confidence, they can be more fully integrated into the hands-on team. Regular refresher training sessions are recommended to ensure that everyone remains familiar with SOPs. These refresher training sessions also provide an opportunity to review and update SOPs as appropriate.

Safety drills

All staff should be trained in appropriate emergency responses to an injury resulting from an

interaction with an elasmobranch. Conducting and documenting practice drills (e.g., an unconscious diver extraction drill) is an excellent way of ensuring that aquarium personnel understand emergency protocols. Both announced and unannounced practice drills should be performed on a regular basis. All drills should be carefully documented and a report issued to all participants indicating areas for improvement or modifications to SOPs.

FIRST AID

All husbandry staff should be trained in first aid and refresher courses run regularly. In addition to standard first aid training, staff should be instructed in techniques to address specific risks represented by their elasmobranch collection and unique work conditions. As medical personnel are unlikely to be familiar with injuries from aquatic animals, including spine envenomation, a reference binder containing the latest information on appropriate medical treatments should be maintained in a convenient location, known to all staff. When an incident occurs, the binder can be taken to the medical facility along with the patient, ensuring that the attending physician has access to up-to-date and appropriate treatment information for an elasmobranch-related injury. The American Red Cross (American Red Cross, Washington, DC, 20006, USA) is an excellent, and regularly updated, first aid resource (www1).

Shark bites may seem superficial, but blood loss can be significant and the patient can quickly go into shock. Should a person be bitten by a shark, the patient should be extracted from the aquarium, blood loss rapidly arrested, the patient reassured and medical attention immediately sought.

Venom associated with elasmobranch spines is generally not lethal unless introduced directly into heart muscle. However, stingray envenomation can be very painful and fragments of the spine may remain lodged in the wound, leading to longer-term complications (refer Reynolds, et al., this volume). It is therefore essential that stung personnel be transferred to a medical facility for treatment and monitored by clinical professionals. Elasmobranch venom consists of heat labile proteins, so it can be denatured and deactivated through the application of heat. Heating the wound, by immersion in hot water, as hot as the patient can tolerate without scalding, will provide immediate pain relief (www2).

All animal-related injuries should be reported to a supervisor and a full report compiled for future reference. What appears, at first, to be a minor incident, may ultimately result in a longer-term and graver health challenge or persistent infection. In addition, a minor interaction with an elasmobranch may be an indication of some change to the dynamics of the animal collection and therefore a precursor to a more significant event.

DIVING WITH ELASMOBRANCHS

Diving regulations

In many countries, diving in an aquarium setting is controlled by occupational diving regulations. Example governing bodies include: Occupational Safety & Health Administration [OSHA] in the USA (www3); Australian Diver Accreditation Scheme [ADAS] managed by the Department of Industry in Australia (Menzies, personal communication); International Marine Contractors Association [IMCA] managed by the Occupational Health and Safety Act in South Africa (McEwan, personal communication); Ministry of Health, Labor and Welfare in Japan (Iwata, personal communication); Ministerio de Fomento in Spain (González Sanz, personal communication); and the Ministério do Desporto in Portugal (Correia, personal communication). It is beyond the scope of this manual to detail the requirements governed by each of these authorities, but it is essential that the diving safety officer (DSO) at each aquarium understand and enforce relevant regional regulations. The Association of Dive Program Administrators (ADPA) has compiled a range of digital resources for institutional diving programs, including “example dive practices” (www3), which can be used as a template for the development of a compliant dive program (Bourbon, personal communication).

Diver vigilance

It is essential for a diver entering an aquarium containing elasmobranchs to be vigilant at all times. If at all possible, the number of elasmobranchs, by species, should be known before entering the water. The diver should not only be aware of the location of each elasmobranch, but also take into account their own position and movements in relation to nearby specimens. Elasmobranchs typically avoid a diver unless being fed. Divers should therefore avoid cornering, or blocking the natural swimming path of, an elasmobranch, as this precaution will substantively reduce the risk of a negative interaction. Similarly, divers should avoid flailing their limbs as they may

inadvertently place their extremities in the pathway of a nearby elasmobranch, where it could be mistaken for a prey item or aggressor and be bitten or stung.

Safety divers

For dive operations that require concerted and directed attention (e.g., underwater repairs to exhibit decor), an additional safety diver is recommended. The safety diver monitors the location and behavior of elasmobranchs in relation to the diver(s) performing the underwater task. The safety diver must be prepared for two important roles: (1) encouraging elasmobranchs to remain at a safe distance from working diver(s); and (2) maintaining non-visual communication with working diver(s).

Elasmobranchs can be encouraged to remain at a safe distance from working divers using a 1 - 2 m length of PVC pipe, called a “shark wand”, wielded by the safety diver (Figure 1). A shark wand should be clearly visible, so it can be used as a visual barrier to ‘guide’ an elasmobranch away from work diver(s) (Hodges and Sabalones, 2004). Black electrical tape wound in a spiral around a white PVC pipe works well. Rarely should a shark wand be used to physically steer an elasmobranch, and they should not be used to poke or strike an animal as so doing may provoke a retaliatory response. The only reason a shark wand should ever be used in this manner would be to intercept an immediate threat to a diver, an extraordinarily rare circumstance. Elasmobranchs typically become accustomed to a shark wand and recognize it as something to avoid.

The simplest method for a safety diver to communicate with a working diver is by physical touch. Whenever possible divers should stay within reach of each other, and have prearranged and recognizable touch signals.

For activities where divers cannot stay in close contact, the use of audio communication is a reasonable alternative. Audio signals can be achieved by tapping on a SCUBA cylinder with a dive knife or other metallic object, or by using a “squawker” (e.g., DiveAlert Plus v.2, Ideations, Seattle, Washington 98139, USA), attached to the

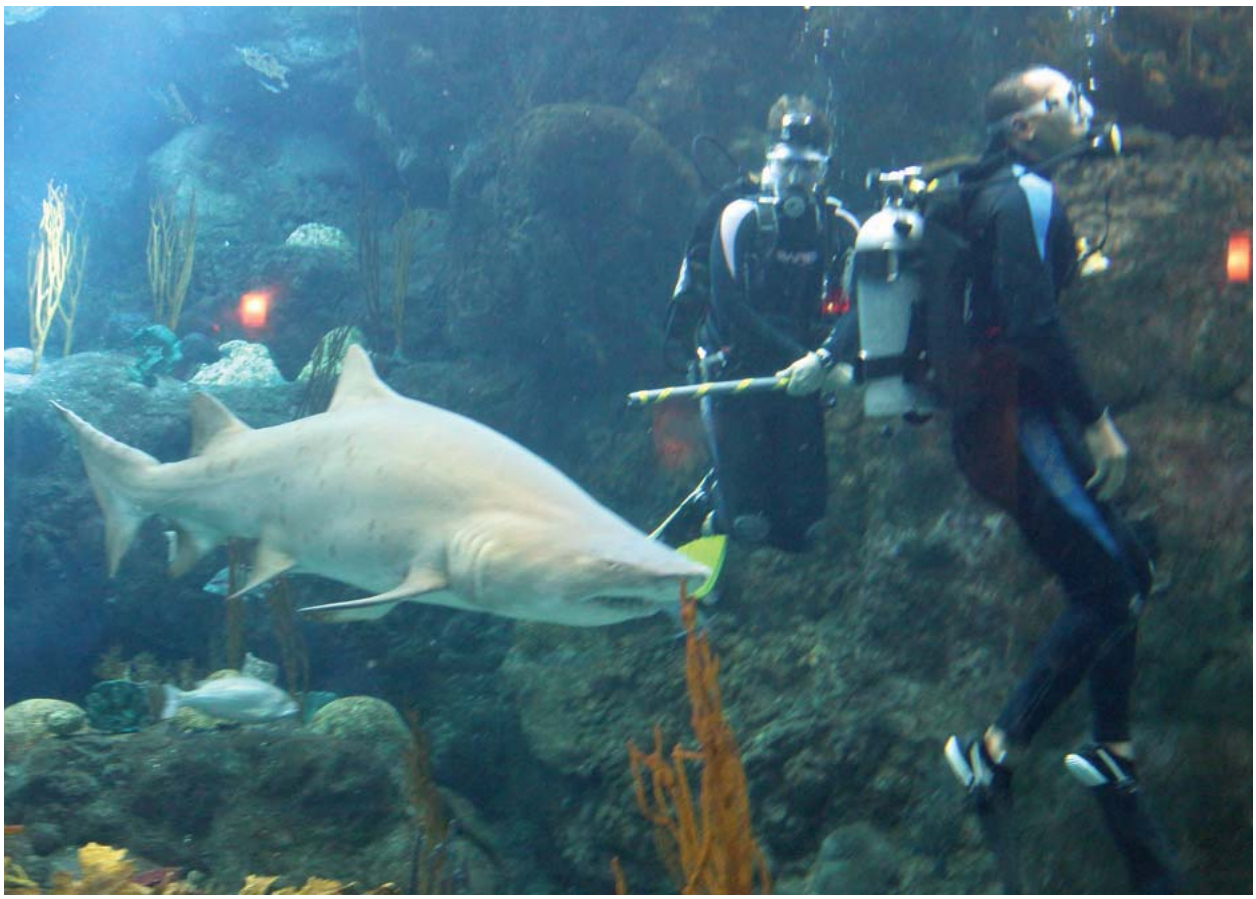

Figure 1. A safety diver with a “shark wand” encourages a sand tiger shark, *Carcharias taurus* (Rafinesque, 1810), to move away from a working diver with his back turned to the approaching animal. Photo courtesy: Mike Terrell, Florida Aquarium, USA.

low pressure supply of a buoyancy compensation device. A simple communication code should be established. For example, a single tap or "squawk" could indicate that an elasmobranch is in the immediate vicinity, while multiple taps or "squawks" in rapid succession could indicate that an elasmobranch poses an immediate threat to the working diver. When alerted to the presence of an elasmobranch, it is the responsibility of the work diver to acknowledge the signal and to identify the exact location of the approaching animal. The work diver should keep their arms close to their body and not make any sudden or jerky movements, or blindly ascend to the surface. When necessary, the work diver should move slowly out of the swimming path of the elasmobranch and remain close to the aquarium wall or floor as the animal passes by. If the elasmobranch poses an immediate threat, the diver should take appropriate evasive action and exit the exhibit as safely and as quickly as possible.

The best option for communication is an umbilical intercom between divers wearing full-face dive masks, including communication with a dive supervisor at the surface. Divers using umbilical communication must take care to manage their surface tethers to prevent entanglement, of themselves, underwater structures or large animals. Wireless communication systems are available but current technology does not work well in the enclosed, reflective environs of an aquarium.

Diver isolation

An alternative to using a safety diver is to isolate the working diver from elasmobranchs. Isolation can be achieved by using a shark cage or by netting off a section of the exhibit (Hodges and Sabalones, 2004). Shark cages can be cumbersome and may not be appropriate for all underwater tasks. A net strung across a section of the aquarium must be monitored to prevent entanglement of divers or animals. A clear extraction plan should be developed and trained in preparation for an accidental diver or animal entanglement. In smaller aquaria, an effective barrier can be provided using semirigid plastic mesh cable tied to a PVC pipe frame. These barriers represent a much lower risk of entanglement than a mesh net across a section of the aquarium.

Feeding elasmobranchs

The feeding of elasmobranchs by divers on SCUBA was covered by Hodges and Sabalones (2004) and the reader is directed to that reference for a review.

HANDLING ELASMOBRANCHS

Approaching elasmobranchs underwater

Some husbandry tasks necessitate interacting directly with elasmobranchs while in the water. Divers should approach sharks from above or below and from behind the gills. Divers place themselves at risk by approaching a shark from the side as the animal can turn suddenly and quickly, when startled, and possibly strike or bite the diver. All movements around a shark should be smooth and deliberate to avoid startling the animal. The diver should always leave an escape route open to the shark should it become startled or agitated.

Desensitization

Some public aquaria have protocols restricting direct contact with elasmobranchs. While this approach can reduce risk during normal operations, it may increase risk during times when contact is necessary for hands-on husbandry procedures. For this reason, other institutions have adopted planned tactile desensitization programs, regularly touching the sharks under controlled conditions. Desensitization reduces stress on the animals, and therefore risk to the handlers, when restraint and interaction is necessary for husbandry procedures.

A desensitization program begins with a diver allowing the target elasmobranch to pass nearby during regular maintenance dives. Deliberate eye contact is avoided. The next step is for the diver to slowly approach the elasmobranch until it shows early signs of avoidance. By approximations, over many days, the gap between the animal and the diver is closed until touching distance is reached. Prior to touching the elasmobranch the diver should practice reaching out, without physically contacting the animal. Only when the elasmobranch shows little interest in these actions should a diver attempt to touch the animal. Touching should be done from above or below the elasmobranch, posterior to the gills and anterior to the ventral fins. When the diver first touches the elasmobranch, it should be with a single finger, and light and fleeting (Figure 2). As the animal becomes increasingly desensitized, touching can be firmer and of longer duration. The desensitization process can take an extended period of time before an elasmobranch is comfortable with regular contact. Once successful, a program of regular touching should be implemented to maintain desensitization.

When a husbandry procedure is necessary, desensitization reduces stress for the elasmobranch and therefore reduces the risk of injury to both animal and to attending staff. Following a hus-

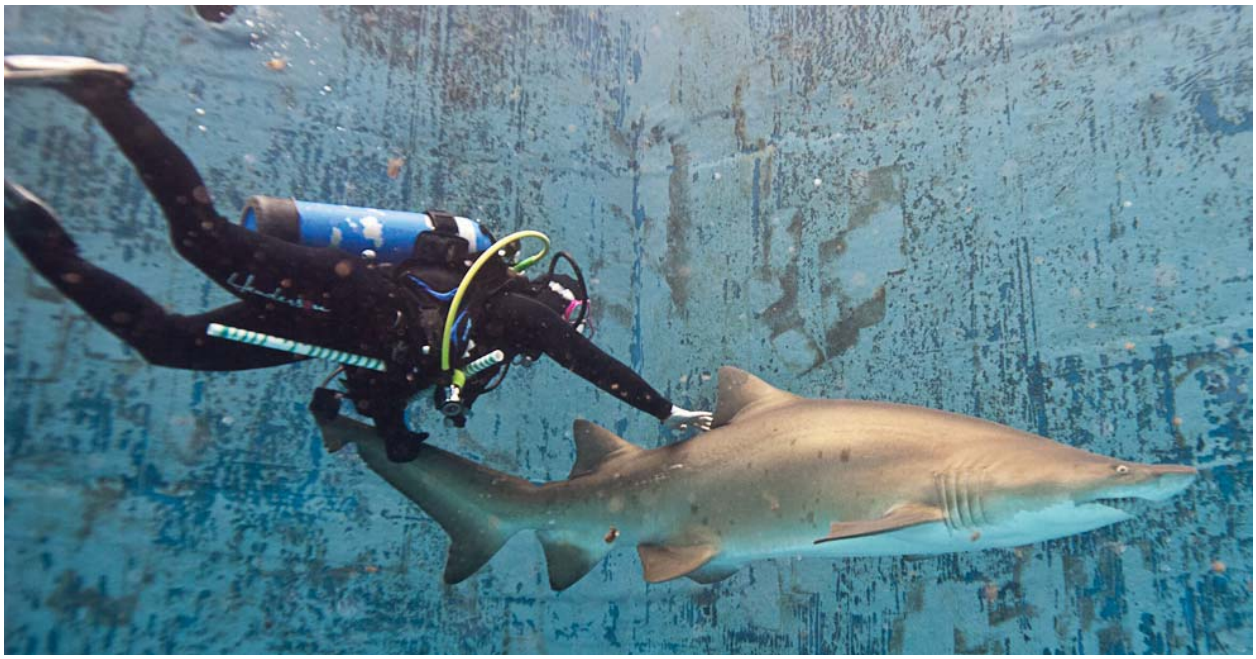

Figure 2. A diver slowly and deliberately reaches out to touch the dorsal saddle of a sand tiger shark, *Carcharias taurus* (Rafinesque, 1810), during the early stages of tactile desensitization. Photo courtesy: Skylar Snowden, Pittsburgh Zoo, Pennsylvania, USA.

bandry or medical procedure requiring restraint, it may be necessary to re-establish and reinforce desensitization using the steps described above.

Physical restraint

At times it is necessary to restrain elasmobranchs for husbandry and medical procedures, including marking or tagging for identification purposes, assisted feeding of inappetent specimens, clipping of spines, in the case of stingrays, and other similar operations. Techniques for the capture and restraint of elasmobranchs have been described by Violetta (2004) and the reader is directed to that reference for a review of the topic. In addition, elasmobranchs can be restrained directly by hand, restrained in a catch net or bag, or secured in a transport box.

For very brief procedures (e.g., the injection of medications or blood draws), elasmobranchs may be held in the capture device (net or bag) for the duration of the task and then immediately released thereafter. Alternatively, smaller animals may be physically restrained directly by hand, with a gentle but firm hold. Gloves are recommended to prevent chaffing from the denticles and to reduce the chances of injury should the animal manage to turn and snap at one of the handlers. For smaller sharks, one hand can be placed over the top of the body and gripped in the region of the pectoral girdle, posterior to the gills and anterior to the pectoral fins. This region of the body has structural cartilage and dense musculature, so the shark can be firmly restrained with little risk of injury. In addition, holding the pectoral girdle provides better control of the shark, as they tend to pivot at this point, minimizing risk of the animal reaching around and biting the handler(s). Further control can be provided by simultaneously holding the shark around, or just behind, the pelvic girdle, another region of firm cartilage and musculature. The pectoral girdle should be grasped before the pelvic girdle, although it is better to restrain both regions simultaneously if at all possible. The abdomen should not be held, especially around the coelomic cavity, as it contains delicate organs and frequently needs to remain clear for inspection and medical procedures. Small sharks are surprisingly strong, so trying to prevent them from struggling can frustrate handlers and potentially injure the specimen. Handlers should maintain a firm grip, but keep their arms loose and flexible, enabling the shark to safely wriggle until it ultimately relaxes.

Tonic immobility (TI) may be used to further aid restraint of smaller elasmobranchs (Henningsen, 1994; Janse et al., 2004; Smith et al., 2004; Stamper, 2004; Kessel and Hussey, 2015). TI is achieved by inverting the shark so that its ventrum faces uppermost, which induces a sleep-like state that can last for several minutes (Figure 3). TI is an excellent technique for handling small to medium-sized elasmobranchs during brief procedures, as

Figure 3. A bonnethead shark, *Sphyrna tiburo* (Linnaeus, 1758), maintained in tonic immobility (TI) while an ultrasound reading is taken. Ventilation is aided by oxygenated water flushed over the gills via a polymer pipe.

it reduces stress on both the animal and the handler(s) (Figure 3). It should be noted that elasmobranchs can spontaneously emerge from TI without warning, so it should not be relied upon for long-duration, complex or fine-scaled procedures.

For longer duration procedures, a larger shark may be placed into a transport box filled with oxygenated water (Smith,1992), where it can breathe unimpeded and is fully supported. The shark may be completely released into the transport box or can be semi-restrained within a shark stretcher and suspended in the water. A submersible pump may be used to flush the gills with oxygenated water throughout the husbandry or medical procedure, aiding respiration. Similarly, rays can be restrained in a stretcher designed to support their large, flat bodies (Smith et al., 2004). Some ray stretchers are fitted with a flap designed to fold over the top of the animal. Once secured with Velcro® tabs, the flap forms a pocket from which the ray cannot easily escape.

Elasmobranchs are strong and injuries can result when a restrained animal struggles. Care should be taken to ensure that staff are positioned where they cannot be struck by the tail of the elasmobranch or be crushed (or have their extremities crushed) against a wall or the restraint device. Given their powerful musculature, it is advisable to allow larger animals to struggle within the confines of the restraint device, as attempting to prevent all movement can lead to injuries of both personnel and the animal. Ideally, the restraint device should be designed to allow some level of safe movement, without the chance of animal escape. An example of an effective restraint device is a shark stretcher, typically built from a sheet of heavy duty vinyl fitted with rigid poles for lifting (Smith, 1992; Gendron & Menzies, 2004; Smith et al., 2004; Violetta, 2004). Once restrained, elasmobranchs typically only struggle for a short time and then remain relatively docile, allowing staff to manipulate the animal safely. However, staff should always be vigilant for the elasmobranch to start struggling without warning.

Additional precautions are required when handling electric rays. Handlers must keep all parts of their body away from the water in which the rays are maintained. Handling tools should be fabricated from electrically insulated materials (e.g., wood, plastic). It is also advisable to wear thick rubber gloves, with long cuffs, as insulated tools may still conduct some electricity when soaked with seawater.

For details on the handling of pristids refer to White et al. (this volume).

Clipping stingray spines

Many rays in public aquaria are displayed in touch tanks, exposing the public to potential injury from

the venomous spines. Some institutions have dive immersion experiences where the public also have exposure to stingrays. While the chances of envenomation are relatively low, many institutions elect to clip the spines of stingrays to remove the risk of injury to the public. Spine clipping is safe and humane for stingrays, but it does expose staff to potential injury while handling animals for the procedure.

Stingrays should be caught with large nets and stretchers, taking care not to get the spine entangled in the mesh netting. If the barbs of the spine get caught, the ray is likely to struggle and tear the entire spine and surrounding tissue out of its tail sheath. Once captured, the stingray should be placed in a shallow tank containing sufficient water to barely cover the animal. Smaller individuals can be managed without sedation, but for greater security an anesthetic is recommended to minimize animal movement.

Both the stingray and its tail should be restrained while clipping the spine. Neoprene and even leather gloves have proven to be ineffective at preventing accidental envenomation during the clipping procedure. Chain mail or Kevlar® boning gloves are recommended as protection against lacerations and puncture wounds. The spine should be cut close to the body with stout shears or wire cutters. Great care must be taken to avoid accidentally cutting the tail. The sharp distal end of the spine may be ejected as it is cut, so all staff must wear safety glasses. As soon as the spine has been severed, it should be recovered with gloved hands and placed in hot water to denature the toxin (Firchau, personal communication). The severed tip of the spine should be considered potentially harmful and handled with great care.

Some species of stingray may be susceptible to TI, but the duration of the sleep-like state is unpredictable and the animal may stir prematurely potentially risking handlers during the procedure.

Assisted swimming

In some cases, sharks may require assisted swimming or 'walking' following excessive hyperactivity, periods of extended inactivity (e.g., a long-duration transport) or anesthesia. This technique has been described by Gendron and Menzies (2004). In addition to 'walking', the trunk and caudal fin of the shark can be flexed to help relieve muscular tetanus and promote circulation. Flexing the trunk will mobilize toxins that have accumulated in the musculature, so they can be further metabolized, helping speed recovery. However, moderation during trunk and tail flexing is recommended, as excess manipulation may force a bolus dose of toxins into the bloodstream having possible deleterious effects (Smith et al., 2004).

Elasmobranchs emerging from an anesthetic should be carefully monitored after "walking" has concluded. Muscular pumping can flush sequestered anesthetic toward the central nervous system and re-anesthetize and disorient the animal (Smith et al., 2004). In addition, elasmobranchs emerging from an anesthetic must be treated with great caution as they can react to stimuli in an unpredictable and potentially aggressive manner, striking randomly at nearby objects (Smith et al., 2004). Some anesthetic reversal agents can cause an excitatory phase, further exacerbating the danger of disorientation during elasmobranch emergence from anesthesia.

Inflating a sand tiger shark

C. taurus periodically swallow air and store it in their stomach to regulate buoyancy. On occasion, *C. taurus* may lose air from their stomach, become negatively buoyant and swim in a labored manner with their tail at a lowered attitude. When prolonged, this swimming posture consumes excess energy and the shark may become exhausted and rest on the bottom of the exhibit. Air loss can occur after a transport or a procedure requiring restraint, and it is not uncommon for a *C. taurus* to lay on the bottom for a short period during recovery. Thereafter, the shark will typically ascent to the surface, gulp down some air and resume swimming normally. However, if the shark does not voluntarily restore its buoyancy, it may be necessary to intervene and actively introduce air into the stomach (Areitio et al., 2001). This procedure should only be attempted by an experienced husbandry and veterinary team once it has been determined that the health of the shark is at genuine risk. Considered a procedure of last resort, extreme care must be exercised during air inflation to ensure the safety of both the animal and attending staff.

Air can be introduced into the stomach of *C. taurus* using a semi-rigid tubing (e.g., cross-linked polyethylene or PEX) of ~10 mm diameter. The semi-rigid tubing should be at least 80 cm longer than the distance from the mouth to the stomach of the shark. The proximal end of the semi-rigid tube is connected to a compressed air supply, with a valve positioned at the junction. The open, distal end of the semi-rigid tube should be rounded and

smooth to prevent injury to the esophagus or stomach of the shark. A clear mark should be placed on the semi-rigid tube, which indicates the insertion stopping point once it reaches the line of the teeth—i.e., when the semi-rigid tube has penetrated sufficiently that the open end is just inside the stomach.

Before the procedure begins, divers must ensure that there is clear space around the shark in case it begins to swim voluntarily. The diver with the tube should position themselves at a safe distance directly in front of the recumbent shark. No diver should be positioned to the side of the shark as they will be at risk should the animal react by lunging sideways. The semi-rigid tube is slowly inserted past the teeth towards the oral sphincter, along the longitudinal axis. It may be necessary to temporarily stop insertion when the shark closes and opens its mouth during its natural ventilation cycle, so the tube does not get snagged on its teeth. The diver will feel some resistance as the end of the tube reaches the oral sphincter and they must use some gentle pressure to push the tube through the opening. The semi-rigid tube is then pushed further through the esophagus until the mark is in line with the teeth of the shark. Once the semi-rigid tube is its final position, small amounts of air can be slowly and carefully introduced using the valve. The amount of required air will vary, so it is important to carefully observe the shark throughout the procedure. As sufficient air has been introduced, the abdomen of the shark will swell slightly and the shark will appear to rise gently from the bottom. Excess inflation should be avoided, although *C. taurus* does have the ability to expel excess air by burping. Once the diver has insufflated an appropriate volume of air, the semi-rigid tube can be carefully removed. The shark will often attempt to swim shortly after inflation. However, if the shark remains on the bottom, it can be encouraged to move using assisted swimming.

If the shark reacts negatively during the inflation procedure, the semi-rigid tube should be rapidly extracted. The fabrication material allows the tube to flex should the shark twist, turn or attempt to swim away, allowing time for the diver to respond and extract the tube.

Injection medications

Intramuscular injections (IM) may be administered to an unrestrained slow-swimming elasmobranch (e.g., *C. taurus*) that has previously been desensitized to tactile stimuli. The injection process can be aided further using an acute process of supplementary conditioning over a period of several minutes. First, a diver reaches out and touches the shark on the region intended for injection (ideally the dorsal saddle) using a short, blunt pole. Initial touches should be gentle. Subsequent touches should be increasingly firm, until they are forceful enough to move (slightly) the shark laterally. Touching is replaced by gentle and then firmer poking, slowly increasing the forcefulness of the strikes. Once the shark appears oblivious to poking, the IM injection can be administered using a pole syringe. If the shark is well conditioned, there is generally little to no reaction from the injection procedure. The pole syringe serves two purposes: (1) it allows injection while maintaining some distance between the shark and the diver; and (2) it can act as a “shark wand” to ward off the animal in the event of a negative reaction. Many benthic species of elasmobranch, e.g., nurse shark, *Ginglymostoma cirratum* (Bonnaterre, 1788), are also good candidates for this technique of IM injection. In addition, this acute desensitization technique may be modified to allow for the taking of small biopsies.

The tough integument of sharks typically demands the use of a heavy gauge needle to ensure effective penetration of the dermis. The low elasticity of shark skin often results in the injected medication leaking from the injection site, making accurate dosing difficult. Dosing accuracy can be improved by injecting directly with a hand-held syringe and then covering the injection site with a finger to reduce medication leakage, until the surrounding tissue has sealed the entry wound. A diver can swim above a shark and cautiously administer an IM injection, by hand, using a process of acute tactile conditioning as described above.

Irrigation anesthetics

Some anesthetics can be administered to an elasmobranch by irrigating the gills—i.e., by directing a stream of concentrated anesthetic into the mouth of the shark so it passes out over the gills. A squeeze bottle or a hand pump spray bottle (e.g., Chapin Lawn and Garden Sprayer, Chapin International, Inc., Batavia, New York, USA) is filled with the anesthetic (e.g., Tricaine-S, Western Chemical Inc., Ferndale, Washington 98248, USA) and some food coloring. A length of flexible tubing is attached to the outlet of the bottle, to which is attached a further 1 m length of rigid tubing. Ideally, the rigid tubing is clear so that it is less visible to the elasmobranch when maneuvered near its mouth. When the tip of the tube is close enough to the mouth of the elasmobranch

to ensure 'inhalation', the solution is 'injected'. The food coloring allows staff to see what proportion of the solution goes into the mouth of the elasmobranch. This technique can be applied by a staff member wading in a shallow aquarium or by a diver in a deeper aquarium. Patience is required when using this technique as it may take several 'injection' attempts for the animal to absorb sufficient anesthetic. Once initial sedation has been achieved, further anesthetic doses can be more readily applied until the desired level of anesthesia is attained. It is important to work quickly when using irrigation anesthesia as the elasmobranch will rouse quickly unless the anesthetic is continuously applied.

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INTERNET RESOURCES

www1 http://www.redcross.org/
www2 http://www.redcross.org/images/MEDIA_CustomProductCatalog/m39740251_FA_CPR_AED_PM_sample_chapter.pdf
www3 http://adpaonline.org/resources/

PERSONNEL COMMUNICATIONS

Bourbon, H. National Aquarium, Baltimore, Maryland 21202, USA.

Correia, J.P. Flying Sharks, Lisboa, 1900-272, Portugal.

Coy, C. Florida Aquarium, Tampa, Florida 33602, USA.

Firchau, B. Virginia Aquarium and Marine Science Center, Virginia Beach, Virginia 23451, USA.

González Sanz, J. Acuario de Zaragoza, Zaragoza, 50018, Spain.

Iwata, M. Fukushima Aquarium, Iwaki, Fukushima 971-8101, Japan.

McEwan, T. uShaka Sea World, Durban, KwaZulu-Natal, 4001, South Africa.

Menzies, S. Reef HQ Aquarium, Townsville, Queensland 4810, Australia.

Terrell, M. Florida Aquarium, Tampa, Florida 33602, USA.

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Chapter 21

Training and conditioning of elasmobranchs in aquaria

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Abstract: Classical and operant conditioning of elasmobranchs has become increasingly common in public aquaria. Of 82 polled insitutions, 39 were housing elasmobranchs in 1993 with 34.1% conducting elasmobranch training and conditioning programs. By 2013, 88.8% of 80 institutions were training and conditioning elasmobranchs. Training programs were considered essential for the care of animal collections by 43% of surveyed institutions. Trained behaviors such as targeting, station feeding, and accepting handling and clinical procedures have all been used to aid captive animal management.

INTRODUCTION

Elasmobranch training and conditioning techniques have advanced considerably in recent years and are being successfully incorporated into standard husbandry practices at public aquaria. This chapter is intended to be an adjunct to Sabalones et al. (2004) and it is assumed that the reader has consulted that work as part of their preparation to develop a training plan for elasmobranchs. This chapter will therefore focus on the following: (1) current trends related to training elasmobranchs in aquaria; (2) recent advances in training in general and identifying underused techniques; (3) tools and techniques for planning, documenting and fine-tuning training sessions to better shape desired behaviors; and (4) areas where training techniques can be more widely used and further developed for the management of elasmobranch collections, especially for advanced husbandry.

Learning and discrimination

Elasmobranchs have long been known to possess the ability to learn, and many public aquaria have used classical and operant conditioning for a variety of applications (Clark, 1959; Clark, 1963; Janse et al., 2004; Sabalones et al., 2004; Corwin, 2012; Collier and Schreiber, 2014; Kimber et al., 2014). As far back as 1966 visual target discrimination in sharks was studied by Tester and Kato (1966), who conducted experiments on two species of carcharhinid. The study demonstrated a capacity for positive discrimination between different colored and different shaped targets by both species. More recently, Hart et al. (2004) demonstrated the possibility for trichromatic color vision in two ray species and Van-Eyk et al. (2011) showed that one of those species was actively able to demonstrate color discrimination.

Conditioning and training

In this chapter, 'conditioning' refers to classical, respondent, or Pavlovian conditioning, where the form and frequency of animal behaviors change passively due to changes in the environment (www1). Similarly, 'training' refers to operant or instrumental conditioning, where a behavior is elicited, on cue, due to an expected desirable outcome (Ramirez, 1999). In the case of elasmobranchs, desirable outcomes are often provided in the form of food as the primary positive reinforcement. Other cues, such as sounds or tactile stimuli, can be paired with the primary reinforcer and, over time, elasmobranchs can be conditioned to recognize these cues as secondary reinforcers. Secondary reinforcers can, in turn, be used to 'bridge' the time-gap between eliciting a desired behavior and receiving the primary reinforcer. Using a bridge is also helpful in pinpointing and rewarding very specific behaviors (Ramirez, 1999). Secondary reinforcers can also be useful in cases where food motivation is decreased or a daily food ration has already been consumed by the target animal (Sabalones et al., 2004). Accurate and consistent use of reinforcers is comparable to a very basic form of communication and it can be helpful to think in this paradigm—e.g., "What exactly am I asking this animal to do?" The basic concepts outlined above are inherently important to understand before creating a training plan.

METHODS

To better understand training and conditioning practices used with chondrichthyan fishes, a survey was sent to pubic aquaria around the world during 2013. Responses to the survey were received from 82 separate institutions, representing 17 countries and five continents. Data was compiled and summarized using software from Survey Monkey (www2) and the Microsoft Excel 2007 Data Analysis toolpak (www3).

RESULTS and DISCUSSION

Current trends

The proportion of aquaria that intentionally train or condition elasmobranchs has gradually increased over time, with a marked increase in recent years from 34.1% in 1993, to 46.4% in 2003, to 88.8% in 2013 (n = 82). Increased commitment to training elasmobranchs may reflect a shift in an understanding of its utility as a tool for aiding routine husbandry activities. This trend may also reflect an increased and broader animal training capacity throughout the zoo and aquarium community. Of institutions that responded to the question (n = 77), 42.9% considered training to be an essential element of the daily care of their elasmobranch collection and proactively incorporated it into their operations.

Training capacity

The proportion of animal care staff that conducted animal training (of any taxa) varied between institutions. In 22.4% of aquaria the entire animal care staff conducted some form of animal training, while "most", "many" and "few" of the animal care team trained animals at 35.5%, 11.8% and 28.9% of the polled institutions, respectively (n = 76). Staff preparation to conduct training of elasmobranchs took a variety of forms across the industry (n = 74 institutions), including: staff mentoring (89.2%), formal instruction (41.9%), study of required reading (32.4%), independent research (31.1%), participation at conferences (20.0%), informal *ad hoc* training, (16.2%), consulting with other institutions (4.1%) and hiring a dedicated staff member (1.4%). Many institutions employed more than one mode of training preparation, but there was a heavy industry-wide emphasis on staff mentoring, which implies a meaningful investment in personnel time and resources, and a commitment on the part of the institution. In 10.8% of cases (n = 83 institutions), respondents concluded that their existing training program was insufficiently resourced to achieve a satisfactory outcome with their elasmobranch collection. Commonly reported resource limitations included time, capacity,

knowledge, tools, infrastructure and institutional support.

Training emphasis

A total of 59 taxonomic groups of elasmobranchs were cited in the survey as trained in aquaria (Table 1). The primary reason cited for training elasmobranchs (n = 67 institutional responses for rays; n = 69 for sharks and chimeras) were diet management (85.3%), followed by managing multi-species feeding sessions (64.3%). Training elasmobranchs primarily to aid veterinary procedures was cited by 33.5% of institutions surveyed. Other reasons for training elasmobranchs included: enhancing education programs and visitor interactions (22.0%); aiding animal transportation and relocation (18.8%); and general behavioral modification (12.0%).

Training implementation

Elasmobranch training "regularly" occurred at the surface (or in shallow water) of the exhibit aquarium at 84% of institutions (n = 75). Training in off-exhibit animal holding or quarantine was conducted at 40% of institutions and 22.7% of animals were trained underwater by personnel on SCUBA. Cues deemed to be "reliably successful" when training elasmobranchs were reported for 73 aquaria as follows: 60.3% for a visual object or motion, 34.3% for an audible rattling or tapping sound, 15.1% for physical contact, and 6.8% each for a change to lighting, or a change to water flow or LSS function.

THE TRAINING PLAN

We advocate using the SPIDER (i.e., set goals, plan, implement, evaluate, and re-adjust) framework for elasmobranch training (Sabalones et al., 2004; www4). This framework is reviewed below, with additional instructions to refine training techniques.

Development of a training plan is simply the process of defining the steps, or successive approximations, needed to achieve a specific behavior on cue. Plans should be designed to ensure the greatest chance for success. However, readers are encouraged to aim for goals that are truly beneficial even if they seem lofty. When setting behavioral goals, it is important to be realistic about the biology, history, morphology and individual plasticity of the animal in question. Other considerations include the age and size of the animal, as well as any limitations imposed by its physical surroundings. Initiating a training plan while an animal is off-exhibit has many potential advantages, such as decreased inter- and intra-specific competition and easier access overall. In general, setting intermediate goals as waypoints to larger or more sophisticated goals is recommended. A sample training plan for ribbontail stingray, *Taeniura lymma* (Forsskål, 1775), is shown in Table 2 and an electronic template can be downloaded from the online resources for this manual (www5).

Training a behavior will be more successful if the number of people involved is limited, as this increases training consistency. If more than one person is training a behavior then communication about progress must be clear and frequent, and a consensus must be reached about how to proceed at the end of each discussion. Once an animal has mastered an intended behavior, the involvement and training of additional people to manage the animal becomes a simpler task. The core principle during training is to maintain consistency—i.e., to ensure that a very precise message is being communicated to the animal. Patience is paramount and plenty of time should be built into the training plan. Timeframes for each behavioral approximation should be established. These target dates should be referenced during training evaluations, with a clear understanding that timeframes are a guide and will likely change during execution of the training plan.

Positive reinforcement (reward-based) training plans require opportunities to provide reinforcers (e.g., food) immediately following the elicitation of a desired behavior. If time and schedules permit, the number of feeding sessions can be increased to provide more training or learning opportunities—e.g., a weekly diet ration could be split into five feeds per week, instead of three. In addition, smaller pieces of food can be offered to the elasmobranch, providing more opportunity for repetitions during a training session, without increasing the weekly dietary ration.

As a SPIDER training plan is implemented, documenting, evaluating and readjusting should be revisited at the conclusion of every session. For each behavioral approximation, be mindful of the next (and successive) planned step as animals can frequently advance more quickly than expected and even skip an approximation. Carefully document each training session using a simple form or log. Record all pertinent details and think ahead to the next session, making observations or recommendations where appropriate. A sample training log for *T. lymma* is shown in Table 3 and a customizable template

Table 1. Elasmobranch training reported from aquaria (n = 71 for rays; n = 73 for sharks and chimera) around the world. Trained behaviors, including frequency trained, as well as number of institutions reporting, are shown for each species reported.

Scientific name	Common name	Start session cue	Target training	Station feeding	Tactile desensitization	Handling desensitization	Stretcher / net trained	Leading A to B	Number of institutions
Aetobatus spp. (Blainville, 1816)	eagle rays	7	15	17	11	5	3	2	(n = 22)
Carcharhinus amblyrhynchos (Bleeker, 1856)	blacktail reef shark	3	1	4					(n = 8)
Carcharhinus falciformis (Müller & Henle, 1839)	silky shark		2	1					(n = 5)
Carcharhinus galapagensis (Snodgrass & Heller, 1905)	Galapagos shark		2	2					(n = 7)
Carcharhinus leucas (Müller & Henle, 1839)	bull shark	2	2	2			1		(n = 8)
Carcharhinus limbatus (Müller & Henle, 1839)	blacktip shark	5	4	10	1	1			(n = 14)
Carcharhinus melanopterus (Quoy & Gaimard, 1824)	blacktip reef shark	11	12	24			2		(n = 41)
Carcharhinus obscurus (Lesueur, 1818)	dusky shark	1		1					(n = 6)
Carcharhinus plumbeus (Nardo, 1827)	sandbar shark	8	9	27	2	1		1	(n = 34)
Carcharias taurus (Rafinesque, 1810)	sand tiger shark	5	9	32	3	1	2		(n = 39)
Carcharodon carcharias (Linnaeus, 1758)	great white shark	1	2	2					(n = 4)
Cephaloscyllium spp. (Gill, 1862)	swell sharks	1	2	1					(n = 14)
Dasyatis americana (Hildebrand & Schroeder, 1928)	southern stingray	7	13	31	9	4	4	1	(n = 36)
Galeocerdo cuvier (Péron & Lesueur, 1822)	tiger shark	1	2	2					(n = 6)
Galeorhinus spp. (Blainville, 1816)	hound sharks	1	1	1					(n = 4)
Ginglymostoma cirratum (Bonnaterre, 1788)	nurse shark	9	21	26	12	6	3	3	(n = 40)
Haploblepharus spp. (Garman, 1913)	shy sharks		1	1					(n = 7)
Heterodontus spp. (Blainville, 1816)	horn sharks	1	5	2					(n = 25)
Himantura chaophraya (Bleeker, 1852)	freshwater whipray			2					(n = 5)
Himantura spp. (Müller & Henle, 1837)	whip rays	3	9	15	3				(n = 19)
Hydrolagus colliei (Lay & Bennett, 1839)	spotted ratfish	1	1	2					(n = 7)
Manta spp. (Bancroft, 1829)	mantas	3	4	4	1	1	1		(n = 7)
Mobula spp. (Rafinesque, 1810)	devil rays	2	3	4	1		1		(n = 6)
Mustelus spp. (Linck, 1790)	smooth hounds		1	2					(n = 13)
Negaprion spp. (Whitley, 1940)	lemon sharks		2	2				1	(n = 9)

Notorynchus cepedianus (Péron, 1807)	broadnose sevengill shark	1	2	3	1	1	1		(n = 6)
Potamotrygon motoro (Müller & Henle, 1841)	ocellate river stingray	2	4	10	1				(n = 20)
Raja clavata (Linnaeus, 1758)	thornback ray		2	1	1				(n = 12)
Rhina ancylostoma (Bloch & Schneider, 1801)	bowmouth guitarfish	4	8	9	1	1	2	1	(n = 16)
Rhincodon typus (Smith, 1828)	whale shark	2	2	3	2	3	1	1	(n = 4)
Rhinoptera spp. (Cuvier, 1829)	cownose rays	6	12	29	12	2	2		(n = 37)
Rhizoprionodon terraenovae (Richardson, 1836)	Atlantic sharpnose shark	1	1						(n = 4)
Scyliorhinus canicula (Linnaeus, 1758)	lesser spotted dogfish			1	1				(n = 13)
Sphyrna tiburo (Linnaeus, 1758)	bonnethead shark	5	5	10					(n = 19)
Squatina spp. (Duméril, 1805)	angel sharks		2	2					(n = 7)
Stegostoma fasciatum (Hermann, 1783)	zebra shark	12	26	33	18	12	8	7	(n = 43)
Torpedo spp. (Duméril, 1805)	torpedo rays		1						(n = 6)
Triaenodon obesus (Rüppell, 1837)	whitetip reef shark	4	3	14			1		(n = 23)
Triakis spp. (Müller & Henle, 1838)	leopard sharks	2	5	10	1		1		(n = 21)
Urobatis jamaicensis (Cuvier, 1816)	yellow stingray	1	3	7	3	1			(n = 19)
Urogymnus asperrimus (Bloch & Schneider, 1801)	porcupine ray			1					(n = 4)
Gymnuridae (Fowler, 1934)	butterfly rays		1						(n = 3)
Hemiscylliidae (Gill, 1862)	bamboo sharks	3	7	12	2				(n = 37)
Narcinidae or Narkidae (Gill, 1862; Fowler, 1934)	electric rays	1	1						(n = 4)
Orectolobidae (Gill, 1862)	wobbegong sharks	2	5	6					(n = 28)
Pristidae (Bonaparte, 1838)	sawfishes	3	8	16	1	1			(n = 20)
Rhinobatidae (Müller & Henle, 1841)	guitarfishes	4	6	14	3	2	1		(n = 28)
Squalidae (Bonaparte, 1834)	dogfish sharks	1	1	3					(n = 9)
Other Carcharhinidae (Jordan & Evermann, 1896)	requiem sharks	4	3	7	1			1	(n = 9)
Other Dasyatidae (Jordan, 1888)	whiptail stingrays	8	18	31	10	4	3		(n = 42)
Other Myliobatidae (Bonaparte, 1838)	eagle rays	3	4	7	2				(n = 11)
Other Potamotrygonidae (Garman, 1877)	freshwater rays	1	4	10	1				(n = 18)
Other Rajidae (Bonaparte, 1831)	skates	1	3	5	3				(n = 20)
Other Rhinidae (Müller & Henle, 1841)	wedgefishes	1	1	2					(n = 6)
Other Scyliorhinidae (Gill, 1862)	catsharks	2	3	2					(n = 19)
Other Sphyrnidae (Gill, 1862)	hammerheads	6	7	11	1	1			(n = 16)
Other Triakidae (Gray, 1851)	hound sharks	1	2						(n = 5)
Other Urolophidae (Müller & Henle, 1841)	stingarees		1	4	1				(n = 12)

Table 2. Sample training plan for obtaining weight and disc width from ribbontail stingrays, *Taeniura lymma* (Forsskål, 1775).

Behavior Training Plan **Trainer/ Coordinator:** Ana (1°) and Ashley (2°)				**Goal:** Collect morphometric data from *Taeniura lymma* with minimum stress when handling (could be applied to young adults on exhibit and juveniles in quarantine)	
		Requirements			
Steps	**Approximations**	**Time**	**Staff**	**Materials / Other**	**Notes**
Collect information about behavior of each animal	Identify individual animals	1 - 2 weeks	Ana or Ashley (2 trainers to cover all week days)	Camera - take photos of animals; print photos with identifiers.	ID2 has only 2 bright blue dots on the dorsal part of the tail; ID5 is the only male.
	Study animal behavior	1 - 2 weeks	All exhibit staff	Daily observations - take note of preferred locations in exhibit, interactions with other animals, feeding behavior, behavior during maintenance.	Bottom dwellers, shy, normally hidden during day, they come out to feed; sometimes aggressive with conspecifics - male is typically aggressor; no predators on exhibit, normally flee when a diver approaches, sensitive to handling and capture; prefer shrimp and capelin.
Station and target feed with a diver (bottom of exhibit)	Associate feeding sessions with a start of session cue and target	1 - 2 weeks	1 dive daily: Ana or Ashley + 1 dive buddy + 1 dive tender	Target (PVC and a red ball). Rattle for auditory cue.	When rays come out to feed, sound the rattle and feed them near target.
	Touch target	1 - 2 weeks	(same as above)	Target and rattle.	Rays come out to feed quicker with the start of session cue. Have them touch the target before reinforcing with food.
Change feeding station location with a diver (bottom of exhibit)	Touch and follow target	1 - 2 weeks	(same as above)	Target and rattle.	For each piece of food, move target to a slightly different location around diver and reinforce only when they touch target. Gradually increase how far target is moved.
	Touch and follow target around exhibit	1 - 2 weeks	(same as above)	Target and rattle.	Gradually move where you start feeding session and continue to reinforce rays as they follow target to new areas of the exhibit.
	Introduce an acrylic platform	2 - 3 weeks	(same as above)	Acrylic platform, target and rattle.	Guide rays with the target to an acrylic platform on substrate and reinforce them on platform.

Transition feeding station to a shallow area near top of exhibit (from the surface; no diver)	Target feed with diver (1.5 m from bottom)	1 - 2 weeks	(same as above)	Acrylic platform, target and rattle.	Guide rays with target to an acrylic platform positioned 1.5 m from bottom and reinforce them on platform. If hesitant to come off bottom, lower platform and increase height with each successful session.
	Target feed with diver (3 m up from bottom)	1 - 2 weeks	(same as above)	Acrylic platform, target and rattle.	Guide rays with target to an acrylic platform positioned 3.0 m from bottom and reinforce them on platform.
	Target feed with diver (shallow area near surface)	1 - 2 weeks	(same as above)	Target and rattle.	Guide rays with target located in shallow area near surface and reinforce them.
	Target feed from surface in shallow area	1 - 2 weeks	Ana or Ashley (2 trainers to cover all week days)	Target and rattle.	No diver. Use rattle for start of session cue (as usual), with target submerged near surface. Pull target into shallow area as rays approach target and reinforce them there.
Measure and weigh animals	Target station: extend time touching target before reinforcement (feeding)	1 week	Ana or Ashley (2 trainers to cover all week days)	Target and rattle.	Gradually extend time that rays stay touching target before reinforcing (feeding).
	Measure disc width during target station	1 week	2 staff per session: Ana or Ashley + 1 staff	Target, rattle, measuring tape.	While rays are on target, disc width is measured dorsally by 2nd person; disc width is measured monthly going forward.
	Target station in shallow area: capture rays using stretcher	1 - 2 weeks	3 staff per session: Ana or Ashley + 2 staff	Target, rattle, stretcher .	Stretcher is placed in shallow area prior to start of session. Rays are targeted into stretcher, weighed and reinforced before and after lifting and release. Rays are weighed every two months going forward.

can be downloaded from the online resources for this manual (www5).

Before initiating a training session, it is important to review exactly what the animal is being 'asked' to do. Cues must be short, clear and unambiguous. Once a desired behavior has been elicited, timing of a reinforcer must be prompt to effectively communicate to the animal that it has achieved the goal. For each successive behavioral approximation, a balance must be struck between reinforcing a behavior that is almost right, versus the animal leaving the area because it has underachieved and not been reinforced. Finding this balance is not always easy, but it is the essence of how desired behaviors are eventually shaped.

Periods of limited progress toward a behavioral goal are not unusual and should be anticipated. For this reason, it is important to include longer-term periodic evaluations within the training plan (in addition to the usual daily evaluations). If an animal is struggling to reach a behavioral approximation, it may be necessary to revert back to the last step in the training plan and strengthen the behavior-reinforcer association. In addition, the training plan should be reviewed to assess any possible need for modification to the proposed next behavioral approximation. Even a small change to the plan may be very effective at aiding the animal in reaching the next step. It is always valuable during a review process to consult trainers experienced with other taxa who may have valuable insight related to training process.

A case study of training using the SPIDER model has been summarized in Table 4. In this case, two female zebra sharks, *Stegostoma fasciatum* (Hermann, 1783), were successfully trained at the Pittsburgh Zoo and PPG Aquarium to achieve two separate goals; intended to aid in their general husbandry and clinical care (Snowden, 2008). A video showing the training of *S. fasciatum* has been made available online and readers are encouraged to view this example (www6).

Table 3. Sample training log for obtaining weight and disc width from ribbontail stingrays, *Taeniura lymma* (Forsskål, 1775).

Behavior Training Log **Animal Name(s)/ID(s):** *T. lymma* - ID2 and ID5					**Step:** Station and target feed with a diver (bottom of the exhibit)		
Date	**Trainer(s)**	**Training session**		**Desired behavior**	**Reinforcement**	**Observations / Results**	**Suggestions for next session**
		Start time	**End time**				
6/1/2013	Ana	10:00 AM	10:10 AM	Associate feeding sessions with a start of session cue and a target	Chopped capelin	Both rays came out right away and ate within 0.5 m of target.	Try to feed rays a little closer to target.
6/2/2013	Ana	10:00 AM	10:20 AM	(same as above)	Chopped shrimp	ID2 came out right away, and started feeding near target. When ID5 came out, he chased ID2 away; ID2 only got 75% of her ration.	Only reinforce ID5 if he 'plays nicely'.
6/3/2013	Ashley	10:00 AM	10:10 AM	(same as above)	Chopped shrimp	Both rays came out quickly. ID5 was being pushy, so I tried to feed them separately, and waited for him to calm down before reinforcing. Both ate well. Got the target a little closer.	Try to feed rays on opposite sides of target.
6/4/2013	Ana	10:00 AM	10:15 AM	(same as above)	Chopped squid and clam	Both rays responded well to rattle. I placed target right in front of me so I could try to get rays on either side of target. Both rays ate well, and came really close to target.	Start trying to get rays to touch target
6/5/2013	Ashley	10:00 AM	10:10 AM	Touch the target	Chopped capelin	Both rays came out right away. I had target in middle (like yesterday), and both rays came really close to target. ID5 made contact with target a couple times.	Keep trying to get rays to contact target during feeding sessions.

Table 4. Using the SPIDER model to train two zebra sharks, *Stegostoma fasciatum* (Hermann, 1783), for husbandry procedures.

SET GOALS

Objective 1 - Control shark diet, supplements and medications by target-feeding at surface.

- Goal 1: Train shark to touch a target at the surface for feeding.

Objective 2 - Reduce shark stress during health exams by conditioning to handling.

- Goal 2: Hold shark still for measurements and dorsal examinations.
- Goal 3: Roll shark over and hold for ventral examinations and blood draws.
- Goal 4: Remove shark from water to obtain weight.

PLAN and IMPLEMENT

Step 1 - Target train shark to a plastic yellow circle affixed to a 20 mm diameter PVC pole.

- Commence training while shark is in off-exhibit holding tank.
- Train during feeding sessions—i.e., three feeds/week at 3% body weight/feeding session.[a]
- Hang target in water during feeding sessions.[b]
- Slowly reduce spatial gap between feeding location and target.
- Once shark feeds near target, show target to shark while primary reinforcer is given.
- Ensure shark associates touching target with the primary reinforcer.[c]

Step 2 - Continue target training until 'target for food' association is solid.

Step 3 - Desensitize shark to human touch to overcome innate flight response.

- During feeding sessions, gently stroke shark dorsum while giving primary reinforcer.[d]

Step 4 - Condition shark to tolerate being held still.

- Increase strength and duration of touching.
- Increase touching until shark can be held in place while giving primary reinforcer.
- Hold shark for up-close examination and measurement without observed stress.

Step 5 - Condition shark to allow staff to roll them with their ventrum uppermost for examination.

- Hold and partially roll shark and give reinforcer.[e]
- Gradually increase roll until shark is lying ventrum uppermost with no observable stress.
- Once ventrum uppermost behavior is strong, attempt blood draw and other clinical procedures (e.g., ultrasonography).[f] [g]

Step 6 - Condition shark to allow staff to remove it from the water to be weighed.

- Strengthen holding shark in place behavior.[h]
- While holding the shark dorsal uppermost, gently lift the animal out of the water for two seconds; return the shark to the water and give reinforcer.
- Gradually increase the duration of time the shark is out of the water.
- Once the shark can be held out of the water for sufficient time, carry the animal to a floor scale and stand on the scale. Weight yourself and the shark, while carefully holding the animal steady. Gently return the shark to the water and give reinforcer. Repeat this procedure each session until there is no observable stress.[i]

Table 4. Using the SPIDER model to train two zebra sharks, *Stegostoma fasciatum* (Hermann, 1783), for husbandry procedures, continued.

DOCUMENT

Training logs to be recorded for each session. The following data to be collected: date and time, trainer(s) name, primary reinforcement (type of food), amount of food given, observations/results and suggestions for the next session.

EVALUATE and RE-ADJUST

Evaluate each session and adjust where necessary. Assess overall progress each week.

The following "evaluate" and "adjust" notes correspond to the superscript letters in the "plan" and "implement" phases detailed above.

a. The feeding regime was modified to increase the amount of training opportunities—i.e., to five feeding session/week, while maintaining the same weekly food ration.
b. The sharks initially avoided the target, so the feeding station was moved to a location where they would feed comfortably.
c. Once the sharks had been moved into the exhibit, target association weakened. A concerted effort to reinforce the association rapidly strengthened targeting behavior.
d. The sharks were initially reluctant to return to the feeding station when touched while feeding. The target was occasionally used to draw the sharks back to the feeding station using a 'come here' gesture, which was introduced as a discriminative stimulus by pairing it with the feeding target and a reinforcer.
e. *S. fasciatum* are not subject to tonic immobility. The sharks were therefore slowly desensitized to rolling, as well as being held ventral-side-up for a period of time.
f. On the first attempted blood draw, from the ventral mid-line, the shark was reinforced throughout and the procedure was successful. In addition, it was possible to perform an ultrasound without the shark struggling.
g. Once a procedure was complete and the shark was released, it would typically swim away. The shark would immediately return if given the discriminative stimulus—i.e., using the target to give the "come here" gesture.
h. On approach, following the discriminative stimulus, the sharks would touch the hand with their rostrum before being lifted from the water—i.e., they began targeting the hand.
i. The weight of the trainer was then subtracted to yield the weight of the shark.

Progress for each goal was as follows:

- Objective 1, Goal 1 - Achieved over an eight week period.
- Objective 2, Goal 2 - Achieved within four weeks of reaching Goal 1.
- Objective 2, Goal 3 - Achieved within three weeks of reaching Goal 2, Phase 1.
- Objective 2, Goal 4 - Achieved within four weeks of reaching Goal 2, Phase 2.

- Total time to achieve all goals was 19 weeks.

A juvenile male *S. fasciatum* was introduced to the exhibit when the two resident female sharks were already target feeding. The same training regime was applied to the male shark, although training progress was slightly slower. When the male *S. fasciatum* became reproductively mature, he began to bite and damage the fins of the female sharks. One of the female *S. fasciatum* was transferred to a holding tank to heal some of the resulting damage. Moving the shark was made easier by virtue of her training. The shark was removed from the exhibit by lifting her out and

carrying her to the holding tank visibly stress free. In the holding tank, the female *S. fasciatum* responded to target feeding with no weakening of the association. Once healed, the female *S. fasciatum* was returned to the exhibit tank. A full training session was conducted with the female shark the day after she was returned to the exhibit and she completed all behaviors with no signs of training regression. On another occasion, due to continued mating aggression, the male *S. fasciatum* was moved off exhibit. The male was easily handled as a result of his training; target feeding, lifting and weighing all occurred without incident or visible stress.

ADVANCING ELASMOBRANCH TRAINING

Infrastructure

As new aquaria are constructed, and established aquaria renovated, infrastructure for training and conditioning animals should be incorporated into their design. Aquarium teams should commit to training their elasmobranch collection and ensure they are sufficiently resourced and qualified to execute a coherent and productive plan.

Proactivity

When animals are first acquired, they are often maintained in a controlled, shallow and easily accessed environment (e.g., quarantine and holding tanks). This situation provides an ideal opportunity to quickly advance training and conditioning, as there is typically less competition for food and there are fewer distractions from visitors to the aquarium.

Target feeding is usually the first major goal when training new elasmobranchs, as it aids dietary and medication management. However, setting goals beyond target training is encouraged—e.g., presentation for health exams, transfers in and out of aquaria, etc.

While in-house clinical procedures are typically brief, they still result in physiological effects (Manire et al., 2001; Charbeneau, 2004). Establishing a training plan that will prepare sharks for routine or emergency clinical situations should be part of a responsible long-term elasmobranch management strategy. Training elasmobranchs to swim into a net or a stretcher, and remain calm during restraint and lifting, can preempt negative associations with catch gear and reduce physiological stress during routine procedures. Aquarists working with large elasmobranch species are encouraged to make the commitment to training their collection. Smaller elasmobranch species should not be overlooked when considering a training plan, even though their size makes them logistically easier to manage.

Documentation

Clear documentation of successes and setbacks while training elasmobranchs is just as valuable as comprehensive feeding and medical care records. In the same way that medical histories are passed on to caretakers when an animal is transferred between institutions, behavioral histories should also be entrusted to the receiving institution. These records provide invaluable information to aid the effective management of the animal(s).

Elasmobranchs in aquaria frequently outlive the tenure of staff members at the same institution. Maintaining comprehensive documentation and lists of trained behaviors for each animal, with relevant cues and training history, can help with the transfer of important information necessary for continuity of care. Searchable digital logs are preferred over more traditional paper records, allowing for rapid searches and access to information about specific behaviors, the remediation of similar behaviors in other animals, etc.

FUTURE DIRECTION

Voluntary blood draws

For blood values to be used as a diagnostic tool, baseline values must be determined and these differ between elasmobranch species (Hadfield and Clayton, this volume). As the reproductive processes of many species are not yet known, baseline blood values, without the artifact of capture stress, may provide valuable clues as to procreation strategies. Henningsen et al. (2004) emphasized the ongoing need for data collection from aquarium specimens, including serum hormone titers. The status of many elasmobranch species in the wild is threatened (Dulvy et al., 2008). Information gleaned from voluntary elasmobranch blood draws can contribute to breeding programs, aid the sustainability of aquarium collections and provide valuable insight into the reproductive strategies of wild conspecifics.

Training elasmobranchs to voluntarily present for a blood draw seems a lofty goal, particularly for

large pelagic species. However, Sodeyama et al. (2013) successfully trained a whale shark, *Rhincodon typus* (Smith, 1828), to position itself and remain calm while divers drew blood, a milestone in how training can be used to aid elasmobranch husbandry. By having an elasmobranch present for a voluntary blood draw, there is reduced stress on the animal, reduced physiological biomarkers of stress in blood samples, and reduced risk and stress for the husbandry staff.

Interactive education

Visitors and supporters of public aquaria have a variety of expectations during their visits, including talking to biologists and connecting with nature (Falk et al., 2007). In education programs where visitors can touch the animals, many of these expectations are fulfilled in a tangible way. When guests feel connected to the animals, they become ambassadors for conservation (Gendron, 2004). Many of the trained behaviors that facilitate elasmobranch handling can also be applied to animal interaction education programs (refer also Firchau, this volume). These and other interactive opportunities should be proactively developed, resulting in animals that are not stressed by interactions with humans but are participating by choice through positive reinforcement.

CONCLUSIONS

Training and conditioning elasmobranchs in public aquaria is no longer a novelty. It is clear that the question of whether to train or not has been replaced by: How can we best use training to care for our elasmobranch collection? There have been noteworthy successes training *S. fasciatum, R. typus,* giant manta, *Manta birostris* (Walbaum, 1792) (Christen and Schreiber, 2010), bowmouth guitarfish, *Rhina ancylostoma* (Bloch and Schneider, 1801), nurse sharks, *Ginglymostoma cirratum* (Bonnaterre, 1788), spotted eagle rays, *Aetobatus narinari* (Euphrasen, 1790), and southern stingrays, *Dasyatis americana* (Hildebrand and Schroeder, 1928).

A key challenge for the future is how to employ some of the training techniques described with pelagic, ram ventilating species, whose welfare is typically more challenged during restraint and handling for transport or clinical procedures. Potential candidates for investigation include sandbar sharks, *Carcharhinus plumbeus* (Nardo, 1827), and blacktip sharks, *Carcharhinus limbatus* (Müller and Henle, 1839), as they are highly represented in aquarium populations. Some institutions are already training advanced behaviors in these species.

Elasmobranch trainers are encouraged to look outward toward work conducted with other animal taxa, from which valuable lessons can be drawn, inspiring new approaches to training and suggesting avenues for further development of underused techniques.

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INTERNET RESOURCES

www1 https://theabma.org/glossary/
www2 https://www.surveymonkey.com
www3 https://support.office.com/en-us/article/Load-the-Analysis-ToolPak-6a63e598-cd6d-42e3-9317-6b40ba1a66b4
www4 www.animalenrichment.org/spider/spider_framework.html
www5 https://sites.google.com/site/elasmobranchhusbandry/home
www6 http://www.youtube.com/watch?v=s52IpLCt6r4

The Elasmobranch Husbandry Manual II: Recent Advances in the Care of Sharks, Rays and their Relatives, pages 223-227.

Chapter 22

Husbandry training of striped catshark, *Poroderma africanum* (Gmelin, 1789)

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Abstract: In December 2011, The California Academy of Sciences acquired four female and two male striped catsharks, *Poroderma africanum* (Gmelin, 1789). These sharks were to be displayed in an existing multi-taxa exhibit with African Penguins, *Spheniscus demersus* (Linnaeus, 1758) and sea stars. While in off-exhibit holding, the nocturnal benthic catsharks were successfully trained to respond to an audio cue and feed at the surface during daylight hours. These trained behaviors were not retained by the *P. africanum* once they were moved into the larger multi-taxa exhibit with the penguins. A number of novel challenges necessitated a re-evaluation and alteration of the training plan, which included SCUBA divers hand-feeding and training the sharks underwater.

INTRODUCTION

The pajama shark, or striped catshark, *Poroderma africanum* (Gmelin, 1789), is a member of the catshark family, Scyliorhinidae, and is endemic to South Africa (Compagno et al., 1989). This genus, as we know it, was first described in a paper presented at the Zoological Society of London by A. Smith in 1837. The species, however, was first described by Gmelin in 1789 as *Squalus africanus* (Compagno, 1988). *P. africanum* are small; males reach approximately 58 - 101 cm total length (TL) and females are slightly smaller at 58 - 93 cm TL (Compagno, 1988). *P. africanum* is whitish, to medium gray, with distinctive dark longitudinal bars. They have short nasal barbs and the second dorsal fin, which is set far back on the body, is much smaller than the first (Ebert et al., 2013). *P. africanum* are oviparous, and lay eggs in pairs, with one egg produced from each of the two oviducts (Compagno et al., 1989). Each oviduct can produce from 15 - 20 eggs per year. However, it is not known whether all eggs laid are fertile (Compagno, 2005). *P. africanum* egg cases are transparent and roughly 10.3 cm long when laid (von Bonde, 1948). Based on the timeline of eggs laid in aquaria, the gestation of the pup is believed to be approximately 165 days, and pups emerge at 14 - 17 cm TL (Ebert et al., 2013).

P. africanum are found in temperate inshore waters off South Africa, from the West Cape to southern Natal, but are centered on Cape

Province (Compagno, 1988). The catsharks favor intertidal rocky reefs but can be found as deep as 100 m, spending much of their time resting in caves and crevices by day (Compagno et al., 1989). *P. africanum* are predominantly nocturnal, hunting for bony fishes such as European anchovy, *Engraulis encrasicolus* (Linnaeus, 1758), and shallow-water cape hake, *Merluccius capensis* (Castelnau, 1861), and also a wide range of invertebrates such as shrimp, crabs, bivalves and polychaete worms (Ebert et al., 2013). In addition, *P. africanum* actively hunt for several species of cephalopods, including cuttlefish, octopus and squid (Compagno et al., 1989).

While most hunting occurs at night, there have been documented cases of *P. africanum* hunting specifically for chokka squid, *Loligo vulgaris* (Lamarck, 1798), during daylight hours (Smale et al., 2001). This behavior appears to only occur during *L. vulgaris* egg-laying and mating season. Smale et al. (2001) observed large numbers of *P. africanum* (approx. 30 sharks in a 10 m radius) around squid egg beds in the Tsitsikamma Coastal National Park during daylight hours. *P. africanum* would settle into the egg beds, with their heads hidden, in order to ambush female squid when they were in the process of laying egg strands. In the absence of squid, the sharks were rarely seen to emerge from their caves and crevices (Smale et al., 2001).

P. africanum is listed on the IUCN Red List as "Near Threatened" (Compagno, 2005). The catsharks are taken as bycatch by trawlers and anglers in locally unregulated waters, and are also caught in gill nets and beach seines in open access areas. When last assessed, *P. africanum* bycatch was not used for human consumption, but has been used for lobster bait by commercial fishers, and returned to the water by recreational fishers (Compagno, 2005). *P. africanum* are a hardy species, so they are also taken in small quantities for the aquarium trade and generally thrive in human care. At present there is no indication of a decline in number of *P. africanum*, or any degradation of their natural habitat, but anthropogenic threats could easily increase without careful monitoring and management. *P. africanum*, and other catshark species typically lay their eggs in benthic spawning areas. These areas could be affected by pollution, egg predation by gastropods, or an increase in other predators (Compagno, 2005).

MATERIALS AND METHODS

Once it was established that the Steinhart Aquarium (California Academy of Sciences, San Francisco, California, USA) would display *P. africanum*, a training plan was developed with a key goal: train the nocturnal benthic catshark to feed from the surface during daylight hours (refer Janssen et al., this volume, for more about training plans). Training and feeding *P. africanum* in this manner would allow biologists to monitor daily food ration, administer vitamin supplements, and facilitate daily health examinations. Feeding during the day would also provide for a better visitor experience. None of these stated goals would be accomplished using a standard broadcast feed.

On 15 December 2011, the Steinhart Aquarium received four female and two male *P. africanum* from the Oceanário de Lisboa (Lisbon, Portugal). The *P. africanum* were surplus to a breeding program operated by the Oceanário de Lisboa. The sharks were transported by air freight in oxygen-filled plastic bags in styrofoam boxes; one per 70 x 40 x 30 cm box. Upon arrival the *P. africanum* were given a visual entry exam and weighed by Animal Health Department staff and biologists. The sharks ranged in weight from 0.37 - 0.56 kg body mass (BM) and measured 40.5 - 48.0 cm TL. Once exams were completed, the animals were placed into a 2.44 m diameter fiberglass holding tank maintained at 13.3°C. Four sections of 30.5 cm long x 10.2 cm diameter schedule 40 PVC pipe were placed into the holding tank for the *P. africanum* to use as habitat.

The *P. africanum* were initially target fed using a feeding pole. The feeding pole consisted of a 91.4 cm long x 1.27 cm wide length of PVC with a black cable tie protruding from the end. Food was attached to the feeding pole by being threaded onto the cable tie.

Different cues were employed to alert *P. africanum* to the presence of food and the feeding pole. The first was a visual cue, consisting of a white plastic circle with black vertical lines affixed to the feeding pole. This visual target was replaced by an auditory cue consisting of "jingle" bells that were attached to the feed pole and rung underwater whenever food was present. The bells were then replaced with a louder electronic sound emitter. The sound emitter consisted of a speaker encased in a watertight PVC capsule connected by wires to an activation button on the dry end of

the feeding pole. When the button was pushed the emitter made a repeated beeping tone.

Once the *P. africanum* were successfully feeding from the pole, the sharks were targeted to feed while lying on a "platform" positioned on the bottom of the tank. The platform consisted of a 30.5 x 30.5 cm piece of white plastic marked with a red "X" using electrical tape. The platform was incrementally raised toward the surface over the course of multiple feeding/training sessions. The platform was then removed during feeding sessions so that the sharks became accustomed to feeding at the surface without any support from underneath.

Training sessions took place three times per week. The reinforcer for training sessions was 30 - 50 g of prawn, capelin or squid. Food fed during the three training sessions constituted the entire dietary ration for the sharks.

RESULTS and DISCUSSION

Training

On 24 May 2012 training began. The *P. africanum* were offered food from the feeding pole, complete with the circular visual cue target attached. It was observed that the sharks did not associate the target with food and that the visual cue was not effective. As a result, on June 2012, an audio cue was implemented using "jingle" bells attached to the end of the feeding pole. The sharks quickly associated the audio cue with food and over the next month roused more and more quickly when the bells were rung, even when food was absent.

Platform feeding

On 1 March 2013, the feeding platform was introduced at the bottom of the exhibit and the sharks targeted to feed while on the platform. The platform was incrementally raised ~30 cm during each feeding/training session. During this process it was observed that the sharks began taking food off the feeding pole, higher in the water column, without regard to the position of the platform. By 31 May 2013 the platform was removed altogether, as it was deemed superfluous. At this time all the *P. africanum* were already taking food from the feeding pole at or near the surface.

Audio cue

On 1 March 2013, in parallel with the introduction of the platform, a plan was initiated to replace the bells audio cue with a waterproof sound emitter. Sharks can hear sounds with frequencies ranging from 20 - 1,500 Hz (cycles per second), but most species studied are more responsive to sounds less than 400 Hz (Nelson and Gruber, 1963). As the *P. africanum* were destined for an exhibit much larger (and more acoustically noisy) than the holding tank, it was proposed that a louder, low frequency, electronic cue would be easier for the sharks to detect. A sound emitter, capable of producing a tone below 400 Hz, was designed and built by a member of the Audio Visual and Electronic Engineering Department (California Academy of Sciences).

To transition the sharks between the two audio cues, each training session was started and concluded with the bells, while the sound emitter was used in the middle of the session when the sharks were feeding. The bells were used for decreasing amounts of time and the emitter tone was increased in duration. Eventually, the bells were removed and only the tone was used for the entire session. During this time, additional cable ties were added to the feeding pole to accommodate several sharks approaching the pole simultaneously. By 13 June 2013 the tone was being used as a start-of-session cue and was only turned on until the sharks roused and start feeding, which was typically a period of ~30 s.

Transfer to exhibit

By 27 July 2013 all six sharks were rousing and feeding at the surface from the feed pole within 30 s of audio cue sounding. It was observed that training had progressed as far as possible in the holding tank and that any further progression would have to take place in the destination exhibit. On 30 July 2013 the *P. africanum* underwent a health assessment and were moved onto exhibit. Blood samples were taken and each shark was marked on their pectoral fin (using silver nitrate) to aid identification of individual animals. Passive integrated transponder (PIT) tags were inserted under the skin of each shark, and weights (BM) and measurements (TL) were taken to help monitor growth. To prepare the sharks for introduction to the penguin exhibit, the temperature in their holding tank was slowly raised over the course of a month to match the exhibit temperature of 18.3°C. Three caves were constructed in the exhibit using small rocks. The caves were positioned near a viewing window, with the intent that the sharks would nestle in an area where the public would see them.

Challenges and solutions

Once the sharks were placed on exhibit, they were given four days to acclimatize before

being offered food. By sharpening their appetite, it was hoped that the sharks would respond well to the first training session. The sound emitter and feeding pole were both used as cues during the first feeding session, but none of the sharks responded to either of the stimuli. *P. africanum* were rarely seen swimming around the exhibit and only one or two animals were observed at any one time (day or night). Training/feeding cues were tried for an additional three days, without response from the sharks. It became clear that biologists, on SCUBA, would have to check on the status of the animals. Only two of the sharks were located on the first dive.

It was hypothesized that the sharks may have entered the life support system (LSS) through PVC pipe supply lines disguised in the exhibit rockwork and not dissimilar to the pipes used within the holding tank as "habitat". A more comprehensive dive was planned and executed the same day. During the second dive, many small cracks and crevices were found in the rockwork. Some of the *P. africanum* had wedged themselves tightly into these spaces. The biologists had a challenging time extracting the sharks from the crevices, but once all six animals had been retrieved, they were removed from the exhibit and placed into a 1.83 m diameter holding tank. The sharks remained in holding until all the LSS supply lines had been screened and barriers erected in front of the crevices. After making the necessary modifications, the sharks were returned to the penguin exhibit.

It became clear that the training approach applied in the holding tank was not easily translatable to the exhibit and needed re-evaluation. Challenges faced in the exhibit included: (1) a much larger space, with many hiding places for the sharks; (2) more ambient noise (from the pumps, LSS equipment, water movement, and penguins), which compromised the audibility of the feeding cue; (3) dietary intake was not controllable, as sharks accessed food dropped by the penguins (as well as food broadcast for the sea stars); and (4) accessing the sharks with a feeding pole was challenging as a result of the exhibit size and topography.

Four solutions were implemented to address these challenges: (1) the biologists created more hiding places for the catsharks in areas more visible to the public; (2) the audio cue was increased in amplitude to make it more audible to the sharks; (3) the penguin biologists made a concerted effort to minimize the amount of excess food left in the exhibit following a feeding session; and (4) feeding and training the sharks was conducted by biologists while diving on SCUBA.

Training while diving

As mentioned, it was challenging to access sharks from the surface of the exhibit with a feeding pole, so control of food ration and supplements was compromised. Visual health checks were similarly impaired. For this reason it was decided to feed and train the sharks from within the water. While on SCUBA, biologists located the *P. africanum*, shook a dive rattle, pulled the sharks from their hiding places, and delivered food by hand. In a short period of time, the sharks started to come out of their hiding places, ready to feed, when the divers entered the water and shook the dive rattle.

CONCLUSIONS

Training *P. africanum* was an ambitious project. However, despite a slow start, the results achieved while the sharks were in holding were remarkable. Unanticipated challenges prevented a successful transition of trained behaviors into the exhibit setting. Nevertheless, initial training successes make us optimistic about successfully training these nocturnal, benthic sharks to feed at the surface during the day. This practice will aid in animal husbandry, health monitoring and, ultimately, enhance the visitor experience by creating a more dynamic and educational exhibit.

Future refinements to the training plan include: (1) introducing a start of session cue; (2) making the existing audio cue louder; (3) using a different, less cryptic, audio cue; and (4) testing the utility of an olfactory cue to start a feeding session. In addition, a strategy of successive approximations will be employed to transition the sharks from hand feeding by SCUBA divers, to target pole feeding from the surface.

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Chapter 23

Rescue, rehabilitation and release of a whale shark, *Rhincodon typus*, in the Arabian Gulf

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Abstract: A stranded 4.9 m total length (TL) juvenile female whale shark, *Rhincodon typus* (Smith, 1829), was rescued from shallow coastal waters of the Arabian Gulf and rehabilitated for 567 days in the Ambassador Lagoon at Atlantis, the Palm (Dubai, UAE). The shark began feeding 12 days after rescue and, with the exception of one 19 day fast near the end of its first year in the aquarium, fed regularly on krill patties and teleost eggs. The shark grew at a rate of 37.5 cm/year, which was higher than previously estimated for juvenile *R. typus* in aquaria. Following rehabilitation, the shark was outfitted with a pop-up satellite archival tag (PSAT) and released back into the Arabian Gulf off Dubai. After 33 days in-place, the tag separated, surfaced and transmitted information from the central Gulf, 320 km west of the release site. The shark had moved ~1,106 km towards, and around, the Al Shaheen oil field, an area of known *R. typus* aggregation about 90 km off the Qatari coast. These results indicate that the *R. typus* rescue, rehabilitation and release efforts proved successful.

INTRODUCTION

Multiple whale shark, *Rhincodon typus* (Smith, 1829), aggregation sites have been identified globally throughout the tropical and subtropical distribution of the species. While reports are limited on *R. typus* presence and movements in the Arabian Gulf, most sightings in the region occur between April and October (Robinson et al., 2016) and a major aggregation of as many as 100 sharks within a square kilometer has been documented in the Al Shaheen oil field in the central Gulf, about 90 km off the coast of Qatar (Robinson et al., 2013).

On 28 August 2008, Atlantis, the Palm, located in Dubai, United Arab Emirates (UAE), was requested by a local marine non-governmental organization to assist in the rescue of a 4.9 m total length (TL) juvenile female *R. typus*. The shark was stranded in the Palm Jebel Ali, a man-made archipelago located approximately 20 km from Atlantis. Intervention and rescue were chosen as the appropriate course of action due to the location of the animal, poor water quality in the area and injuries sustained during the stranding event. In addition, an *R. typus* stranding event the previous year, in the same location, resulted in mortality, influencing the decision to intervene.

The shark was transferred by boat to the Lost Chambers Aquarium and Ambassador Lagoon of Atlantis, the Palm, where the animal was successfully rehabilitated over 19 months. During this period, different feeding techniques and changes to diet and habitat were adopted, as necessary, to maximally support the needs of the animal. Following rehabilitation, the shark was tagged and released back into the Arabian Gulf. Information transmitted from the tag confirmed immediate post-release survival and suggested that the shark successfully re-acclimated to its natural environment.

STRANDING, RESCUE AND TRANSPORTATION

The Palm Jebel Ali (25.00°N / 54.99°E) is a series of man-made islands containing a shallow, open-ended lagoon with a depth averaging about 2 m. At the time of rescue, the *R. typus* was facing extreme seawater temperature (38°C) and salinity (47 g/L) conditions in the lagoon. How the animal stranded in this location, and the exact duration of the stranding event, remain unknown, as the Palm Jebel Ali is largely unpopulated.

When Atlantis staff arrived at the stranding site, the animal was lethargic and, at times, motionless on the bottom of the lagoon. This enabled Atlantis team members to restrain the animal with relative ease and guide it into a stretcher, attached to a boat derrick, for lifting into a 5.5 m long x 4.0 m wide x 2.0 m deep holding tank aboard the vessel. The tank contained recirculating natural seawater. Transportation time from the Palm Jebel Ali to Atlantis, the Palm, was approximately 3.5 h. Upon arrival at Atlantis, the animal was transferred from the holding tank on the boat to a second tank on a truck, again using a stretcher attached to the boat derrick. The truck transportation tank was 5.0 m long x 1.2 m wide x 1.5 m deep. The animal was transported to the entrance of the aquarium, which took approximately 10 min. The transportation tank was transferred from the truck to two dollies connected to an electric cart via a forklift. The tank containing the animal was then raised to the top of the exhibit via a 6 T elevator. Once at the top of the exhibit, the TL of the shark was measured and a brief assessment of its condition was made. Based on behavioral and externally visible criteria, the shark was judged to be suitable for release into the aquarium. The container was lifted from the dollies by a winch, lowered into the aquarium, and completely flooded. With assistance, the

animal swam out of the transportation tank into the exhibit.

THE EXHIBIT

The Ambassador Lagoon exhibit was an open-air 70 m x 30 m aquarium with a maximum depth of 9.25 m (Figure 1). The themed exhibit was based on the lost city of Atlantis, with artifacts extending a maximum of 8 m high inside the tank. The exhibit had a 3 m shallow ledge at one end, with a substrate composed of 7 cm deep, fine white sand. The 11,000 m^3 semi-open system received natural seawater pumped from the Arabian Gulf in front of the Atlantis property. The exhibit had a water make-up rate of 1.89 - 2.27 m^3/min, or 27.2% per day, with a total volumetric turnover time through the life support system of 70 min. Water temperature in the exhibit ranged from 23 - 26°C, and was heavily regulated during the summer to compensate for the extreme temperature of local intake seawater. The exhibit had a moderate counterclockwise current.

Diving activities, including exhibit maintenance and animal feeds, were temporarily suspended for 24 h following the introduction of the *R. typus* to minimize stress imposed on the animal. Following this temporary suspension, diving activities resumed, but were limited to certain areas of the tank, allowing the animal opportunity to avoid human contact.

SWIMMING PATTERNS

Initial observations of the behavior of the animal indicated that it quickly became aware of the boundaries of the exhibit. The shark established a predominantly counterclockwise swimming pattern at an average depth of about 2 m. Efforts were made to vary the swimming pattern by lowering some of the taller artifacts and columns inside the tank, including a prominent 8 m obelisk. Following the removal of these obstructions and the acclimatization of the animal to the exhibit, its swimming pattern began to vary and it swam deeper in the water column, at times only ~60 cm above

Figure 1. Aerial photo of the Ambassador Lagoon at Atlantis, the Palm aquarium, Dubai, showing (highlight) the rescued female whale shark, *Rhincodon typus* (Smith, 1829).

the substrate. To encourage this behavior, further action was taken to relocate artifacts and afford the shark an unobstructed swimming area.

From late March through July, 2009, the shark was observed spending more time swimming towards the bottom of the exhibit, sometimes very close to the substrate and frequently with its mouth agape as if feeding. As the exhibit was open to the air, and used natural seawater, it is possible that some naturally occurring prey may have become available in the water column, potentially explaining this behavior.

When food was offered to the animal, it would immediately break its typical swimming pattern and assume a near vertical position during feeding, approximately 4.5 m away from the feeding platform.

Due to the irregular shape of the exhibit, further measures were taken to prevent the animal from coming into contact with the perimeter walls. These measures included hanging 7.5 m long pieces of free-swinging PVC pipe into the water, approximately 1 m away from the walls, to deter the shark from swimming against the abrasive surfaces of the exhibit perimeter.

FEEDING AND GROWTH

Initial attempts to feed the shark krill (*Euphausia pacifica* and *E. superba*) were first made on 29 August 2008. Attempts were made each day at 10:00, 14:00, 16:00, and 18:00. Small patties of krill were thrown into the water ~60 cm in front of the mouth of the *R. typus*, in an effort to entice the shark to feed. These attempts continued until the animal started to feed on 8 September 2008. The number of feeds was then reduced to two feeds per day, at 10:00 and 16:00. In general, a total of 10 kg of food was fed per day, split over the two feeding sessions, each krill patty weighing ~200 g. Alterations to dietary ration were based on the behavior and appetite of the shark. If the shark was seen actively searching for food following a feeding session, more food was offered to a maximum of 16 kg per day.

On 29 October 2008, fish eggs, or tobiko roe (family: Exocoetidae), were introduced into the diet. Initial trials with the roe alone were unsuccessful (the shark spat them out), so the roe was added to the krill mix. This modification proved to be successful, with the animal consuming approximately 1 kg of roe per day, until its release. Daily vitamin supplementation was provided in the form of one Elasmobranch Tablet (International Zoo Veterinary Group, Keighley, West Yorkshire BD21 1AG, UK) per 500 g food. Tablets were cut into small pieces to minimize rejection by the shark.

A period of inappetence was observed from 25 July to 12 August 2009. Feeding slowed two days prior to the start of the fast, with the animal entering the feeding area but showing little interest in food. During this period of inappetence, the shark maintained its regular swimming patterns but did not enter the feeding area when food was offered. The shark was put on a 24 h watch by aquarium staff during this period. No fecal material was observed coming from the cloaca of the shark during this time, until 11 August, when a large amount of excrement was observed. On 13 August, the animal again started to show interest in food. Upon resumption of feeding, a maximum of 5 kg of food was offered per day, with the ration gradually increased to a maximum of 6 kg per day for the remainder of the shark's time at Atlantis, the Palm.

In the 567 days the shark was in the aquarium, it grew from 4.9 m to 5.5 m TL. This growth rate (38.6 cm/year) was higher than the rate of 29.5 cm/year estimated by Uchida et al. (2000) for juvenile *R. typus* under human care.

REHABILITATION

At the time of initial rescue, significant tissue damage was present on the leading edges of the pectoral fins and the lower lobe of the caudal fin of the *R. typus*, likely a result of damage sustained during entrapment in the shallow lagoon of Palm Jebel Ali. This damage was particularly prominent on the pectoral fins, where large areas of subcutaneous tissue and cartilage had been exposed. Despite the extent of these injuries, no secondary infections were observed at the time of rescue or during rehabilitation. When first introduced into the exhibit, the shark preferentially employed a counterclockwise swimming pattern, and the left pectoral fin deteriorated due to constant contact with the aquarium wall. However, the installation of the PVC 'bumper' pipes reduced this behavior and injuries immediately began to heal. As the wounds granulated, a thick layer of skin and scar tissue formed around the exposed areas of cartilage, which created an uneven leading edge along the pectoral fins.

When the *R. typus* was rescued, the bottom tip of the lower lobe of its caudal fin was splayed

out, creating a flat edge, probably due to contact with the bottom of the Palm Jebel Ali lagoon. During rehabilitation of the shark in the Ambassador Lagoon, tissue grew over the bottom edge of its caudal fin, covering the exposed area. As this area healed, the tip of the lobe curled to the left, with the bottom edge remaining flat. On the flat surface of the lower lobe, medium-sized carbuncle-like lumps appeared. These lumps were firm to the touch and did not appear to contain any fluid. In addition, there was some superficial damage to the skin tissue on the lateral ridge between the second dorsal fin and the anal fin, which completely healed during rehabilitation.

A small (approximately 10 cm^2) patch of unpigmented skin appeared on the upper side of the head of the *R. typus*, while on exhibit. The dorsal location of the patch made it unlikely to be a contact abrasion, as there were no overhead obstructions in the exhibit. Over time, the patch darkened and was nearly unnoticeable at the time of release. A couple of less conspicuous patches appeared in other places, but followed the same pattern of change.

RELEASE

The shark was released back into the wild on 18 March 2010. Assessment of suitability for release was based on the improved condition of the wounds sustained during entrapment in the Palm Jebel Ali lagoon, prevailing sea conditions and confirmation of the annual return of wild *R. typus* to the region. March offered the best opportunity to release the animal, with sea temperatures similar to those found in the Ambassador Lagoon. Plans to release the animal earlier in the month were temporarily postponed, due to the presence of a severe harmful algal bloom in the area.

At 04:00 on 18 March, a watertight stretcher supported by a 5.9 m x 1.5 m metal frame, based on a design used by the Georgia Aquarium, was lowered into the water just below the surface. As the animal swam towards the frame, it was guided towards the open end of the stretcher by a team of eight aquarists in the water. A 500 T crane, with an extended reach, was used to lift the frame and stretcher, containing the animal, into a rig on the back of a waiting truck. A constant flow of oxygen was fed into the water, inside the stretcher, maintaining the dissolved oxygen concentration at a constant 16 mg/L.

Transportation time to the release site was approximately 15 minutes. The animal was released at 05:30 on the ocean side of Palm Jumeirah. A 700 T crane was used to lift the animal over a 30 m wide breakwater and lower it into the water. The location was chosen due to the narrow width of the breakwater and the relatively low amount of human activity. Prior to release, the shark was tagged with a pop-up satellite archival transmitting tag (PSAT) (Mk10-PAT; Wildlife Computers, Redmond, Washington, USA) to document its post-release behavior.

POST-RELEASE TRACKING

On 23 April 2010 (33 days post-release), the PSAT detached and began transmitting at the surface off the coast of Qatar, a straight-line distance of 320 km from the release site (Figure 2). The maximum likelihood track (MLT; see Hueter et al., in press, for methodology) demonstrated movement in a northwesterly path through late March and early April. By 6 April, the shark reached the Al Shaheen oil field area and remained in this vicinity for a period of approximately 11 days (Figure 2). The shark then moved in a southerly direction along the east coast of Qatar before the tag detached about 50 km northwest of Doha. During the tracking period, the *R. typus* moved an estimated distance of 1,106 km, at an average rate of 33.5 km/day, reached a maximum depth of 72 m and experienced water temperatures ranging from 22.6 to 26.8°C.

CONCLUSIONS

The growth of the *R. typus* in the aquarium and the subsequent track of its movements after release indicate that it was successfully rehabilitated. Injuries sustained by the shark prior to rescue healed while on display. The shark fed regularly (with one exception) and grew at a rate that exceeded a previous estimate for juvenile *R. typus* in aquaria. Upon release, the shark traveled to an area of the Arabian Gulf known for aggregations of *R. typus*, further indicating a healthy condition. Therefore, we conclude that our rescue, rehabilitation and release efforts were successful in preventing the death of the shark from stranding and enabled it to continue its natural behavior in the Arabian Gulf. We hope that photographic records of the spot pattern on the animal, from its time at the aquarium, may also allow for subsequent monitoring of the shark in the wild, using photo-ID techniques (Robinson et al. 2016).

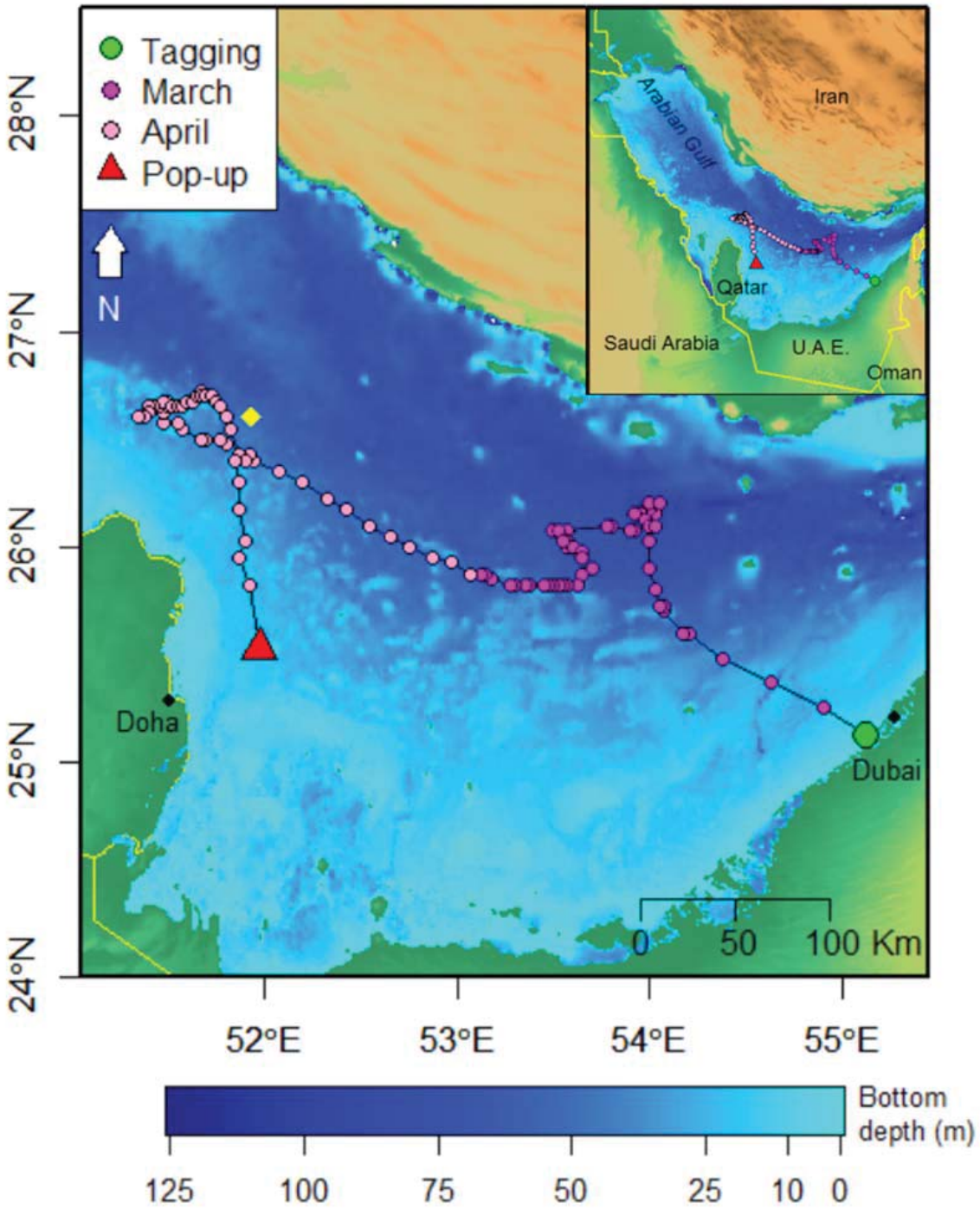

Figure 2. Tagging, pop-up and maximum likelihood track locations for a satellite-tagged female whale shark, *Rhincodon typus* (Smith, 1829), after release from rescue and 1.6 years of rehabilitation at Atlantis, the Palm aquarium, Dubai. The yellow diamond marks the location of the Al Shaheen oil field.

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Chapter 24

Elasmobranch Deaccession

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Abstract: Over the past few years, advances and improvements in the capture, transportation, life support systems for, and husbandry of, elasmobranchs, have permitted a dramatic broadening of the sizes and species of sharks and rays able to be maintained in aquaria. One easily overlooked aspect of these more diverse and longer-lived collections is an appropriate deaccession plan. In addition to the "...'til death do us part..." paradigm of holding animals indefinitely, there are only three other options available: transfer to another facility, release to the wild or euthanasia. Each has its own set of advantages, disadvantages and opportunities, all of which warrant consideration. Regardless of the preferred option, a well-considered deaccession plan must be part of any accession plan.

INTRODUCTION

Humans have an undeniable interest in elasmobranchs, verified by the daily media attention dedicated to these species. The public expects to see sharks and rays in every aquarium they visit. The capability of the aquarium industry to improve all aspects of live animal management has advanced exponentially over the past few decades. No longer are aquariums limited to exhibiting the hardiest of species. Indeed, some species that were once considered impossible to maintain in a display setting are becoming increasingly common in public aquaria worldwide.

Not only is the species list expanding, but the longevity of individual animals in aquaria is also increasing. As biologists gain a better understanding of the life history and basic biology of different elasmobranch species, aquarium professionals are integrating this information into their husbandry practices. Veterinary medicine is also playing an increasingly important role, bringing newer medical technologies into the aquarium world. The net result of increased elasmobranch longevity is not only the potential for increased animal size, but also potential changes in behavior, habitat requirements, reproductive activity and diet, as they progress through various developmental stages. Responsible animal management dictates that these changes be considered, as plans to exhibit various elasmobranch species are developed.

DEACCESSION PLANNING

During animal acquisition planning, the specifics of an animal management plan are developed. For facilities accredited by the Association of Zoos and Aquariums (AZA) (www1), these specifics are found in the Institutional Collection Plan (ICP). Institutional Collection Plans should contain information justifying the inclusion of the species within the institution's collection, details about the *in situ* and *ex situ* status of the species, and captive management or husbandry details. Methods for acquiring exhibit animals, while beyond the scope of this manuscript, are also typically contained within an Institutional Collection Plan. While not specifically mandated, it is incumbent upon facilities holding wildlife, including elasmobranchs, to have deaccession plans in place before accession efforts are initiated. Failure to do so may inadvertently place these animals in physically, psy-

chologically or socially unhealthy environments, not only inappropriate for the individual animal (or others within the collection), but also reflecting poorly on the institution and the aquarium industry as a whole.

Options available for the deaccession of elasmobranchs maintained under human care are: (1) maintain the fish for the duration of its life; (2) transfer the fish to another institution; (3) release the fish back into the wild; and (4) euthanasia. There are advantages and disadvantages to each alternative. Additionally, there may be several options available for each circumstance. As a plan is developed, one must bear in mind that there may be legal considerations for transfer, release or euthanasia. And, not surprisingly, these considerations are subject to ongoing modification. Therefore, an institution needs to validate assumptions made during planning inception, to assure accuracy, prior to implementation. Some incorporated flexibility and a well-conceived back-up plan is highly recommended.

LONG-TERM HOLDING

One might make a compelling argument that the best elasmobranch deaccession plan is to never have the need to deaccess specimens at all. Advances in our understanding of the basic biology and natural history of the various species in our care, coupled with modern life support systems, aquarium designs and advances in medical care, enable a more predictable trajectory of an animal's physical, behavioral and dietary development over time. As a result, one can more readily and accurately predict how and when deaccession may become necessary. However, animal behavior can be unpredictable and exceptions to anticipated outcomes may arise.

For obvious reasons, the best long-term holding option for elasmobranchs is the complex, enriched habitat in which they are typically displayed to the public. Certain circumstances, however, may dictate that animals be moved into off-exhibit holding areas for long periods of time. Even though these animals are removed from the view of the general public, it is essential that the holding area meet species-appropriate social, physical, behavioral and nutritional requirements. Simply placing animals into monochromatic circular tanks, without appropriate environmental or behavioral complexity (Figure 1), may not be ethically justifiable and may mean that maintenance of the animals under human care until natural mortality is untenable. If circumstances are such that an animal cannot remain on exhibit indefinitely, the need for the installation, management and maintenance of a suitable long-term holding facility may prove challenging, expensive or even impossible, and therefore that the species should not be acquired at all.

Figure 1. An example of off-exhibit holding, including: a 9.1 m (30 ft) diameter round tank, a 12.2 m (40 ft) diameter round tank and a 12.2 m x 18.3 m (40 ft x 60 ft) oval tank. Even with relatively large volumes, monochromatic tanks, without significant environmental complexity, may not meet species-appropriate requirements for long-term holding. Photo by Michael J. Murray.

TRANSFER TO ANOTHER FACILITY

Transferring elasmobranchs that no longer meet the requirements of the institution's collection plan, from one facility to another, may be a suitable deaccession strategy. This situation not only fosters a cooperative culture among institutions, but also decreases possible pressure on *in situ* populations by aquarium wild collection efforts. There are some pre-requisite considerations for lateral transfers, however, that warrant specific attention.

For institutions accredited by a voluntary professional group, such as the AZA (for public display facilities) or the Association for the Assessment & Accreditation of Laboratory Animal Care International (AAALAC) (for research facilities), there are specific requirements for deaccession to other institutions. In most cases, lateral movements between accredited facilities are straightforward. Nevertheless, it remains incumbent upon the transferring facility to assure that the recipient has the physical facility and professional expertise necessary to care for the species under consideration. A suitable risk assessment must be conducted in regards to compatibility between the animal being transferred and the existing population within the recipient institution's exhibit, taking into account individual specimen size, developmental stage and behavior.

The degree of scrutiny necessary for deaccession to non-accredited facilities should be even greater. Not only is it important to consider the status of the physical plant and the experience of staff, but the recipient's mission and long-term plans must also be evaluated to assure compatibility with the transferring facility. It is incumbent upon the institution deaccessing the animal to make every effort to assure that transferred animals do not fall into the hands of unqualified carers, not only in the primary transfer, but in all subsequent transfers. Pre-transfer investigations may include site visits, written application materials and testimonials provided by colleagues. All of this information should be recorded and documented within archived animal records. A written, formal transfer agreement, in which expectations, responsibilities and limitations are specifically documented, is strongly encouraged.

In addition to institution-to-institution considerations, there may be regulatory mandates for animal transports established by local, regional and/or federal governments. Both the shipping and receiving institutions should investigate regulatory implications for animal transfers to assure compliance. It is important to note that regulatory requirements change frequently and unexpectedly, so a thorough investigation should be conducted prior to each shipment.

Figure 2. Shipping large elasmobranchs requires robust and complex transportation plans and vehicles. In this case, a 12,000 L tank, equipped with biofiltration, oxygen supplementation, closed-circuit cameras and water quality sensors, is mounted on a flatbed trailer. Transport tanks of this type allow for fishes to swim freely, but rapid access to the animal may be impaired. Photo by Michael J. Murray.

For the most part, local regulatory impact on animal transfers is limited to the management of transport vehicles (Figure 2). While not intending to downplay the importance of compliance with these regulations, their discussion is beyond the scope of this manuscript. The reader is directed to Choromanski (2004) for a fuller review.

Many elasmobranchs maintained in aquaria are initially acquired from *in situ* populations. As such, they were likely acquired under the auspices of some form of scientific, commercial or recreational collecting permit. Often, permits contain language that restricts the transfer of collected animals from one institution to another. In the case of the California Scientific Collecting Permit, the statement is: "...the specimens you collect under this Permit must be used solely in and by your institution and not sold or traded to other institutions without prior written authorization...". As stated, such a mandate does not preclude inter-institutional transfers, however, it does add an additional layer of complexity to the process.

Over the past several years, there has been a significant change to the manner in which government departments interpret "fishes" in zoos and aquariums. These animals, including elasmobranchs, are now considered "aquaculture" by some, including the United States (US) Department of Agriculture, and are, therefore, subject to a broader scope of oversight and regulation than historically applied. Movements of elasmobranchs across intra-national borders are often subject to oversight by state, provincial, and/or regional departments of agriculture and the US Fish and Wildlife Service (www2). Not surprisingly, regulatory requirements are not consistent between regions, or over time. It is important that all parties involved in lateral animal transfers make direct contact with the relevant agricultural agencies before initiating transport. In the case of transfers within the US, the offices of the various state veterinarians (www3) are the most appropriate starting point. However, at their discretion, other agencies such as resource or wildlife agencies may be asked to weigh in on decisions about animal transfers.

The deaccession of elasmobranchs across international borders is complex and expensive. Not only are local, regional and federal regulations applicable, but there are further international trade implications, notably CITES (www4) permitting, that must be considered. Navigating the complex network of export regulations involving wildlife and customs, coupled with analogous wildlife and customs import rules, is often tedious, convoluted and seemingly illogical. For that reason, institutions will benefit from hiring professional animal transport brokers for international shipments, if for no other reason than to aid in the navigation of the associated paperwork.

Despite the morass of rules and regulations associated with deaccession through inter-institutional transfer, this method is still valid and suitable for managing a collection of elasmobranchs.

RELEASE TO THE WILD

Releasing elasmobranchs into wild populations is a third, albeit controversial, option for the deaccession of aquarium specimens. Application of this deaccession strategy mandates that there

Figure 3. Release of a juvenile white shark, *Carcharodon carcharias* (Linnaeus, 1758), into the eastern Pacific Ocean, after several months on exhibit. Plans for release must include extensive risk assessment for both the individual animal and the population into which the animal is being released, as well as consideration for the regulatory requirements associated with re-introduction. Photo by Randy Wilder, Monterey Bay Aquarium.

is consideration not only for government regulations, similar to those described for transferring animals, but also includes evaluation of the risks inherent in the release of fishes from human care back into the wild (Figure 3).

In nearly every case, a decision to release aquarium specimens to the wild is only marginally supported by data demonstrating a positive impact on wild populations. In the absence of this data, it must be acknowledged that most benefit derived from a release-to-the-wild strategy is gained by the individual animal and by the institution, and that releasing an animal to the wild is contrary to guidelines established by the International Union for Conservation of Nature (IUCN / SSC, 2013). Benefit can accrue, however, by implementation of a scientifically sound post-release monitoring or tagging study. Such efforts to better understand survivorship, movements and/or growth, better justify release-to-the-wild and may assuage concerns raised by external commentators.

A significant amount of risk is inherent in release-to-the-wild deaccession programs. Release of aquarium animals may result in the introduction of novel, or modified, disease or parasite agents into wild populations. Such infectious disease may be transmitted among tank mates in aquarium settings or through feeding strategies. The unnaturally supportive husbandry practices of human care may allow pathogens to gain a foothold in host fishes without causing significant clinical disease. Thereafter, stress associated with the harsher realities of wild conditions can facilitate pathogen amplification, and may result in disease expression and introduction to conspecifics or other organisms. Such concerns exist not only for novel infectious diseases, but also those that may be cyclical in nature. It is naïve to think that it is possible to determine an animal to be pathogen-free and, therefore, the degree of risk is unpredictable. For that reason, deaccession plans, which include release-to-the-wild strategies, must be subjected to critical multi-disciplinary review.

Since most aquarium elasmobranchs are wild-caught, there is little to no information on individual genealogy. Additionally, genetic evaluations of *ex situ* individuals and *in situ* populations, into which they may be released, are rarely carried out. As a result, the relatively random introduction of genetic material into a wild population may represent a risk of out-breeding or in-breeding depression.

Sound husbandry management of fishes under human care includes medical management, which often dictates the use of chemotherapeutics. Unfortunately, drugs used in elasmobranchs are non-specific in their application; dose, frequency, and duration are frequently extrapolated from other species and indications. More importantly, there is no data to support or evaluate withdrawal periods for various drugs in different species or in varying water temperatures. As a result, there is potential for human consumption of drug-contaminated food fishes, including elasmobranchs. As release plans are considered, a deliberate and defensible risk assessment should be performed when drugs have been used.

Another concern arising from release-to-the-wild strategies is the introduction of drug-resistant populations of potential pathogens, following artificial selection in the human-managed environment. The genes responsible for drug resistance can spread, representing a material risk to the wild population.

Beyond the population-level risk, there are significant concerns for an individual animal should it be released into a wild population. Modern husbandry protocols are designed to maximally support the needs of aquarium inhabitants. Food is plentiful, nutritious, and delivered without the effort and risk associated with hunting. Few, if any, predators exist for elasmobranchs in aquaria. Such is not the case for wild elasmobranchs. Released animals need to immediately adjust to their new environment, start hunting effectively, avoid predation and adapt to a water column that varies in depth, light intensity and temperature. Additionally, sharks and rays in aquaria have little to no need to maintain the athletic capacity required to survive in the wild, a situation that must change quickly for post-release survival. As release options are considered, institutions should contemplate not only oceanographic parameters, but also life history considerations, such as migration behavior and food availability, and the physical condition of animals maintained in aquaria.

While one may debate the cognition of sharks and rays, there is no doubt that their behavior can be deliberately or accidentally changed through the use of training methods. As such, consideration of the degree to which an elasmobranch has been imprinted or trained to associate food with humans is necessary when establishing suitability for release into the wild. An unnatural attraction to humans as a food source may place people, and also the fish, in harm's way. If release-to-the-wild

is part of the deaccession strategy, training programs must be managed accordingly.

Movement of elasmobranchs associated with release activities may attract many of the same considerations described for inter-institutional transfers. In addition to the associated agricultural agencies, local, regional, federal and international wildlife management regulations may need to be addressed prior to releasing an elasmobranch.

The ideal candidate for potential release to the wild would be an animal that had been collected and housed locally, with cohorts from the same geographic location, which had spent minimal time in captivity, was fed using locally collected species and had not been exposed to medications. Release should occur within the same locale and season, abiding by all relevant regulations and allowing for post-release monitoring. These conditions rarely exist. Therefore, a thorough risk assessment must be conducted prior to employment of a release-to-the-wild deaccession strategy.

The ability of an institution to employ a release-to-the-wild strategy may change depending upon the species' conservation importance, population status, market value as a food fish, and the real or perceived human health risk. Regulations change over time, and therefore a case-by-case evaluation is required. Final confirmation with local, regional and federal resources agencies should be conducted before elasmobranchs are released into the wild.

EUTHANASIA

The last, and arguably least desirable, deaccession strategy is euthanasia. Euthanasia, a term combining the Greek words for "good" (eu) and "death" (thanatos), has an important role in veterinary medicine. While uncommon, medical justification for this action, due to concerns surrounding quality of life, is occasionally encountered. In some more fecund species, institutions may be forced to use euthanasia for the management of population size. A third, relatively rare, reason for euthanasia is a specific stipulation in the collection permit. This situation can be the case for research animals that, due to the nature of non-lethal experimental procedures or housing requirements, cannot be transferred externally, or may be unsuitable for incorporation into open aquarium systems, or co-habitation with conspecifics or other species. Outside of these three relatively uncommon situations, euthanasia as a primary, pre-determined deaccession strategy is generally considered unethical and unjustifiable.

Euthanasia protocols should be consistent with the ethical tenets and accreditation standards of the industry, and it is recommended that they be developed in accordance with guidelines published by the American Veterinary Medical Association (www5) and/or the American Association of Zoo Veterinarians (AAZV, 2006). These guidelines include both chemical and physical methods of euthanasia.

Whenever possible, euthanasia should be scheduled and performed in a manner that provides the opportunity for post-mortem use of the carcass. A complete necropsy examination will provide direct feedback about the husbandry and health protocols employed, and access to a fresh, non-autolyzed specimen is valuable to a host of scientists working on elasmobranch biology. It is irresponsible to euthanize an elasmobranch and simply discard the carcass, without taking advantage of the opportunity to further the body of knowledge about of these amazing fishes.

SUMMARY

An institution's decision to keep and care for elasmobranchs commands significant responsibility. As Institutional Collection Plans are developed, it is important that the deaccession of display sharks and rays is considered prior to acquisition, yet, this aspect of an Institutional Collection Plans is commonly overlooked. There are essentially four options for deaccession: (1) lifetime holding; (2) transfer to another facility; (3) release to the wild; and (4) euthanasia. Euthanasia is inappropriate as a primary deaccession strategy for healthy animals. If euthanasia is the only option, then in almost every case the elasmobranch(s) should not be acquired.

As specific deaccession plans are developed, it is important to engage local, regional, federal and possibly international agencies, to assure compliance with regulatory and permit-based requirements. Moving animals out of a public setting is often an emotional event for institutional supporters. The use of experienced, trained communications staff to assure constructive public messaging, and appropriate degrees of transparency is an underused but worthwhile addition to deaccession protocols.

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INTERNET RESOURCES

www1 http://www.aza.org

www2 http://www.fws.gov/offices/statelinks.html

www3 http://agr.wa.gov/FoodAnimal/AnimalHealth/statevets.aspx

www4 https://www.cites.org/

www5 https://www.avma.org/issues/animal_welfare/euthanasia.pdf

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Chapter 25

Shark Health Management

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Abstract: Advances in the health management of elasmobranchs over the past two decades have enhanced longevity for many species, and provided the basis for earlier recognition of several frequently occurring syndromes. The pathogenesis of some of the common maladies suffered by sharks and rays in aquaria are becoming better understood. Improved knowledge of elasmobranch physiology and anatomy, combined with better approaches to husbandry, routine monitoring, and more reliable delivery of therapy to elasmobranchs, provides insight into the prevention and mitigation of these conditions.

INTRODUCTION – PROGRESS IN THE FIELD

Many key advances made in the decade since the publication of the first *Elasmobranch Husbandry Manual* have dramatically affected the approach to maintaining elasmobranchs in aquaria. These amazing animals are no longer considered disposable, and a great amount of social and ethical responsibility accompanies their husbandry. Many elasmobranch species are now listed as endangered or threatened and are under formal regulation and protection, and their importance to the stability of marine ecosystems is now much more widely understood and recognized. With these changes, our understanding of the natural biology of several key species has been greatly improved through comprehensive field studies, providing a stronger basis for re-evaluation of husbandry approaches.

Surprisingly, even the basic anatomy of many commonly maintained species was lacking. Recent studies, ranging from dissection-based traditional anatomical studies, to more complex imaging studies, now provides a much better understanding and basis for better clinical sampling and biopsy approaches to facilitate health assessment and management. Considerable study of lateral line systems and pit organs, particularly of batoids, has augmented Heuter and Maruska's work (Maruska, 2001; Hueter et al., 2004; Jordan, 2008; Wueringer and Tibbetts, 2008; Jordan et al., 2009a; Jordan et al., 2009b; Peach and Marshall, 2009). Other work on sensory anatomy and physiology has provided us with a better understanding of how elasmobranchs perceive their environment. Detailed anatomy of the ear (Evangelista et al., 2010), eye (Harahush et al., 2009; Harahush et al., 2014; Litherland et al., 2009a; Litherland et al., 2009b; McComb et al., 2010), and olfactory system (Schluessel et al., 2008; Theiss et al., 2009), as well as published insights into the neuroanatomy of elasmobranchs (Yopak et al., 2010; Yopak, 2012), provide a better foundation for clinical and preventative management of conditions that affect these organs and systems. Furthermore, this information helps inform the design of holding facilities. Other key systems critical to elasmobranch health maintenance have benefited from further study, including the respiratory (Wegner et al., 2010) and cardiovascular systems (Speers-Roesch et al., 2012). Studies on the anatomy of the jaw, mouth and feeding apparatus (Wilga, 2005; Wilga et al., 2007; Gerry, 2008; Abel et al., 2010; Wilga et al., 2012; Rygg et al., 2013), and detailed work on

thyroid histology (Borucinska and Tafur, 2009), have also provided useful insights into key challenges in the health maintenance of some aquarium species.

Similarly, solid basic science studies in other areas have informed our clinical management of elasmobranchs. The depth and range of work on metabolics (Skomal and Bernal, 2010; Wood et al., 2010; Bernal et al., 2012; Dove et al., 2012), osmoregulation (Anderson et al., 2010; Mori et al., 2010; Reilly et al., 2011), endocrinology (Anderson, 2012) and reproduction (Henningsen et al., 2008; Waltrick et al., 2012; Prohaska et al., 2013), all of which are relevant to the human care of elasmobranchs, has opened up new areas of health assessment and diagnostics.

Unfortunately, basic science studies have far outnumbered clinical studies of elasmobranchs over the past decade. Many of the more clinically focused efforts either fail to reach the literature, or may languish in somewhat obscure 'grey literature'. Consequently, it remains important to encourage clinicians to publish even straightforward case reports on these important species in peer-reviewed literature. Pharmacokinetic studies for elasmobranchs remain relatively few in number, and are focused on a limited number of drugs. Yet, these studies give us better insight into the metabolism and pharmacology of therapeutic agents in elasmobranchs, and by extrapolation and modeling, these studies also provide the basis for better prediction of the kinetics and dynamics of a wider array of related drugs. This knowledge in the hands of skilled clinicians significantly improves our basis for determining appropriate drug dosing, treatment intervals and therapeutic duration, when administering chemotherapeutic agents to elasmobranchs. Scientific assessment of the impacts of other therapeutic techniques, including endoscopy (Carrier et al., 2003; Murray, 2010), imaging (Carrier et al., 2003; Preziosi et al., 2006; Anderson et al., 2010; Grant et al., 2012) and biopsy techniques (Robbins, 2006; Daly and Smale, 2013), can also strengthen our understanding of how best to deliver elasmobranch health management.

The important work of scientists and clinicians working to characterize hematologic, serum chemistry and other key clinical pathology parameters of elasmobranchs, has provided a better scaffolding for diagnostic evaluation, with some papers presenting interesting conundrums that need further investigation (Cain et al., 2004; Arnold, 2005; Mylniczenko et al., 2006; Mylniczenko et al., 2007; Ferreira et al., 2010; Otway et al., 2011; Naples et al., 2012). This work, in turn, has contributed to improved therapeutic outcomes when aquarium elasmobranchs must be treated for illness. Particularly useful is a recent compilation of pathology findings, which gives clinicians a solid overview of a wide range of pathologies impacting aquarium animals, which is destined to be a landmark paper in elasmobranch health management (Garner, 2013).

Of equal importance to the health management of elasmobranchs under human care is new insight into normal behavior in the wild and in aquaria (Casper and Popper, 2010; Skomal and Mandelman, 2012). These studies, in combination with broader natural history studies of species commonly maintained in human care (Baremore and Hale, 2012; Speed et al., 2012), give us a much better foundation for identifying the early onset of disease, assessing the impact of therapeutic approaches and for preventing a number of health impacts that can be avoided by addressing normal behavioral needs with management manipulations.

KEY CHALLENGES REMAIN

Despite the many advances in elasmobranch husbandry over the past decade, a great deal of information is still outstanding. In some ways, the strides of advancement emphasize how much more there is to learn, and demonstrate how valuable a better understanding of elasmobranchs would be toward improving their health management in aquaria. Unfortunately, most of the literature in this area is found in this manual. It is, therefore, hoped that this work will inform future design and implementation of elasmobranchs holding facilities, allowing access to elasmobranchs, whether on exhibit, in quarantine or in holding. In addition, it is hoped that clinicians at aquaria will continue to investigate the efficacy of emerging clinical techniques and redouble their efforts to publish their findings in peer-reviewed literature.

FACILITY DESIGN ISSUES

Aspects of facility design offer, perhaps, the greatest potential for high impact improvement in aquarium elasmobranch health. Still today, insufficient or suboptimal access to animals for routine examinations, clinical sampling, or even routine husbandry manipulations, contribute to frustrations for husbandry managers and clinicians,

alike, who are unable to act when necessary to ameliorate challenges identified for the animals in their charge. New facilities are being built that replicate a deficiency that has long plagued elasmobranch management. Relatively little work has gone into the development of 'aquatic chutes' or restraint systems, or infrastructure to execute the behavioral modifications that would facilitate routine access to these animals. Many of the techniques used for marine mammals, for example, could be expected (with appropriate modifications) to be successful when applied to elasmobranchs. Some of this work is ongoing (Schleussel and Beckman, 2012; Guttridge and Brown, 2013), but much more is needed. Improved access to animals for routine examinations and diagnostics is key to our ability to recognize maladaptation, or the early onset of disease, and when correction may be feasible.

Many established institutions continue to struggle with inadequate holding for animals that need to be separated from display exhibits or the rest of the collection for various reasons. Relatively little innovation has occurred in the incorporation of sufficient off-exhibit holding space, particularly for large elasmobranchs, to allow proper management to meet their natural history, behavioral and health management needs. Quarantine practices have been collated across many institutions (Hadfield and Clayton, 2011), but are largely unsuitable, primarily because of issues of adequate, truly isolated holding space. There is a strong need for development of improved holding facilities and systems that allow easy manipulation of animals. These systems must permit true separation, based on concerns for animal safety, as well as the potential for infectious disease transmission. A critical point is that these facilities need to be designed and implemented with an understanding that animals may occupy them for extended periods of time.

Though there have been important advances in understanding the matching of tank configuration to elasmobranch behavioral and biomechanical needs, there is still much room for improvement in our understanding of these issues. Again, a literature search generally turns up the excellent papers presented during the first Elasmobranch Husbandry Symposium, but unfortunately little else. The questions of absolute size, depth, configuration and complexity of space in holding facilities for various elasmobranch species are far from reconciled. Collaborations between curators, design professionals, and physiologists will be critical to answering these complex questions across the breadth of elasmobranchs held in aquaria.

Planned maintenance is now a standard procedure in major aquariums, but there is significant room for improvement across the aquarium industry in this regard. Many of the conditions that impact captive elasmobranchs appear to have a relationship with deteriorating facilities, although isolating this from other causes remains incomplete and speculative. The sensitivity of elasmobranchs to stray micro-voltages that can occur either from flaws in basic exhibit construction or when water intrudes through disrupted membranes into concrete holding structures, setting up reactions with metal reinforcing, is well-documented. This topic is covered in more detail elsewhere in this volume (McCarthy and Levins, this volume). Stray voltages are a concern to the health management of elasmobranchs in aquaria. Better methods of detection of these voltages, either immediately post construction, or as a monitoring program while exhibits are in use, are needed, and such monitoring needs to occur routinely for elasmobranch exhibits. Less is known about toxicity challenges that can occur as membranes fail and oxidation products of structural and other materials leach out into system water. Whenever membranes are breached, or rust is evident, the assumption should be that potentially toxic species are reaching the water where elasmobranchs are being held. When this situation is occurring, there is significant risk of 'acute' mortality or disease events due to the sudden release of residue toxicants in the face of relatively minor, acute pH drops of only one- to two-tenths of a pH point.

MEDICAL ISSUES

There are a number of conditions that occur frequently enough in sharks in aquaria to make one consider that they may be related to human care management, although a detailed understanding of the etiology, pathogenesis or prescriptive preventative or therapeutic options are not available. I colloquially refer to these conditions as "mystery syndromes", even though considerable research and study has developed our understanding of some, more than others.

Sand tiger shark, *Carcharias taurus* (Rafinesque, 1810), scoliosis/kyphosis is one of the more studied "mystery syndromes" in aquarium sharks. A debilitating condition of *C. taurus*, which has frequently resulted in severe compromise and death in animals where it has been detected. This con-

dition has been well characterized radiographically and histologically (Preziosi et al., 2006), the result of which suggested that nutrition and/or trauma might have been causative. Subsequent studies have provided additional information documenting abnormalities in zinc, vitamin C, and potassium concentrations in blood (Anderson et al., 2012). More recently, controversial studies have suggested relationships with tank design and swimming behavior (Tate et al., 2013). Unfortunately, efforts to conduct a prospective study in this work relied on evaluating and comparing the swimming patterns of affected sharks to sharks potentially not yet known to be affected. In addition to the small sample size, this assumption notably weakened the conclusions drawn from the study, which failed to adequately account for the impact of the condition itself on the ability of sharks to sustain their height within the water column. This syndrome is a great example of where considerable information has been gathered to point research in a direction, but additional studies and tools will be necessary to help tease out whether exhibit design parameters, capture methods or underlying nutritional issues are in, and of themselves, sufficient to generate the problem, and what, if any, steps can be taken to mitigate the condition in animals in aquaria.

Some of the evidence in the *C. taurus* scoliosis studies suggested that the condition may be related to another problem with wider species impact; the syndrome of 'tank exhaustion', otherwise known as generalized maladaptation to aquaria. It is probably a misnomer to list this as a "mystery syndrome", as the basic underlying energetics involved in this problem have been largely understood for decades. On the other hand, we do not yet have good diagnostic approaches for early identification of this condition, monitoring its progress, or amelioration by husbandry adjustments. Thus, significant opportunities for advances in our understanding of the condition exist.

Post-capture collapse syndrome, and the likely very similar post-transport collapse, seen with larger shark specimens of a variety of species has been characterized over the years (Smith, 1992; Stoskopf, 1993; Haulena, 1999), and our understanding of the dynamics of serum glucose, potassium and other factors related to these conditions has led to important improvements in decision-making in the capture and transport of larger shark specimens (refer Smith et al., 2004 for more detail). Measures to avoid, or mitigate, the precipitous depletion of circulating glucose and potassium in sharks under stress have greatly improved survival for several key display species. It may, again, seem odd to list these problems as "mystery syndromes", but our advances have not eliminated the condition, even when great care is taken to manage the events with attention to what is known about the impact of these stresses on shark physiology. It is likely that other factors not monitored on routine blood and serum chemistry panels are perturbed and/or play an important role in the pathogenesis of these acute collapses. New approaches to investigating these "mystery syndromes" are needed.

It is interesting that the controversy about the necessity to remove hooks from elasmobranchs continues to color discussion of post-capture management of large species of elasmobranchs. The belief that long line hooks will rust away and not cause problems persists. In part, this belief has come about because of the failure of veterinarians to report on the complications of hook retention in the peer-reviewed literature. Long delayed hook migrations, even through the body wall, are not uncommon. This challenge is also generally fairly straightforward to diagnose once the hook begins to emerge through the skin. More work on this question seems necessary to convince shark managers of the value of accepting the risk of hook removal at time of capture, or shortly thereafter. The success of these procedures goes hand-in-hand with the challenge of improving handling techniques and access to newly caught animals.

Other "mystery syndromes" are very deserving of the category name. Gastric eversion entrapment occurs frequently enough to be presented in the clinical literature as case series (Tuttle et al., 2008), but the pathogenesis, and how to mitigate the challenges the syndrome presents, remain a mystery. A separate syndrome, *C. taurus* drop jaw syndrome, occurs when a shark suddenly loses the ability to close its mouth and the upper dental arcade appears to have permanently dropped into a pre-bite posture, below the normal resting position of the arcade. Pathology examinations implicate localized infections of the base of a key ligament involved in the upper jaw stay apparatus. This apparatus varies among elasmobranchs, but in *C. taurus*, the ligamentum levator palatoquadrati is key to maintaining the upper jaw in a retracted position, while at rest. It remains a mystery how such a localized impact would be seen in multiple cases, in one species of shark, over different facilities. Another syndrome of abnormal rapid tooth loss in *C. taurus* has been speculatively attributed to

nutritional challenges, but no real understanding of the mechanism or how to treat the condition is known. Finally, the frequent postmortem finding, in female *C. taurus* with a history of chronic progressive debilitation of degenerate ova/embryos in the reproductive tract, emphasizes the need for us to better understand elasmobranch physiology in general, with a view to applying this knowledge to better husbandry methods.

HUSBANDRY/MANAGEMENT ISSUES

A number of the opportunities available to us for improving the management of elasmobranchs in aquaria relate to basic husbandry issues. In addition to those mentioned under facility design issues (see above), there is significant opportunity to increase our understanding of the nutritional needs of the various species at different times in their life cycle. The issues of abnormally slow growth rates, balanced by those of obesity and lack of condition, represent the opposite poles of our relative ignorance of the nutritional demands to support elasmobranch health. Recent developments in the understanding of the neurocontrol of feeding behaviors (Demski, 2012), advanced basic work establishing markers for dietary composition of wild sharks (Beckmann et al., 2013), and studies of stable isotope dynamics in elasmobranchs (Logan and Lutcavage, 2010; Malpica-Cruz et al., 2012) are helping to form the foundation needed to apply modern metabolomic techniques to nutritional evaluation of elasmobranchs under human care.

The relationship between goiter in elasmobranchs and animal management continues to be elucidated. First posited long ago as a theory with little experimental support (Stoskopf, 1993), work in the past 10 years has firmly linked the presence of nitrogenous waste in closed systems, exacerbated by the use of ozonation for disinfection, to the syndrome (Morris et al., 2011; Morris et al., 2012). A better understanding of the mechanisms of decrease in bioavailability of dietary and environmental iodine, thought to be the underlying cause of the condition, should help explain the variability in species susceptibility to this problem and point to better management methods for controlling the disease.

The detailed understanding of the physiology and biochemistry of elasmobranchs and their adaptations and maladaptations to environmental conditions, being pioneered by scientists from a broad range of disciplines today, is critical to the development of successful management techniques for these species in aquaria. This knowledge will play an important role in solving the "mystery syndromes" experienced under human care, as well as contributing to our understanding of how climate and environmental changes may impact these species in the wild. There is a very strong need for improved non-lethal and minimally invasive diagnostics for elasmobranchs to further these investigations and to support clinical and management efforts across the board. One emerging area that focuses on the use of stable isotopic dynamics is the use of nuclear magnetic resonance (NMR) metabolomics as a diagnostic tool.

METABOLOMICS AS A DIAGNOSTIC AND MANAGEMENT TOOL

Metabolomics is an emerging technology offering major opportunities for advances in clinical and husbandry diagnostics (Dove, 2012; Lankadurai et al., 2013). It has already been applied in elasmobranch health assessment, albeit for a limited number of species and with relatively restricted application (Dowd et al., 2010; Dove et al., 2012). Long applied throughout veterinary medicine, traditional clinical chemistry panels have proven themselves in mammals and are time-tested. However, to be carefully objective, in the realm of elasmobranch health, they have proven to be of relatively minor value. The old analytes that are traditionally examined tend not to be particularly useful in elasmobranchs, nor are they good at detecting subtle deviations from normal baselines, a characteristic critical for effective diagnostic use in preventative programs. By the time traditional serum chemistry parameters are perturbed to the point of revealing abnormalities for elasmobranchs, the animal is often untreatable. We are in need of much more subtle endpoints that allow earlier detection and correction of problems with aquarium sharks and rays. The relative lack of meaningful literature, where routine serum chemistry has been instrumental in the diagnosis or evaluation of treatment in elasmobranchs, in the face of so many frequently occurring mystery syndromes, supports this clinical opinion, which is based on nearly 40 years of practice.

Metabolomics represents a diagnostic tool of great potential; and the development of simpler sampling and sample handling techniques, along with the development of better access to animals, are key to the success of using metabolomics routinely in the assessment of aquarium elasmo-

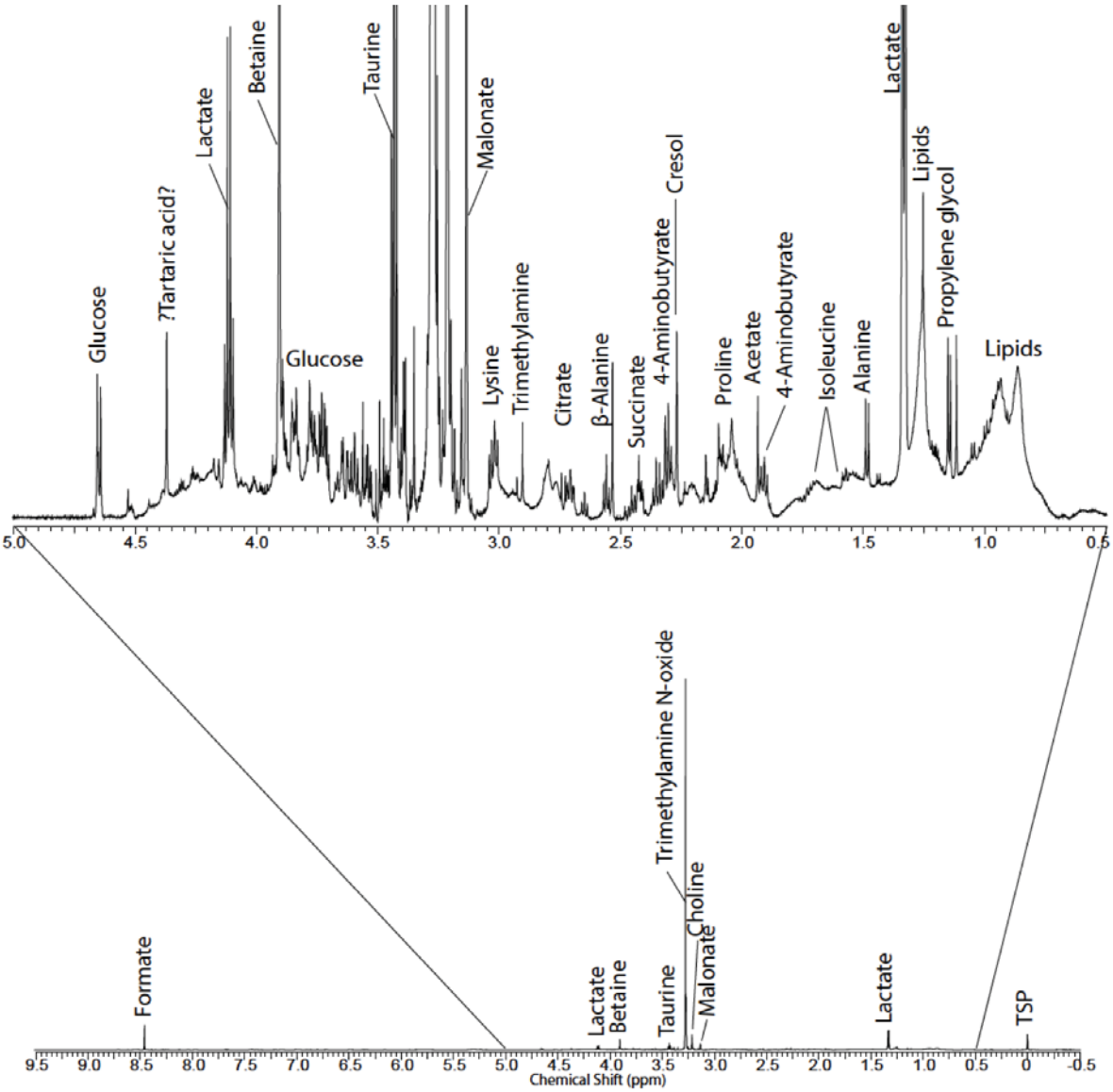

Figure 1. One dimensional proton spectrum of whole blood from an aquarium female bonnethead shark, *Sphyrna tiburo* (Linnaeus, 1758), affected with dystocia. Whole blood frozen on dry ice and held at -80°C was thawed, then centrifuged at 13k RPM for 5 min. Supernatant was mixed 9:1 with D2O containing 1 mM TSP, 10 mM formate, and 100 mM phosphate buffer, to achieve final concentrations of: 0.1 mM TSP, 1 mM formate, and 10 mM buffer. The spectrum was zero filled to 32,000, and 0.5 Hz line broadening was applied. Figure created by Andrey Tikunov, 2013.

branchs. The development of methods that can be accomplished from a whole blood sample using relatively simple rapid quenching techniques in dry ice or liquid nitrogen is making it feasible to gather metabolomic data simultaneously with the more routine evaluation of hematology and serum chemistry data. Though there remain a number of controversies about extraction methods and preparation of samples, as well as the issue of how to handle the vast amount of data that is obtained from a single metabolomic sample, these are being rapidly resolved as the methods are used more routinely. The significant complexities of baseline determination for such sensitive analyses, and developing an understanding of the impacts of very minor manipulations in animal handling or sampling on the data sets, are being worked out. An example of a typical NMR-based metabolomic spectrum of a shark is shown in Figure 1. The areas under the peaks labeled in the figure are quantitative, and can be related to standards added to the sample in known amounts, such as tri-sodium phosphate (TSP) and formate, which were used in the example shown.

One of the earliest uses of NMR-based metabolomics on elasmobranchs was to look at health issues in the largest and one of the more

challenging elasmobranch species to maintain in aquaria, the whale shark, *Rhincodon typus* (Smith, 1828) (Dove et al., 2012). Used in combination with advanced mass spectroscopy techniques, the authors were able to distinguish among six individual animals and separate 'healthy' and 'unhealthy' animals—based on routine clinical assessments—by differences in the metabolites in serum. This study was done without careful attention to quenching of metabolic activity and demonstrates the potential usefulness of these methods in clinical situations with elasmobranchs in aquaria. The differences found in homerine and trimethylamine N-oxide (TMAO) serum concentrations between 'healthy' and 'unhealthy' animals open two interesting avenues of pursuit of the basis of health in *R. typus* under human care. Though this work is impressive, it represents only a small fraction of the potential for metabolomic testing to improve our management of elasmobranch health.

Clearly, the identification of specialized diagnostic markers for particular health conditions is a valuable potential result of metabolomic investigations of elasmobranchs. Beyond quantitative assessment of marker concentrations in biofluids, the technique provides the potential to actually localize metabolites and metabolite accumulations and depletions to organs, and even within individual organs, using advanced techniques to generate spectral data from magnetic resonance imaging data sets. The techniques have application across a wide range of situations and conditions, but one very obvious subset of questions—i.e., how to provide optimal nutritional support for elasmobranch's in aquaria—stands out as a major use for advanced metabolomic assessments.

Baseline determinations

Baseline studies of the metabolite profiles of elasmobranchs in different environmental situations can help to identify key markers that may be useful in assessing wellbeing in sharks and rays. These types of studies are optimally conducted when data from a number of aquarium animals, successfully maintained for a reasonably long duration, under reasonably standardized conditions, can be compared with animals in the wild. Studies limited to aquarium animals can also provide insight, particularly when a species is maintained in numbers, under different conditions, allowing questions about differences in holding conditions to be explored.

Impacts of capture and transport

Studies focused on evaluating the impacts of capture and transport techniques for a given species of elasmobranch can provide important management understanding. A significant collection from the wild offers a particularly valuable opportunity for investigation of the relative importance of different handling techniques. Similarly, when numbers of animals will be transported, between institutions or into holding for renovations, blood sampling for metabolomic assessment using standardized quenching can provide important insight into the impact of these moves, as well as set baseline data for evaluation of the impact of various other changes over time.

Impacts of diet and evaluation of pathogenesis of recurrent syndromes

Prior and post diet change studies can give important information about the biochemical changes that may be occurring as a result of diet manipulation.

Also of extreme clinical interest is the study of metabolism underlying key clinical syndromes (detailed above as "mystery syndromes") through periodic health assessments of animals throughout their tenure under human care. A better understanding of the pathogenesis of *C. taurus* drop jaw, stomach eversion, *C. taurus* scoliosis and kyphosis, goiter, or other syndromes and diseases where the impact of husbandry, facilities or nutrition are suspected to play a role, would be exceptionally valuable to designing prevention and therapeutic options.

SUMMARY

The health management of elasmobranchs has improved greatly due to the hard work of many people over the past decade, but there is still a long way to go before we can reach the level of understanding we enjoy in mammalian health management. There is an important need to gain further understanding of the impacts of holding facilities on the health of animals. In particular, there is a need to improve access to elasmobranchs and options for isolation, therapeutic delivery and routine sampling, through innovative exhibit and holding facility design. Numerous syndromes occur sufficiently frequently in various captive elasmobranch species to drive the need for further basic and clinical research and the adoption of advanced diagnostic and research technologies. This need includes the emerging field of metabolomics, which offers important op-

portunities to improve the wellbeing of elasmobranchs under human care.

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Chapter 26

Physical examination, blood sampling, and sedation of large elasmobranchs

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Abstract: Physical examinations and clinical procedures are difficult to perform on large elasmobranchs. As a result, these procedures are rarely carried out in the field or in aquaria. The Okinawa Churaumi Aquarium (Okinawa, Japan) has been drawing and analyzing blood, and employing a range of anesthetics to sedate and examine large elasmobranchs, such as the whale shark, *Rhincodon typus* (Smith, 1829), and the manta ray, *Manta alfredi* (Krefft, 1868), for many years. Intramuscular midazolam has been employed successfully as a sedative, and has been combined with intravenous propofol in cases where immobilization is required. Techniques employed, and data obtained, have contributed significantly to the diagnosis and medical management of large elasmobranchs.

INTRODUCTION

Sharks and rays are maintained for display in aquaria worldwide (Clark, 2004). Whale sharks, *Rhincodon typus* (Smith, 1828) and manta rays, *Manta alfredi* (Krefft, 1868) and *Manta birostris* (Walbaum, 1792), attract large numbers of visitors to aquaria.

The oldest record of an *R. typus* maintained in an aquarium dates back to 1934, at the Nakanoshima Aquarium (currently Izu Mito Sea Paradise, Japan), where a single specimen was maintained in a semi-open seawater pool for four months (Clark, 1963; Uchida, 1986). Fifty years later, the first long-term husbandry project involving *R. typus* and *Manta* spp. was introduced at the Okinawa Expo Aquarium (currently Okinawa Churaumi Aquarium, Japan) (Uchida, 1982; Sato et al., 2010; Matsumoto et al., this volume). At present, *R. typus* and *Manta* spp. are currently on exhibit in at least seven aquaria.

R. typus and all *Manta* spp. are listed in Appendix II of CITES. As a consequence, their conservation and sustainable use should be the highest priority, and any techniques for *ex situ* maintenance and breeding in aquaria are warranted. Due to the relative lack of opportunity to develop techniques, practices for capturing, transporting and maintaining large elasmobranchs are still being developed. Clinical procedures for larger elasmobranchs have only been attempted in a few cases (Stamper et al., 2004; Dove et al. 2010; Dove et al., 2012). Improvements to clinical procedures for these larger elasmobranch species are essential for their successful long-term husbandry and reproduction in aquaria, and for the advancement of wildlife medicine. This chapter focuses on methodologies used for physical examination, sedation, and immobilization of large elasmobranchs at the Okinawa Churaumi Aquarium and the resulting data yielded by these efforts.

PHYSICAL EXAMINATION

Blood sampling

In order to advance clinical procedures, standardize protocols and establish baseline blood parameters for large elasmobranchs, we have developed techniques for collecting blood samples underwater.

Hematological values in elasmobranchs, and procedures for blood collection, have been well demonstrated by Walsh and Luer (2004). According to their methods, blood samples are collected from the caudal artery or vein, or through cardiac puncture for some batoids. However, blood sampling from the caudal artery or vein may not always be possible for large elasmobranchs, as the distance between the skin surface and the vessels can be too great.

Ideally, blood samples should be collected from: (1) the caudal vein (if available), (2) the pectoral fin radial vein or artery, (3) the dorsal cutaneous vein or (4) the ventral aorta (cardiac puncture). To reduce stress on the animal, it is better to use distal parts of the body for injection. Therefore, we have not collected blood samples from the ventral aorta or through cardiac puncture, except from dead or dying individuals with low blood pressure. If the caudal vein or artery is accessible by needle, this should be the first choice for sampling. Since the caudal vein is located on the ventral side of the centrum, and is enclosed within the hemal arch, the needle must penetrate the thin cartilage of the arch to reach the vein. The use of spinal needles is recommended as they are less susceptible to blockages with cartilaginous substances. The radial vein of the pectoral fin is easily accessible, but special attention is required to avoid hypertrophy of the vein from frequent punctures. Inserting a needle from the axil of either the first or second dorsal fin allows penetration of the dorsal cutaneous vein. Blood

samples from the dorsal cutaneous vein are often contaminated with other fluid, so caution should be exercised when assessing observed blood values.

Blood sampling from live sharks and rays is usually done under immobilization or sedation (see next section). However, blood for this study was collected by veterinary staff swimming alongside, or temporarily restraining, animals while they were in the water (Figure 1A). Two syringes, connected by a 3-way stopcock and an extension tube (Figure 1B) were used for underwater sampling. Although both syringes were filled with blood samples, only the sample from the second syringe (filled during the middle of the draw by switching the stopcock) was analyzed, as the first syringe was likely to be contaminated with seawater and other body fluids. When plasma was required for analysis, tubes were coated with an anticoagulant. Although different anticoagulants have their respective advantages and disadvantages (Stoskopf, 1993; Violetta, 2004) we preferred lithium heparin, because EDTA lowers the concentration of calcium and other divalent ions as a result of its chelating activity.

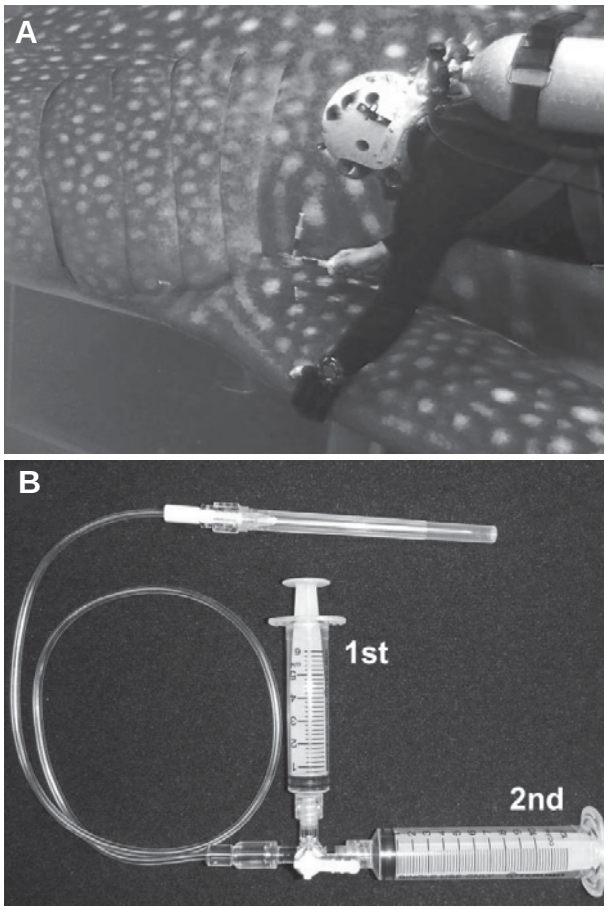

Figure 1. Underwater blood collection from a whale shark, *Rhincodon typus* (Smith, 1829), at the Okinawa Churaumi Aquarium, showing: (A) a veterinary staff member sampling the radial vein of the pectoral fin to fill both syringes with blood; and (B) the blood collecting device, with 2 syringes, extension tube, needle and 3-way stopcock.

Blood analysis and parameters

Blood samples were collected from healthy specimens, both from the aquarium (aquarium-bred, where possible) and wild-caught. No damage to the animals or abnormal behavior post-sampling was observed. Sampling opportunities from large wild elasmobranchs were limited, so we included specimens caught incidentally by local fishers. Collected blood included: 151 samples from 11 *R. typus*, 47 samples from six *M. alfredi*, six samples from five bull sharks, *Carcharhinus leucas* (Müller and Henle, 1839), and 10 samples from eight tawny nurse sharks, *Nebrius ferrugineus* (Lesson, 1831).

Blood analyses determined enzymes, electrolytes, general chemistry, hematology and osmolality (summarized in Table 1). Biochemical and enzyme markers were analyzed using a Fujifilm Dri-chem 7000 V (Fujifilm Co., Tokyo, Japan) and pH was analyzed using a Rapid Lab System 1265 (Siemens Co., Erlangen, Germany). Plasma osmolality was measured using a Vapor Pressure Osmometer Vapro 5520 (Wescor Inc., South Logan, Utah, USA). A comparison of blood parameters from wild and aquarium *R. typus* is provided in Table 2.

SEDATION AND IMMOBILIZATION

When performed correctly, sedation or immobilization of large elasmobranchs allows time to examine, clinically treat and/or safely transport animals. Although immersion anesthesia has been used extensively on many fish species, it has rarely been used on large elasmobranchs because of the large quantity of anesthetic required. Injectable anesthetics have many advantages over immersion anesthetics for large elasmobranchs.

Drug administration

Typically, drug delivery to large elasmobranchs is by intramuscular (IM) injection. The recommended site for IM injection is generally the dorsal saddle (dorsolateral side of body below the first dorsal fin) (Stamper, 2004; Stamper, 2007). Methods of delivery include hand injection

Table 1. Blood parameter mean values ± standard error (S.E.) for whale sharks, *Rhincodon typus* (Smith, 1829), manta rays, *Manta alfredi* (Krefft, 1868), bull sharks, *Carcharhinus leucas* (Müller and Henle, 1839), and tawny nurse sharks, *Nebrius ferrugineus* (Lesson, 1831). Parameter ranges are given in parentheses. Samples were collected from aquarium and wild specimens regarded as being in good conditioned.

	Whale shark *Rhincodon typus* (n = 151)		Alfred manta *Manta alfredi* (n = 47)		Bull shark *Carcharhinus leucas* (n = 6)		Tawny nurse shark *Nebrius ferrugineus* (n = 10)	
	mean ± S.E.	**range**	**mean ± S.E.**	**range**	**mean ± S.E.**	**range**	**mean ± S.E.**	**range**
Enzymes								
ALT (IU/L)	5.75 ± 0.16	(2.00 - 14.00)	5.00 ± 0.10	(4.00 - 6.00)	7.60 ± 2.39	(2.00 - 17.00)	6.56 ± 0.83	(3.00 - 10.00)
ASP (IU/L)	12.88 ± 0.47	(6.00 - 51.00)	10.00 ± 0.55	(7.00 - 29.00)	27.50 ± 10.29	(9.00 - 82.00)	19.67 ± 3.10	(9.00 - 33.00)
LDH (IU/L)	36.30 ± 2.75	(3.00 - 289.00)	99.0 ± 24.11	(13.00 - 842.00)	1,358.00 ± 1,169.16	(48.00 - 7762.00)	118.44 ± 20.71	(43.00 - 225.00)
CPK (IU/L)	122.30 ± 13.39	(6.00 - 865.00)	759.00 ± 222.33	(65.00 - 8,667.00)	391.00 ± 47.73	(266.00 - 573.00)	409.67 ± 144.13	(4.00 - 1499.00)
ALP (IU/L)	46.60 ± 1.47	(17.00 - 142.00)	66.00 ± 5.61	(32.00 - 191.00)	20.00 ± 2.35	(13.00 - 30.00)	273.11 ± 48.49	(68.00 - 525.00)
General chemistry								
NH_3 (µg/dL)	99.47 ± 3.66	(21.00 - 302.00)	149.38 ± 14.94	(19.00 - 425.00)	216.00 ± 75.02	(32.00 - 589.00)	199.33 ± 29.69	(49.00 - 334.00)
Na (mEq/L)	291.26 ± 0.96	(255.00 - 336.00)	266.00 ± 2.53	(228.00 - 336.00)	308.00 ± 6.54	(282.00 - 330.00)	280.22 ± 5.54	(252.00 - 306.00)
K (mEq/L)	3.96 ± 0.04	(3.00 - 5.00)	3.30 ± 0.06	(2.40 - 4.20)	4.50 ± 0.50	(3.00 - 6.90)	3.67 ± 0.20	(3.00 - 4.80)
Cl (mEq/L)	242.83 ± 1.22	(124.00 - 282.00)	219.00 ± 2.44	(184.00 - 285.00)	255.00 ± 6.36	(231.00 - 279.00)	233.44 ± 6.42	(198.00 - 258.00)
Mg (mg/dL)	4.20 ± 0.14	(1.90 - 12.50)	3.56 ± 0.12	(2.50 - 6.00)	4.66 ± 0.21	(3.80 - 5.10)	4.55 ± 0.21	(4.00 - 5.00)
Ca (mg/dL)	15.30 ± 0.15	(10.90 - 19.30)	15.79 ± 0.20	(9.10 - 18.00)	20.37 ± 0.80	(17.90 - 22.70)	17.57 ± 0.39	(16.00 - 19.20)
Phosphorus (mg/dL)	2.94 ± 0.09	(1.10 - 14.80)	3.64 ± 0.12	(1.40 - 5.80)	6.45 ± 1.10	(3.70 - 11.70)	4.51 ± 0.36	(3.00 - 6.30)
GLU (mg/dL)	51.40 ± 0.57	(35.00 - 75.00)	62.26 ± 2.61	(44.00 - 171.00)	77.33 ± 10.94	(49.00 - 129.00)	31.78 ± 3.20	(17.00 - 49.0)
ALB (g/dL)	1.47 ± 0.01	(1.00 - 1.90)	1.58 ± 0.03	(1.00 - 2.00)	1.80 ± 0.14	(1.10 - 2.10)	1.74 ± 0.06	(1.40 - 2.10)
Cholesterol (mg/dL)	67.38 ± 2.11	(9.00 - 206.00)	67.79 ± 20.42	(3.00 - 717.00)	65.83 ± 7.18	(38.00 - 85.00)	61.11 ± 12.15	(11.00 - 145.00)
Triglyceride (mg/dL)	29.17 ± 1.21	(3.00 - 105.00)	65.65 ± 10.89	(17.00 - 352.00)	99.50 ± 12.70	(54.00 - 157.00)	47.89 ± 12.13	(3.00 - 131.00)
TP (g/dL)	2.29 ± 0.04	(1.20 - 3.90)	3.33 ± 0.09	(2.00 - 4.50)	3.65 ± 0.17	(3.00 - 4.20)	3.42 ± 0.27	(2.40 - 4.80)
CRE (mg/dL)	0.12 ± 0.02	(0.10 - 3.00)	0.11 ± 0.01	(0.10 - 0.30)	0.30 ± 0.18	(0.10 - 1.30)	0.14 ± 0.02	(0.10 - 0.20)
UA (mg/dL)	0.13 ± 0.00	(0.10 - 0.30)	0.37 ± 0.02	(0.20 - 0.60)	0.35 ± 0.06	(0.20 - 0.60)	0.11 ± 0.01	(0.10 - 0.20)
TBIL (mg/dL)	0.32 ± 0.01	(0.10 - 1.00)	0.27 ± 0.01	(0.10 - 0.50)	0.37 ± 0.03	(0.30 - 0.50)	0.24 ± 0.02	(0.20 - 0.30)
BUN (mg/dL)	870.47 ± 2.55	(778.1 - 932.40)	1,052.40 ± 5.69	(957.40 - 1,132.30)	995.72 ± 12.89	(945.80 - 1,041.80)	1,005.38 ± 10.53	(957.60 - 1,043.20)
Hematology								
Hb (g/dL)	7.11 ± 0.11	(3.60 - 9.80)	9.28 ± 0.25	(6.10 - 13.10)	8.35 ± 0.69	(5.10 - 10.00)	6.24 ± 0.13	(5.90 - 6.90)
Hematocrit (%)	23.31 ± 0.30	(13.50 - 30.00)	29.10 ± 0.70	(21.00 - 40.00)	25.70 ± 2.00	(17.00 - 31.00)	20.86 ± 0.75	(18.00 - 24.50)
Erythrocytes per µL ($\times 10^4$)	28.25 ± 0.44	(14.30 - 43.80)	37.80 ± 1.01	(19.40 - 53.90)	55.00 ± 2.90	(42.00 - 64.00)	40.91 ± 2.55	(32.00 - 54.30)
Leukocytes per µL ($\times 10^3$)	4.27 ± 0.13	(1.78 - 8.80)	2.48 ± 0.20	(1.06 - 6.59)	9.93 ± 2.14	(5.96 - 20.93)	4.03 ± 0.69	(2.16 - 8.86)
Plasma								
pH	7.622 ± 0.008	(7.061 - 7.747)	7.663 ± 0.014	(7.367 - 7.841)	7.280 ± 0.094	(6.932 - 7.534)	7.678 ± 0.056	(7.458 - 7.802)
Osmolality (mmol/kg)	1,003.00 ± 4.10	(865.00 - 1,089.00)	990.40 ± 7.26	(888.00 - 1,084.00)	1,059.80 ± 12.64	(1,029.00 - 1,120.00)	1,021.00 ± 48.08	(953.00 - 1,089.00)

Table 2. A comparison of mean (± S.E.) biochemical and hematological parameters from aquarium and wild whale sharks, *Rhincodon typus* (Smith, 1829). * indicates significant difference between wild and aquarium specimens.

	Captive whale shark *Rhincodon typus* (n = 151)	Wild-caught whale shark *Rhincodon typus* (n = 12)	Significant difference (t-test)
Parameter	**mean ± S.E.**	**mean ± S.E.**	
Hb (g/dL)	7.11 ± 0.11	6.98 ± 0.13	p > 0.05
Hematocrit (%)	23.31 ± 0.30	23.04 ± 0.46	p > 0.05
ALT (IU/L)	5.75 ± 0.16	6.42 ± 0.96	p > 0.05
AST (IU/L)	12.88 ± 0.47	11.58 ± 0.80	p > 0.05
LDH (IU/L)	36.30 ± 2.75	42.17 ± 4.75	p > 0.05
GLU (mg/dL)	51.40 ± 0.57	57.50 ± 7.42	p > 0.05
Cholesterol (mg/dL)	67.38 ± 2.11	31.50 ± 3.50	p < 0.01 *
Triglyceride (mg/dL)	29.17 ± 1.21	16.40 ± 5.98	p < 0.01 *
BUN (mg/dL)	870.47 ± 2.55	831.32 ± 12.28	p < 0.01 *

for *R. typus* and *Manta* spp., or a jab stick syringe (similar to a pole syringe) for large carcharhinids and large skates with dangerous spines (Figure 2).

Sedatives and anesthetics can be used either individually, or in combination. Midazolam has been used successfully with *R. typus* and *Manta* spp. to facilitate transportation of individuals. Midazolam is also widely used in humans to induce sedation and anesthesia before medical procedures. The advantages of midazolam are: (1) it can be administered to elasmobranchs via both IM and intravenous (IV) injection, and is quickly absorbed when administered intramuscularly, (2) sedated animals have a rapid recovery time and (3) it has a relatively reduced effect on cardiac function compared to other drugs.

Midazolam dosage for elasmobranchs is generally calculated as follows: estimated body weight (kg) × 4.5 mg/kg × 0.9 (the "0.9" multiplier is an optional safety factor to minimize risk of overdose). However, our experiences have demonstrated that required dosage rates can vary between elasmobranch species (e.g., 0.5 - 2.5 mg/kg is more appropriate for *Manta* spp.), but is generally much higher than that recommended for mammals by Walsh and Bossart (1999). For large elasmobranchs, we have typically used a concentrated midazolam solution (i.e., 3 or 4 times standard solution), in order to reduce the volume

Figure 2. Intramuscular drug administration to a sicklefin lemon shark, *Negaprion acutidens* (Rüppell, 1837), using a jab stick syringe (similar to a pole syringe). Sedatives (e.g., midazolam) are typically administered via this method to larger species considered to be potentially dangerous.

required for IM injections. Large elasmobranchs typically succumbed to midazolam within 10 - 20 min of an IM injection, but responses to the drug varied, even within species. We have found that midazolam is safe and effective for the sedation of large elasmobranchs, and it has enabled us to catch, restrain or transport animals safely for periods of up to several hours (Figure 3A).

Anesthetic combination

In cases where longer immobilization is required for the clinical treatment of a large elasmobranch, we have used a combination of drugs with propofol as the anesthetic. Propofol, which is administered IV, is a strong drug that depresses respiratory and cardiac function, so careful monitoring of the anesthetized individual is essential.

We used propofol and midazolam, in combination, to facilitate the removal of parasites from the oral cavity of a *R. typus*, where the animal needed to be anesthetized and restrained ventrum-side up (Figure 3B). Midazolam (4.5 mg/kg, IM) was administered for initial sedation and then propofol (1.5 mg/kg, IV) was given for the deeper anesthesia. Within 10 min of administration of the anesthetic, the shark was immobilized and could be treated while in an inverted posture. Immobilization was maintained for over 2 h, and the shark had completely recovered 4 h after propofol administration.

Monitoring the cardiac cycle and respiration

When an anesthetized shark is immobilized and stable, the cardiac cycle should be monitored constantly using ultrasonography. Immobilized individuals often stop buccal pumping, thereby requiring artificial water flow through the mouth to the gill filaments to supply adequate concentrations of dissolved oxygen (DO). When executing a clinical procedure on a sedated large elasmobranch, it is possible to monitor respiratory effectiveness using blood gas analysis, which measures DO, CO_2, pH and lactic acid concentrations. However, it is also possible to monitor respiration in real time using two oxygen meters to measure the difference in DO concentration from in front of the mouth and in the 5th gill opening.

Comparison of DO at the mouth and in the 5th gill opening for a brownbanded bambooshark, *Chiloscyllium punctatum* (Müller & Henle, 1838) sedated with midazolam (Figure 4A), and a *R. typus* anesthetized with propofol (Figure 4B), both show a marked decrease in oxygen once the water had passed the gill lamellae. The difference between the values reflected oxygen consumption by the sharks while sedated. A useful diagnostic tool, during a clinical procedure, is the extent to which DO concentrations at the 5th gill opening increase, which can indicate a reduction in gas exchange and therefore reduced blood circulation (Figure 4A).

DISCUSSION

By using objective physical indices and advanced clinical techniques, the survival rates of *R. typus* and *Manta* spp. in aquaria have improved greatly over the last decade. Uchida et al. (2000) reported that the longest survival time of a *R. typus* at the Okinawa Expo Aquarium, between 1980 and 1998, was 2,056 days. However, a specimen currently maintained at the Aquarium has survived for more than 20 years.

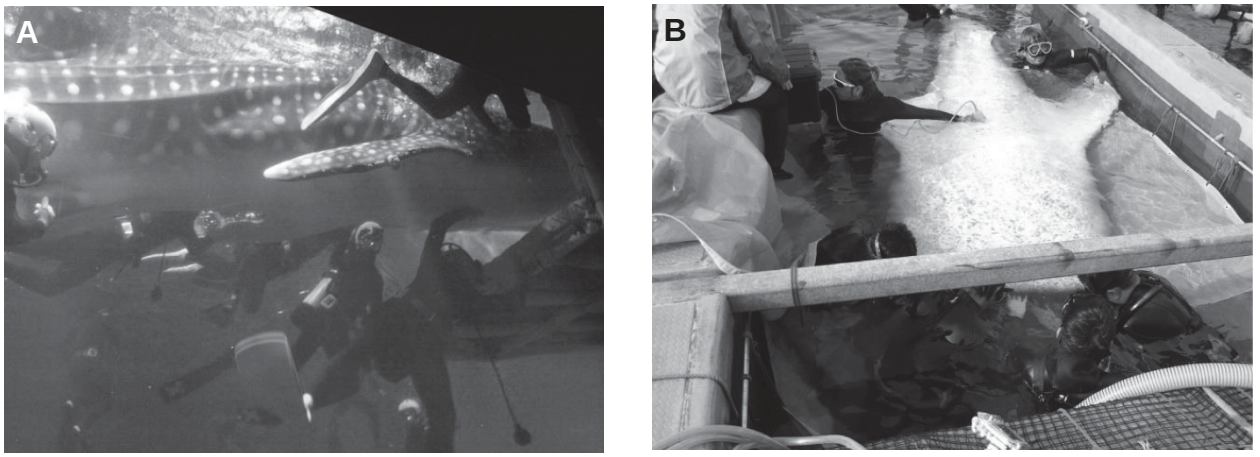

Figure 3. A sedated whale shark, *Rhincodon typus* (Smith, 1829), being guided into a restraint container (A). Once IV anesthesia (i.e., propofol) takes effect, the *R. typus* can be immobilized and turned over for medical procedures while its pulse is monitored using ultrasonography (B).

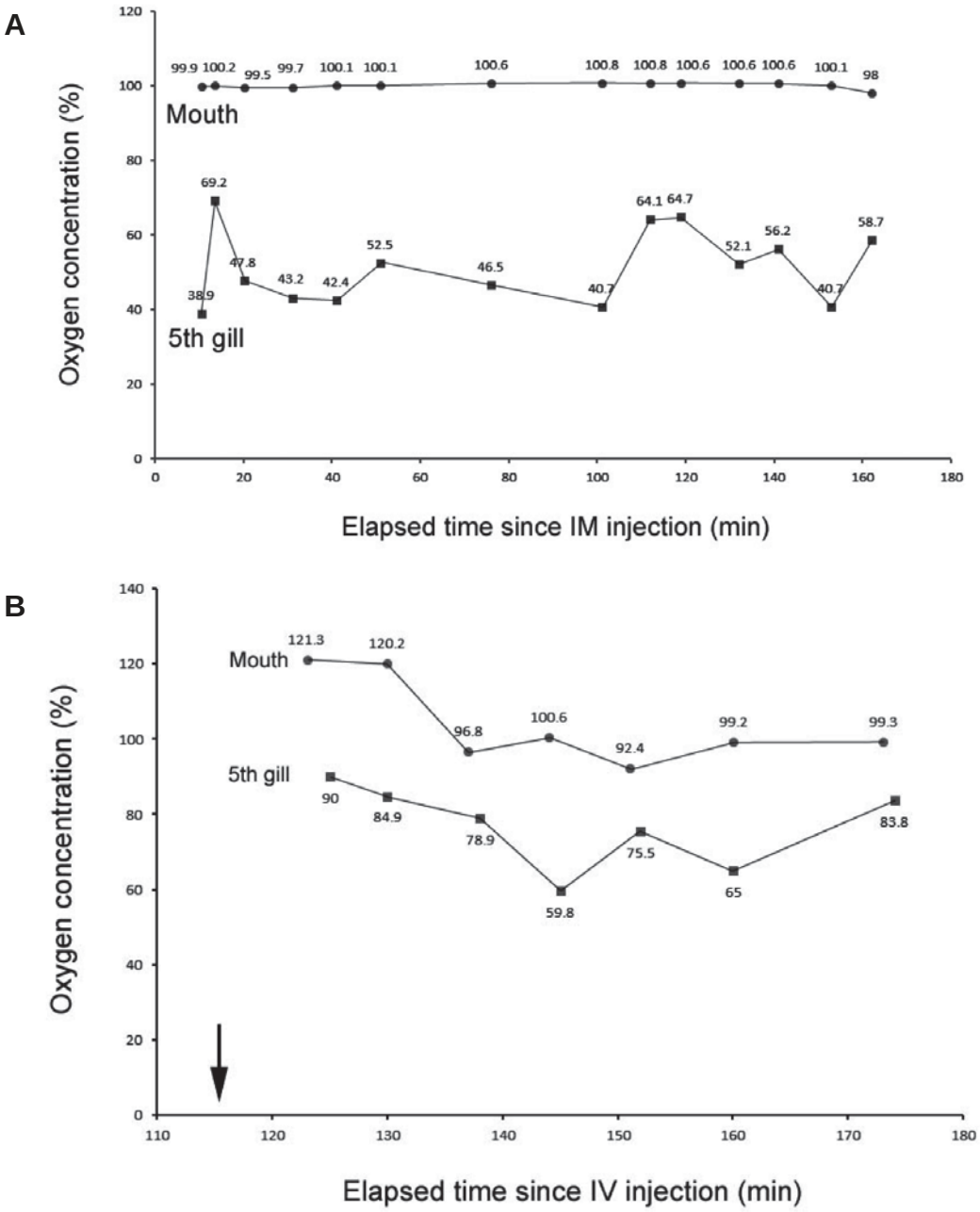

Figure 4. Comparison of dissolved oxygen concentration at the mouth and in the 5th gill opening of a brownbanded bambooshark, *Chiloscyllium punctatum* (Müller & Henle, 1838) sedated with midazolam (A), and a *R. typus* anesthetized with propofol (B). The arrow indicates when buccal pumping stopped. The oxygen concentrations at mouth (solid circle) and 5th gill (solid square) are expressed as a percentage of dissolved oxygen. These graphs show that both sharks respired throughout immobilization.

Based on our findings, concentrations of lipids (i.e., cholesterol and triglycerides) were slightly elevated in aquarium *R. typus* compared to wild conspecifics. This finding may indicate that elasmobranchs under human care were slightly overfed, or that the wild-caught specimens were suffering from nutritional deficiency. Additional data from wild specimens, in good condition, is required before further conclusions can be drawn. Overall, a better understanding of *R. typus* nutritional requirements may allow for the formulation of a more appropriate aquarium diet.

To improve the clinical treatment of large elasmobranchs in aquaria, sedation and immobilization techniques are essential. Tonic immobilization is widely used for small sharks and rays. However, anesthetic administration is the

only safe course for large or potentially dangerous elasmobranchs. Sample sizes represented in this article were too small to obtain robust dosage rates for midazolam and propofol, but starting points for future study are provided. Additional studies using anesthetics to sedate and immobilize large elasmobranchs are required to advance this area of aquarium science.

If regular physical examinations and timely medical treatments are provided to large elasmobranchs, their survival rate in aquaria is likely to dramatically improve. Techniques described in this article may be developed further and employed in the field to advance *in situ* conservation of large elasmobranch species.

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Chapter 27

Emerging diseases of elasmobranchs in aquaria

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Abstract: Parasitic diseases of aquarium-held elasmobranchs were well covered in the Elasmobranch Husbandry Manual by Benz and Bullard (2004), while bacterial and fungal diseases were covered by Terrell (2004) and protistan diseases by Goertz (2004). Five emergent diseases not covered in detail in the Elasmobranch Husbandry Manual include the coccidian *Eimeria southwelli* in myliobatid rays, the capsalid monogenean *Benedeniella posterocolpa* on myliobatid rays, the leech *Branchellion torpedinis* on demersal elasmobranchs, fungal infections in sharks, and the nematode *Huffmanela* in the skin of bonnethead sharks, *Sphyrna tiburo* (Linnaeus, 1758), and sandbar sharks, *Carcharhinus plumbeus* (Nardo, 1827). It is likely that clinically-significant diseases will continue to emerge as aquaria push the boundaries of elasmobranch husbandry with new and challenging species.

INTRODUCTION

Infectious pathogens have significant capacity to cause disease, and sometimes death, in elasmobranchs (Poynton et al., 1997; Benz and Bullard, 2004; Goertz, 2004; Terrell, 2004; Mylniczenko et al., 2007; Kik et al., 2011; Marancik et al., 2011). The largely unquantified, but undoubtedly huge, diversity of pathogens, combined with a host diversity consisting of dozens of routinely-maintained sharks, skates and rays, leads to a potentially staggering number of potential host-pathogen combinations. Many of these infections or infestations may not occur in nature, but are a function of housing animals together at higher-than-natural density, and in some cases, housing species together that would otherwise not occupy the same habitat. Thankfully, mechanisms that reinforce some degree of host-specificity (Miller et al., 2011), be they derived from host or parasite, serve to winnow down the combinations such that, in practice, there are a suite of 'usual candidates' that cause the majority of husbandry challenges (Benz et al., 2001). Nonetheless, novel problems continue to emerge, as aquariums expand the boundaries of elasmobranch care and display, and this chapter seeks to characterize some of these emerging infectious diseases and how they may be managed.

Benz and Bullard (2004), Goertz (2004) and Terrell (2004) did an admirable job providing systematic coverage of the state of knowledge regarding pathogens common in aquarium elasmobranchs. Benz and Bullard, in particular, provided extraordinary depth in their summary of metazoan parasites and commensals of sharks and rays. The other two chapters were necessarily shorter, because there was simply less literature to review at that time regarding protists, bacteria, fungi and viruses in elasmobranchs, certainly compared to the expansive literature on metazoan parasites of sharks and rays. As Terrell (2004) put it: "the study of infectious diseases in this group is in its infancy."

We aim to characterize several problematic pathogens that have emerged since, or were not otherwise covered in, the Elasmobranch Husbandry Manual, and also to provide figures on how to recognize, and advice on how to manage, these problems in aquarium elasmobranchs. The selection of these pathogens was necessarily biased by our experiences in assembling and maintaining the collection at the Georgia Aquarium, which opened in 2005, and has since maintained one of the most diverse elasmobranch collections of any aquarium. Serendipitously, the species of pathogen we discuss cover a wide range of taxa: a protist, a monogenean, a leech, a nematode and several fungi. In each case, we have organized the text as a miniature literature review to provide context of prior knowledge, a section on presentation, including gross appearance and histopathology (if relevant) with figures, some notes on pertinent biology, and a section on management and treatment strategies that have been attempted, or proven successful. We strongly recommend that this chapter be read as a complimentary accompaniment to the three disease-related chapters in the Elasmobranch Husbandry Manual, which remain an excellent systematic treatment of the disease problems of aquarium-held elasmobranchs.

EMERGING DISEASES

Eimeria southwelli (Halawani, 1930)

Literature review

There are few peer-reviewed references to the coccidian parasite *Eimeria southwelli* (Halawani, 1930). The original description was from spotted eagle rays, *Aetobatus narinari* (Euphrasen, 1790), and so was a subsequent synonymous re-description (Boulard, 1977). A new host record was later published from cownose rays, *Rhinoptera bonasus* (Mitchill, 1815) at the New York Aquarium (Cheung, 1993). The first paper documenting the pathogenicity of this organism in aquarium animals was for *R. bonasus* (Stamper et al., 1998). Goertz (2004) also mentioned several of these papers in her brief review of protistan parasites of elasmobranchs, as did Garner (2013) in his retrospective study of pathologies in elasmobranchs.

Presentation

Unlike most eimeriid coccidians infecting other animals, *E. southwelli* occupies a parenteral site: the peritoneum lining the coelomic surfaces of organs, such as the liver, spleen and spiral valve intestine, as well as the inner surface of the abdominal body wall (Cheung, 1993). There may be few signs of infection, other than the presence of distinctive oocysts in coelomic flushes. Alternatively, the serosa of organs may appear rough or ruptured (Cheung, 1993) and there may be a serous or sero-sanguinous coelomic effusion or ascites (Stamper et al., 1998): a slightly cloudy or bloody liquid in the body cavity that, when

examined directly under microscope, is replete with oocysts. Infection can be lethal in *R. bonasus* (Cheung, 1993; Stamper et al., 1998). While all published literature refers to either *R. bonasus* or *A. narinari*, all myliobatid rays should be considered susceptible to infection with *E. southwelli*.

The oocysts of *E. southwelli* are extremely distinctive (Figures 1 and 2). They possess the typical 1+4+2 oocyst formula common to all the Eimeriidae, that is, 1 oocyst contains 4 sporocysts, each of which contains 2 sporozoites. They differ radically from other eimeriids, however, in that the oocyst is not remotely spherical. Indeed, it is elongate or semi-cylindrical, such that the sporocysts align in a fixed tandem or serial configuration. Stamper et al. (1998) reported that the oocysts are polymorphic, and this may be true to a degree but probably also reflects the presence of a range of developmental stages in the coelomic effusion.

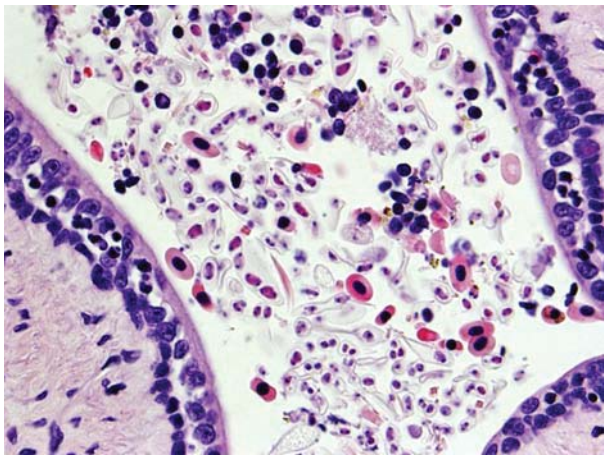

Figure 1. Oocysts of *Eimeria southwelli* in the coelom of a spotted eagle ray, *Aetobatus narinari* (Euphrasen, 1790), along with erythrocytes, lymphocytes and cellular debris. 400x total magnification.

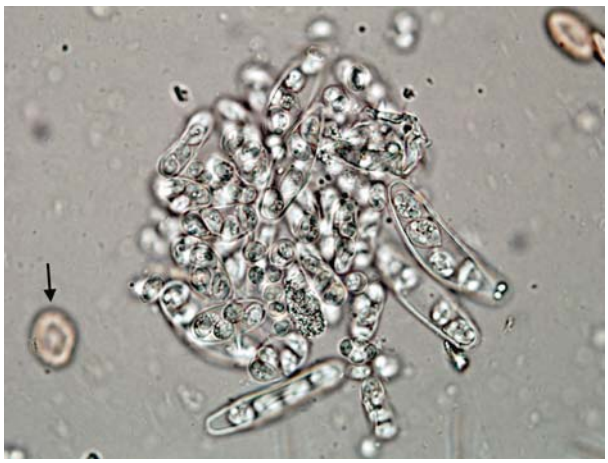

Figure 2. Wet preparation of oocysts of *Eimeria southwelli*. Note the variation in size and shape of the oocysts with developmental stage of sporulation, increasing elongation as the oocyst matures. Note also the red blood cell (arrowed) for scale. Photo taken at 400x total magnification.

Mature oocysts are approximately 60 µm long and 15 µm wide; they are therefore barely distinguishable using a 10x objective lens (i.e., 100x total magnification), and are much better appreciated using a 40x objective (i.e., 400x magnification). They are 2-3 times the size of the host's red blood cells, which are a handy gauge when determining approximate pathogen size. Like most protists, the oocysts of *E. southwelli* are most easily detected using a good quality phase contrast or Nomarski DIC (differential interference contrast) microscope. In the absence of these diagnostic tools, a regular bright field microscope can suffice, as long as the light path is properly aligned and the field and condenser irises are partially closed to introduce a degree of artificial contrast. A handy technique to introduce additional contrast, is to wave the edge of one finger in and out of the light path between the light source and stage condenser while observing down the eyepiece. Refringent objects, like coccidian oocysts, should visually 'pop' as the light path is interrupted. In histological sections, coccidians are always shown in good contrast by using acid-fast stains typically applied to discriminate mycobacteria, under which the *Eimeria* will be acid fast positive (red) against a blue or green counterstain background.

Published literature regarding this parasite focuses on its presence in the serosa of visceral organs, but we have also seen it in the testes and within the tubules of the kidney.

Biology notes

Stamper et al. (1998) noted that oocysts were often abundant in the coelomic effusion, but totally absent in feces. It seems that this coccidian does not use the gastrointestinal system of the host in the life cycle, which removes one convenient way of diagnosing infection (fecal cytology). It should be possible to diagnose the infection by flushing sterile saline in through one of the coelomic pores that rays possess, and then aspirating the mixture back and using it as the basis for cytological examination.

The life cycle of *E. southwelli* is unknown but presumed to be direct, as it is with most eimeriid coccidians. We can speculate that oocysts pass through coelomic pores to the exterior, and

perhaps this explains their elongate shape, but how the ray acquires the infection remains a mystery. Stamper et al. (1998) noted that oocysts maintained structural integrity in seawater at 21°C for two years.

Treatment and Management

Frequently, despite parasite 'absence' in coelomic flushes (or fecal flushes), suspicion of an *Eimeria* infection arises based on the host's poor body condition, pale or blotchy skin coloration, lethargy and general failure to thrive, despite reportedly eating quite well. In these cases, even if no oocysts are detected, we typically treat with an anti-coccidial drug, such as sulfadimethoxine (Albon, Pfizer Animal Health), sulfadimethoxine & ormetoprim (Primor, Pfizer Animal Health), toltrazuril (Baycox, Bayer Pharmaceutical) or ponazuril (Marquis, Merial Inc.). Treatment with toltrazuril has been subjectively more effective than sulfadimethoxine alone, or in combination with ormetroprim. Much like management and treatment of coccidiosis in livestock, the goal is not necessarily to rid the animal(s) or the environment of the parasite, but more to manage the infection and allow the animals to develop immunity naturally. One of the pitfalls of treating with any of the anti-coccidial drugs listed above is that they all require oral administration. Many batoids, and especially *R. bonasus* or *A. narinari*, tend to thoroughly grind or masticate whatever they eat, thus making it challenging to hide medications in food items. Due to poor success with opportunistic food delivery, staff at the Georgia Aquarium prefer to deliver medications via oro-gastric tube, which obviously requires catching and restraining the animals multiple days in a row, and typically for two separate rounds of the medication 10 - 14 days apart (refer Mylniczenko and Clauss, this volume, for dosing). Administration of the drug can occur with animals either manually or chemically restrained. The method of restraint often depends on the size and type of elasmobranch in question.

Benedeniella posterocolpa (Hargis, 1955)

Literature review

The Capsalidae is one of the major families of monogenean platyhelminths (Whittington, 2010); they fall in the group parasitologists refer to as Monopisthocotylea or Polyonchoinea. That is, they are characterized by a posterior holdfast organ for attachment, or haptor, which relies mostly on suction, and an array of hooks and other sclerotized parts, rather than clamps (Whittington, 2010). They eat mucus and skin, not blood, and are typically clear to milky white in color, although they can sequester pigments from the skin of their hosts and use them for camouflage, presumably against cleaner symbionts (*Stenopus* spp., *Labroides* spp., etc.). Many aquarists are familiar with the capsalid *Neobenedenia melleni*, which is one of the more problematic metazoan parasites of tropical teleosts in aquaria. Capsalids may also parasitize elasmobranchs (see Schell, 1985), and one host-parasite combination, in particular, can cause problems and is difficult to manage: *Benedeniella posterocolpa* on *R. bonasus* and other myliobatids. To date there are no publications describing *Benedeniella* infections in elasmobranchs under human care.

Presentation

Capsalids are relatively large monogeneans, and *Benedeniella* more so than most. Even though *B. posterocolpa* adults are large, at up to 1.0 cm in diameter, their thin translucent body can make it very hard to spot them on the skin of affected *R. bonasus* (Figure 3). A common trick used to detect *N. melleni* on teleosts can work for this parasite too, which is to hold a flashlight next to your eye and look at the eye of the ray. The worms may reveal themselves in outline or movement against the cornea of the ray. Typically if you can detect infections this way, then the intensity is heavy enough to require treatment. Worms also may show up on diagnostic skin scrapes during quarantine or routine health exams. Detection is greatly enhanced by scraping into a petri dish of salt water, where the worms will actively move.

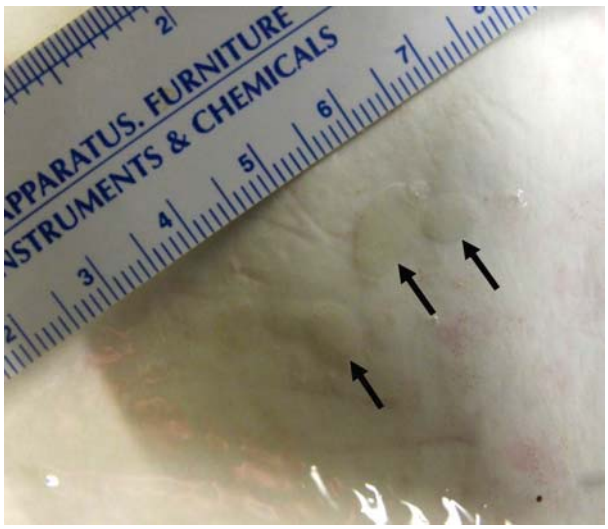

Figure 3. *Benedeniella posterocolpa* on the skin of a cownose ray, *Rhinoptera bonasus* (Mitchill, 1815). Several adult worms are indicated (arrows), showing that despite their large size, they can be quite hard to discern.

The worms will certainly show up if the scraping is made directly onto a microscope slide, and evaluated using a compound microscope. One other common way that infection is recognized is when rays are given a prophylactic freshwater or praziquantel (Biltricide, Bayer) dip, as the worms will quickly turn opaque white and can be seen falling away from the ray.

Benedeniella can be distinguished from other capsalids by the presence of a long vagina that opens on the left margin of the body just into the posterior half (Figure 4). Otherwise *Benedeniella* resemble most capsalids, having an oval shaped body, with a round to oval posterior attachment organ or haptor (Figure 4), with two prominent anchors or hamuli and some associated accessory sclerites, and two anterior attachment organs or lappets that resemble "Mickey Mouse ears". The worms are sufficiently thin and translucent that the internal anatomy will be evident on a dissecting microscope (Figure 5).

Biology notes

Like all monogeneans (Schell, 1985), *B. posterocolpa* has a direct life cycle, i.e., with no intermediate host. Adult worms are hermaphrodites, but are not capable of self-fertilizing; they require a mate for reciprocal copulation. Microscopic quinone-tanned eggs are laid one at a time and immediately shed from the host into the water column (there is no uterus for egg storage as in digeneans), where they are negatively buoyant and eventually sink to form an egg bank in the substrate. From the egg hatches a ciliated larva called an oncho-miracidium, which actively seeks a host in response to light and chemical cues. The length of the life cycle is not known, but based on similar species can be estimated at 3 - 5 weeks (egg to egg) at 24°C.

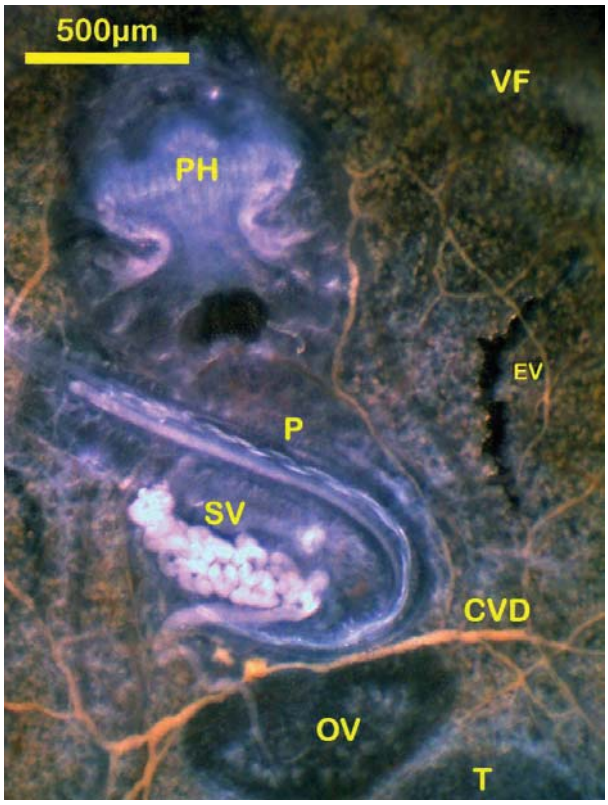

Figure 5. Internal anatomy of *Benedeniella posterocolpa*. CVD, common vitelline duct; EV, excretory vesicle; OV, ovary; P, penis; PH, pharynx; SV, seminal vesicle; T, testis; VF, vitelline (egg shell/yolk) follicles.

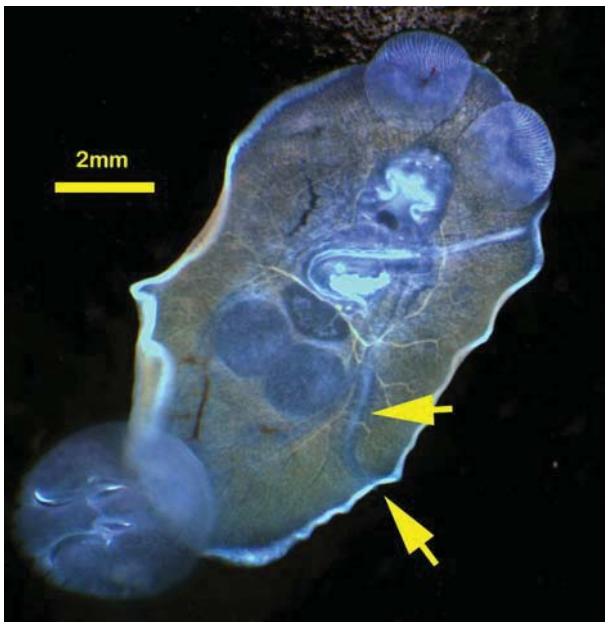

Figure 4. Adult *Benedeniella posterocolpa* from the skin of a giant manta, *Manta birostris* (Walbaum, 1792), showing the two anterior accessory suckers or lappets ("Mickey Mouse ears"), the large posterior haptor, or holdfast, with hamuli or hooks, and the diagnostic long vagina opening on the left body margin in the posterior half of the body (arrows).

Treatment and Management

Thoney (1990) described treatment protocols for *B. posterocolpa* infection in *R. bonasus*. Praziquantel at 20 mg/L effectively cleared 100% of adult worms, whereas trichlorfon (Dylox, Bayer) only cleared 81% of adult worms. Copper sulfate was less effective. Practitioners may also wish to consult Janse and Borgsteede (2003) for a praziquantel protocol for monogenean infections in *A. narinari*.

Benedeniella on *R. bonasus* has been the most commonly encountered capsalid at the Georgia Aquarium. Despite prolonged quarantine periods,

with multiple praziquantel treatments and repeated negative exams, of all fishes, prior to leaving quarantine, the parasite was introduced to our primary exhibit. Typically, heavier infestations occurred on *R. bonasus* that were compromised by other parasites, illness or trauma. *Benedeniella* has been found on two other batoid species (both mobulids) in an exhibit with a known and very active *Benedeniella* infestation of *R. bonasus*. Eradication of this parasite from a primary multi-species exhibit poses challenges, due to the presence of substrate and sand filters, limitations on the duration of praziquantel treatments and constraints imposed by praziquantel concentration sensitivities among the various animals in the exhibit.

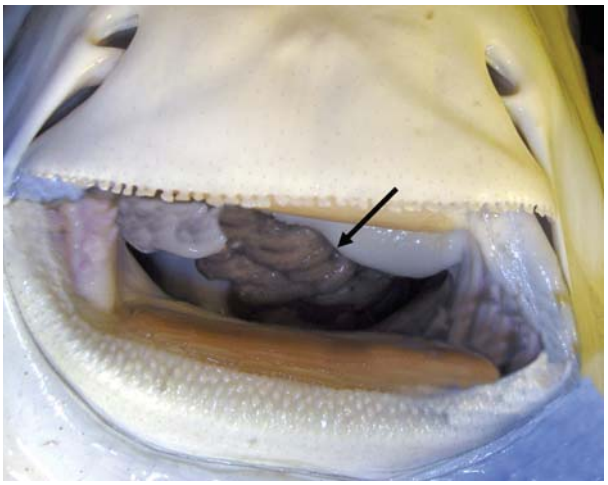

Figure 6. A clump of *Branchellion torpedinis* (arrow) in the orobranchial chamber of a cownose ray, *Rhinoptera bonasus* (Mitchill, 1815).

Branchellion torpedinis Savigny, 1822

Literature review

Sawyer et al. (1975) provided a synopsis of marine leeches in the United States (US) and Gulf of Mexico and discriminated *Branchellion torpedinis* from *B. ravenelii*, with the former occurring in northeastern US states and the latter occurring south of North Carolina into the Gulf of Mexico. *B. torpedinis* also occurs off the western coast of Africa (Sawyer et al., 1975) and Pauls and Provenzano (1999) recorded it from wild-caught *A. narinari* in Venezuela. Specimens from the Georgia Aquarium were identified as *B. torpedinis* (Burreson, personal communication) and, although their geographic origins are unknown, they are suspected to have arrived with wild-caught *R. bonasus*. Host-specificity of *Branchellion* is typically low; between the two species mentioned above infections in natural settings are known previously from the genera *Dasyatis*, *Raja*, *Squatina*, *Rhina*, *Torpedo*, *Aetobatus*, *Gymnura*, *Narcine*, and the teleost genera *Scopthalmus* and *Labrus.*

Presentation

B. torpedinis is a very large and obvious leech (Figures 6 and 7) that can infect a wide range of demersal elasmobranchs in aquaria, which is consistent with its wide host range in nature. Host specificity in the aquarium is apparently more determined by host behavior (bottom-dwelling) than host phylogeny (i.e., relatedness). *B. torpedinis* is identified by the presence of 33 pairs of frilly gill-like appendages that line the lateral margins of the trunk of the peach, tan or khaki colored body. Mature leeches can be 5 - 8 cm in length (Figure 7), and may be readily visible from

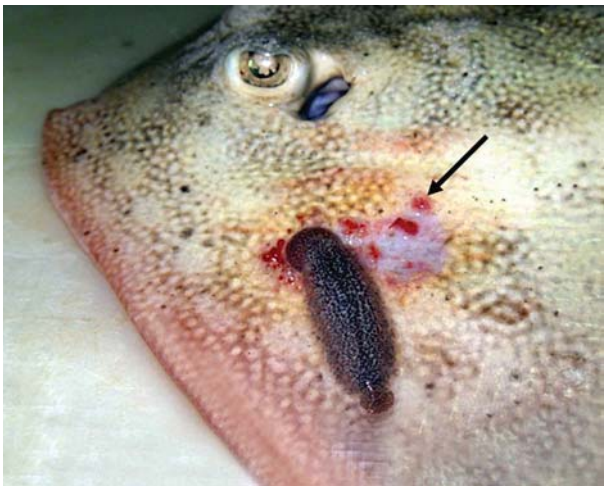

Figure 7. A single, large *Branchellion* attached to the dorsal surface of a yellow ray, *Urobatis jamaicensis* (Cuvier, 1816). Note the ulceration and hemorrhage (arrow) surrounding the attachment site of the posterior sucker.

outside the exhibit. We have observed *Branchellion* infections on the genera *Pristis*, *Stegostoma*, *Orectolobus*, *Eucrossorhinus*, *Manta*, *Rhynchobatus, Urobatis, Dasyatis, Urogymnus, Rhinoptera* and even, occasionally, on whale sharks, *Rhincodon typus* (Smith, 1829).

Leeches may present in obvious external locations, such as on the rostrum of sawfishes (*Pristis* spp.), or they may be quite cryptic. *B. torpedinis* seems to show a special affinity for hiding in the orobranchial chamber of *R. bonasus*. Regardless of the host, *Branchellion* can be gregarious, and may form large clumps of dozens, or even hundreds, of leeches. Grossly, the leech causes a raised and often reddened button-like

lesion up to 1.0 cm wide, where the posterior sucker is attached to the skin, usually surrounded by a halo of smaller red feeding lesions, each around 1.0 mm in diameter. In heavier infections, whole regions can become reddened and hemorrhagic. Histologically, edema, lymphocytic and granulocytic infiltration are common (Figure 8), and it is possible to determine the traces of proboscis tracks through the dermis at sites of feeding.

The primary feature of morbidity from *Branchellion* infection is a profound hemorrhagic anemia. It is not unusual to see heavily-infected animals with packed cell volumes (hematocrit) in the single digits, even as low as 2%. In experimental infections using yellow stingrays, *Urobatis jamaicensis* (Cuvier, 1816), even a single adult *Branchellion* (Figure 7) was sufficient to cause a substantial drop in packed cell volume in a few days; infected animals also stopped eating immediately (Marancik et al., 2012a). It is sometimes possible to diagnose *R. bonasus* with heavy oro-branchial infections, even from outside the exhibit, by their pronounced pallor. In heavy infections, where extensive areas of skin have been compromised, it seems likely that osmotic imbalance also plays a role in pathogenesis.

Biology notes

This group of leeches differs from the more familiar terrestrial leeches in possessing well developed anterior suckers, but lacking jaws of any kind. Instead, they have muscular, glandular proboscises that are used to feed on hosts that lack a keratinized epidermis. The salivary glands of *Branchellion* secrete a litany of proteolytic, and other chemical agents, that interact with the host immune system (Marancik et al., 2012a; Marancik et al., 2012b).

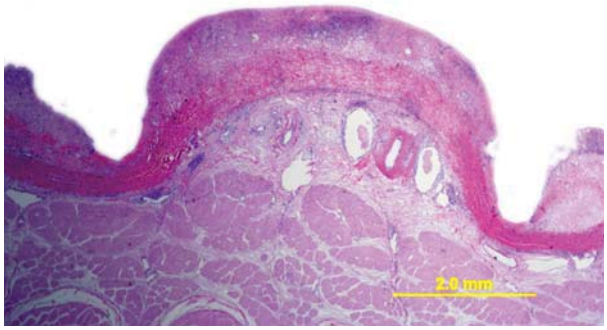

Figure 8. Low power photomicrograph of a button-like raised lesion from the posterior sucker of a *Branchellion torpedinis* on a yellow ray, *Urobatis jamaicensis* (Cuvier, 1816). Pressure necrosis, interstitial hemorrhage, edema, secondary bacterial invasion and lymphocytic infiltration are all evident at higher magnifications.

The life cycle of *Branchellion* has not been fully-characterized, but it differs from known leech life cycles in one important aspect. Most leeches deposit fewer than 10 cocoons, each containing one to a few embryos, and they adhere them to a hard surface using glues secreted from the clitellum region. By contrast, *Branchellion* releases its relatively small cocoons freely into the water, and each contains only a single embryo. In this sense, the reproductive mode of *Branchellion* is more akin to that of monogenean flatworms than it is to other leeches, and this has important management implications. The cocoons are discus-shaped, golden to brown in color, and sticky, and they soon become encased in sand grains and debris once they come in contact with the substrate. The average hatching time has not been determined, but we have observed viable-looking cocoons after several months in seawater at 23°C. An adult leech removed from its host may lay several dozen cocoons over the course of a 24 hr period, but it is difficult to determine if this is reflective of the rate of cocoon production on hosts, or a form of 'panic laying' that is triggered by separation from the host.

Treatment and Management

Managing leech infections is problematic, and this is perhaps more so for *Branchellion* than for any other species because of its monogenean-like reproductive mode, which confers extremely high intrinsic population growth. This challenge is exacerbated by the relative complexity of leech anatomy and physiology; they present a near-vertebrate level of tissue organization and, indeed, are more closely related to vertebrates than are many targets of chemotherapy like protists or platyhelminths. As such, there are no drugs available that are robust in the sense of being profoundly toxic to the leech without being toxic to the host as well. This lack of effective chemotherapeutic agents doubtless also reflects a lack of research on anti-leech therapies because so few species cause problems in aquaculture. Even if there were effective drugs for adult leeches, the quinone-tanned cocoons are extremely resistant to chemical agents, including peroxide and bleach, so breaking the life cycle remains very difficult. The cocoons are probably susceptible to heat and desiccation, but these strategies are impractical as management tools.

The net result of the lack of drugs for use against *Branchellion* and the resistant nature of the

cocoon stage is that management occurs primarily through aggressive exhibit maintenance and physical removal of leeches whenever they are observed on collection animals. Removal of leeches can sometimes be done by divers, but more often necessitate capture and restraint of the host. Hemostats or forceps are used to grasp leeches near the posterior sucker and then pull them from the skin of the host. Handling of this sort is labor intensive and stressful, but must be balanced against the stress and risk of leaving an escalating infection unchecked. Extermination of the organism from an aquarium exhibit is unlikely because of the long-lived and resistant cocoon stage, so the goal must be management for enzootic stability—i.e., keeping infection rates low and fluctuations to a minimum. There is anecdotal and limited experimental evidence (Marancik et al., 2012b) that elasmobranchs can and do develop acquired partial immunity to *Branchellion*; newly-added animals generally display greater susceptibility and this is reflected in differing antibody titers between naïve and exposed hosts.

In severe cases, rapid manual removal of as many leeches as possible is recommended, followed by a bath treatment of trichlorfon (Dylox, Bayer) at 0.25 - 0.33 mg/L of active ingredient for 1 hour to kill juveniles and adults that may have been missed. The elasmobranch is pretreated with injectable atropine at 0.04 - 0.1 mg/kg prior to bath treatment with the organophosphate. Additional supportive care is warranted in severe anemia cases and may include antibiotic therapy, corticosteroids, vitamin and mineral supplementation (e.g., vitamin B complex, vitamin C, vitamin E, selenium, iron, Yunnan paiyao), fluid therapy, blood transfusion or erythropoietin.

Huffmanela spp.

Literature review

Four species of tissue-dwelling capillariid nematodes in the genus *Huffmanela* are known to infect elasmobranchs: *H.* cf. *carcharhini* (Bullard et al., 2012), *H. lata* (Justine, 2005), *H. markgracei* (Ruiz and Bullard, 2013) and a currently unidentified *Huffmanela* species (MacLean et al., 2006). Nematodes in this group are characterized by their tiny, limp, thread-like bodies and thick-walled elliptical eggs with distinctive polar plugs. There are at least 17 named and six putative species of *Huffmanela* in teleosts and elasmobranchs (Bullard et al., 2012), but only two of the named species have included characteristics of the adults in their taxonomic description, the rest are based on egg measurements alone. Bullard et al. (2012) described infections with (presumptively) *H. carcharhini* in sandbar sharks, *Carcharhinus plumbeus* (Nardo, 1827), based on the measurements of eggs in tissues, but were unable to find adult nematodes in any tissue, despite an exhaustive search. Similarly, *H. lata* was described from eggs only in the gills and skin of a blacktail reef shark, *Carcharhinus amblyrhynchos* (Bleeker, 1856) (Justine, 2005), and *H. markgracei* was described from eggs in the skin of Atlantic sharpnose sharks, *Rhizoprionodon terraenovae* (Richardson, 1836), in the Gulf of Mexico. This practice of describing parasites from their eggs, rather than adults, is unusual and undesirable, but not against the tenets of taxonomy, as long as each new type can be distinguished from others based on consistent features and/or consistently different measurements. In capillariids, the eggs are so distinctive, and the adults so elusive, that it makes some sense to use the eggs as a taxonomic indicator because they are the stage most likely to be encountered by the diagnostician.

Presentation

Huffmanela eggs cause curious, unique and highly distinctive lesions in the skin of sharks, where they form scribble-like patterns that may have the appearance of a filigree tattoo (Figure 9). These lesions are apparently the result of an adult worm meandering through the dermis, leaving behind a serial chain of heavily-tanned eggs that are visible through the epidermis, representing an unmistakable signature. Skin scrapings reveal darkly pigmented brown elliptical eggs and polar plugs

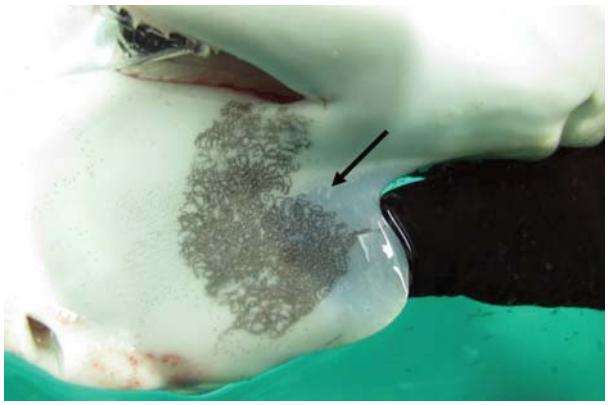

Figure 9. *Huffmanela* infection in the ventral cephalofoil of a bonnethead shark, *Sphyrna tiburo* (Linnaeus, 1758). Note the distinctive tattoo-like lesion (arrow) consisting of darkly pigmented eggs in the dermis.

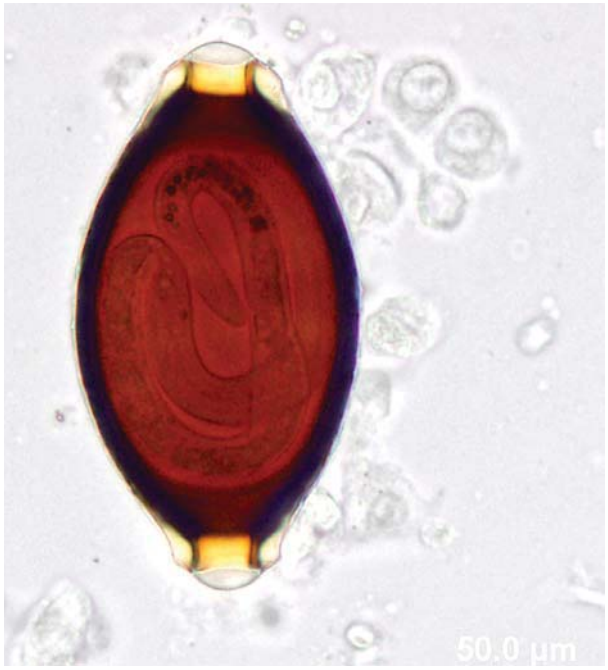

Figure 10. Egg of an unidentified *Huffmanela* species recovered from the skin of a bonnethead shark, *Sphyrna tiburo* (Linnaeus, 1758). The egg is heavily pigmented and houses a fully-embryonated L1 larval stage.

at opposing ends (Figure 10), which are also obvious at low power in histological sections (Figure 11).

We have observed *Huffmanela* infections in a bonnethead shark, *Sphyrna tiburo* (Linnaeus, 1758), where the lesions were indistinguishable from those previously described in *C. plumbeus* (Figure 9). There were no other abnormal signs or indicators of irritation or pathology. The animal had no history of abnormal behavior and no abnormal behavior was noted after examination. Full thickness biopsies of the skin of the lateral cephalofoil were obtained and examined histologically. The decision was made not to treat the shark with any anthelminthics, antibiotics or medications. The shark was routinely administered vitamin C in food. Within three weeks of observed integument hyperpigmentation, there was a significant decrease in the surface area of the skin that was discolored. Additional biopsies were obtained for further identification and characterization of the *Huffmanela* infection. Upon recheck of the biopsy sites, the lesions were no longer visible. At the time of de-accession more than one year later, there were no visible discolorations or defects in the skin, suggesting that the infection was self-limiting. No other *S. tiburo* acquired at the same time, or since that time, have presented with similar lesions, suggesting transmission from shark to shark is unlikely.

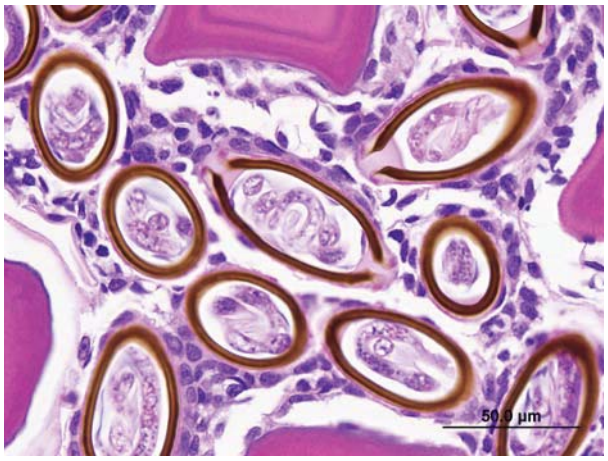

Figure 11. Eggs of the capillariid *Huffmanela* sp. in a histological section of the dermis of a bonnethead shark, *Sphyrna tiburo* (Linnaeus, 1758), stained with hematoxylin and eosin. 400x total magnification.

Biology notes

The life cycle of *Huffmanela* is unknown, but may involve a crustacean intermediate host (Cox et al., 2004), which might help to explain why transmission does not seem to occur within the aquarium environment.

Treatment and Management

MacClean et al. (2006) describe clearance of an extensive *Huffmanela* infection in a *C. plumbeus* within 21 days of treatment with 10 mg/kg levamisole (Ergamisol, Carbone Scientific Co., Ltd.) injected intramuscularly. As noted above, however, the infections may be self-limiting, due to the lack of appropriate intermediate hosts.

Emerging fungal infections

Literature review

Terrell (2004) presented a synopsis of a fungal disease he termed “bonnethead shark disease”, caused by *Fusarium solani* (Von Martius, 1842) infection in *S. tiburo* and scalloped hammerhead sharks, *Sphyrna lewini* (Griffith & Smith, 1834). Smith et al. (1989) and Muhvich et al. (1989) both described a fatal *F. solani* infection in the same brood of *S. tiburo* from the National Aquarium in Baltimore, a case where some pups died *in utero* and all were dead by seven weeks post-parturition. In that case, the fungus had penetrated hyaline cartilage and caused chondritis, as well as myositis in the surrounding muscle. Crow et al. (1995) described granulomatous exudative dermatitis of the lateral line

canal system as a result of *F. solani* infection in two *S. lewini*. The disease resulted in mortality in both cases. The authors interpreted the fatal nature of the infection, combined with pronounced behavioral changes but no systemic involvement, as evidence that a fungal toxin may be involved in pathogenesis with *F. solani*. Certainly, some *Fusarium* species are known to be toxigenic (Döll and Dänicke, 2011).

Fusarium is not the only fungus to cause disease in elasmobranchs in aquaria. Gaskins and Cheung (1986) described a fatal mycosis of smooth dogfish, *Mustelus canis* (Mitchill, 1815) pups, caused by the pigmented fungus *Exophiala pisciphila*. Marancik et al. (2011) described two cases involving three species of fungi: *Paecilomyces lilacinus* Thom, 1910 in a great hammerhead shark, *Sphyrna mokarran* (Rüppell, 1837), and concurrent infection with *E. pisciphila* and *Mucor circinelloides* Tiegh., 1875, in a juvenile zebra shark, *Stegostoma fasciatum* (Hermann, 1783). Both cases documented by Marancik et al. (2011) involved animals within the collection at the Georgia Aquarium, and warrant mention as potential emerging pathogens in aquarium elasmobranchs.

Presentations

The *Paecilomyces* case mentioned above presented in a female *S. mokarran* during quarantine, after collection off the west coast of Florida. The animal had shown agitated, self-injurious behavior shortly after collection and had been provided with treatment and supportive nutrition and was showing steady and consistent improvement when it unexpectedly died. There were few findings at gross necropsy, but on histological evaluation, fungal hyphae were revealed using special stains (Grocott's methenamine silver stain and Periodic acid-Schiff), associated with multifocal areas of hemorrhage and coagulative necrosis in the liver. Fungal hyphae were associated with a focal lesion in the compact myocardium of the heart and were present in lower numbers in gill sections. Culture samples of kidney, spleen and spinal fluid were all culture positive for *P. lilacinus*, suggesting that infection was systemic.

The *S. fasciatum* case involved a juvenile female shark, laid and hatched in the aquarium. The animal displayed abnormal behaviors and altered blood work profiles, but did not respond to treatment and was euthanized. Similar to the hammerhead case, there were few findings at gross necropsy, but multi-focal coagulative necrosis of the liver associated with fungal hyphae, of two distinct morphotypes, was evident on histological evaluation (Figure 12). The hyphae invaded hepatic blood vessels and were also present among the fibers of the heart muscle and in the ventricular lumen. Liver, kidney and spleen were all culture positive for two colony types of fungi. *E. pisciphila* was subsequently identified by DNA sequence homology, whereas *M. circinelloides* was identified by fruiting body morphology.

In addition to the cases reported in Marancik et al. (2011), we have also observed *Fusarium*-like infections in *U. jamaicensis* and fungal encephalitis in *S. fasciatum*, so nervous system involvement is definitely possible.

Biology notes

There are some common features to the emerging mycoses of elasmobranchs in aquariums. Ante-mortem behavioral alterations were present in all the cases detailed above. In the absence of any detectable lesions in the nervous system, the inference of a role in pathogenesis for toxins of fungal origin seems reasonable. Multifocal necrotizing hepatitis was a common histo-pathological presentation, with invasion into blood vessels and associated lesions in the heart.

The morphology of vegetative hyphae is important for taxonomy in fungi and basic characteristics may prove useful for animal care staff when forming diagnoses (Table 1).

Treatment and Management

Since all cases were fatal, we are unable to recommend successful treatment strategies at

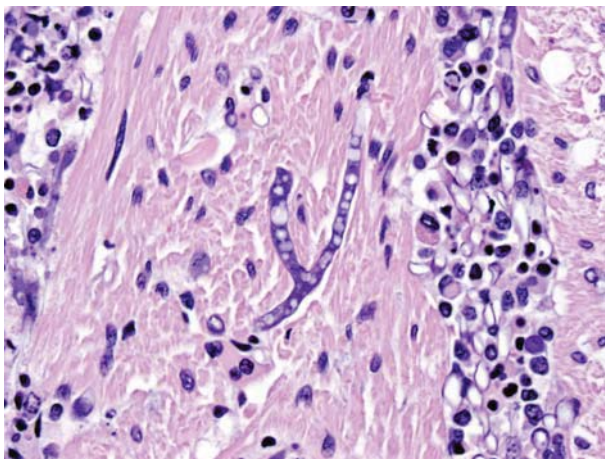

Figure 12. Branching fungal hyphae in brain tissue of zebra shark, *Stegostoma fasciatum* (Hermann, 1783). Little tissue reaction is evident. Total magnification 400x.

Table 1. Characteristics of fungal hyphae recorded from diseased elasmobranchs in aquariums.

Fungal genus	Hyphae (diameter)	Septae	Walls	Branches	Pigment
Paecilomyces	3 - 6 µm	Regular	Thin, parallel	Infrequent, 90°	No
Exophiala	3 - 5 µm	Irregular	Thin, parallel	Frequent, acute angle	Lightly gold/grey
Mucor	10 - 12 µm	Infrequent	Unparallel, dilations	90°	In spores
Fusarium	2.5 - 3.5 µm	Regular	Hyaline, parallel	Acute angles	No

this time. Animal care staff should watch closely for behavioral alterations, like disorientation, loss of equilibrium, collisions or nystagmus (eyes darting unjustifiably), and consider preemptive treatment strategies for fungal infections, because waiting for culture results will likely take too long to be clinically helpful. Antifungal sensitivity tests conducted for *Paecilomyces* suggested resistance to amphotericin B (Amphotec, Alkopharma USA, Inc.), fluconazole (Diflucan, Pfizer) and itraconazole (Sporonox, Janssen), and sensitivity to voriconazole (Vfend, Pfizer), posaconazole (Noxafil, Merck) and terbinifine (Lamisil, Novartis) (Marancik et al., 2011).

DISCUSSION

The pathogens described in this chapter cover a wide taxonomic range, but all are capable of causing clinically significant disease in aquarium elasmobranchs and all but *Huffmanela* can ultimately be fatal. With the possible (but undetermined) exception of *Huffmanela*, these organisms are united by their relatively simple life cycles; all can complete their life cycle using just the elasmobranch host. This phenomenon is common in aquarium diseases, where assembling the correct combination of intermediate and definitive hosts to achieve successful transmission of more complex life cycles, like those of digenean platyhelminths ("trematodes"), would be difficult. The species covered here are marked by a certain lack of host specificity, especially *Branchellion*, which is also a common feature of pathogens/parasites that are 'successful' in aquariums and aquaculture facilities. The combination of simple life cycle and low host specificity allows for a degree of risk analysis for newly discovered/emerged parasites and pathogens. Provided with some basic life history knowledge, it should be possible to make a good estimate of whether a given pathogen is likely to become a persistent problem.

We would be remiss not to address the lack of viral diseases in our list. This taxonomic bias may reflect a real biological pattern wherein viral diseases are genuinely less common in aquariums than bacterial, fungal and parasitic diseases, but it also almost certainly includes a component of diagnostic bias. In the absence of widely available elasmobranch cell lines for cytopathic effect (CPE) studies, it is difficult to diagnose viral infections, unless the virus in question produces large nuclear inclusions that are evident on histopathological evaluation. One possible result of this bias is that viral diagnoses are underrepresented. Future application of molecular tools, such as high throughput metagenomics, will help overcome this problem and accelerate the identification of viral pathogens of sharks, as they are almost certainly relevant.

Advances in materials science, life support systems, husbandry and veterinary care are allowing public aquariums to collect and house an ever-larger diversity of elasmobranch species in larger and more complex community exhibits, exemplified by the "Ocean Voyager" system at Georgia Aquarium. As these new elasmobranch species and new community combinations are explored, it seems inevitable that we will continue to encounter new pathogens and new clinically-significant diseases in aquarium collections of sharks and rays. We expect that this will not be the last time that emergent parasites and pathogens are the subject of a husbandry manual.

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Chapter 28

A review of pathologic findings in elasmobranchs: a retrospective case series

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Abstract: International Zoo Veterinary Group Pathology documented histopathologic findings for 632 elasmobranch fishes received from Merlin Entertainments Group's Sea Life attractions during the period of May 2003 to June 2013. Fishes received represented 72 different species. The most frequently received species were blacktip reef sharks, *Carcharhinus melanopterus* (Quoy and Gaimard, 1824) (10.8%); cownose rays, *Rhinoptera bonasus* (Mitchill, 1815) (9.5%); thornback rays, *Raja clavata* (Linnaeus, 1758) (7.3%); bonnethead sharks, *Sphyrna tiburo* (Linnaeus, 1758) (6.6%); small-spotted catsharks, *Scyliorhinus canicula* (Linnaeus, 1758) (6.0%); zebra sharks, *Stegostoma fasciatum* (Hermann, 1783) (5.4%); and nursehound sharks, *Scyliorhinus stellaris* (Linnaeus, 1758) (5.1%). For all elasmobranchs, across all years, the distribution of cases by pathologic process was: no pathologic diagnosis (19.6%); infectious/inflammatory (41.0%); nutritional (32.6%); traumatic (2.4%); suspected toxicosis (2.3%); degenerative (1.9%); deposition (0.8%); environmental/mechanical plant failure (0.8%); neoplastic (0.5%); and reproductive (0.4%). Etiologic agents identified included: bacterial infection (12.2%); nematodes (7.5%); trematodes (6.0%); scuticociliates (4.6%); coccidia (4.3%); myxozoans (2.4%); fungi (2.0%); epitheliocystis-like organisms (1.7%); other protozoa (0.8%); and suspected viral infection (0.5%). These results supplement previous analyses of disease in aquarium elasmobranchs, and provide an evidence base to improve husbandry and future disease investigations.

INTRODUCTION

Few published studies address overall patterns of pathology in zoo and aquarium elasmobranchs, although compilations exist based on case studies and individual unpublished observations (Stoskopf, 2010). A recently published article by Garner (2013) represents the first systematic, large-scale retrospective study of aquarium elasmobranch pathology, incorporating histopathologic findings from a range of zoos and aquaria, and included findings from 1,546 individuals representing at least 60 species. The aim of the current study was to supplement that information by examining a comparable population of elasmobranchs from Merlin Entertainments Group's Sea Life aquarium facilities in Europe.

Overall, the patterns of pathology in our study resemble (with minor differences of emphasis in species, process and etiology) those reported by Garner (2013).

MATERIALS AND METHODS

All elasmobranch histopathology cases from European Sea Life attractions referred to International Zoo Veterinary Group (IZVG) Pathology between November 2003 and June 2013 were retrieved and assessed. A total of 632 separate animals were assessed. Histopathologic examinations were undertaken by a single pathologist (MFS) on routine hematoxylin and eosin-stained histology slides, prepared from biopsies or formalin-fixed post-mortem tissue sets submitted by aquarists at each aquarium site. Original reports were evaluated by the same pathologist, with re-examination of the original slides, or slides of retained archival tissues (where necessary) for clarification. Histologic special stains were used as required. A small proportion of cases included supporting microbiology from swabs or body fluids. A combined analysis across Sea Life attractions, as a whole, was considered most beneficial, due to small case numbers from individual attractions.

Findings were simplified into major disease categories, corresponding broadly with those used in the study of Garner (2013), to provide an indication of the most common underlying processes and probable causes. The following categories were used: no pathologic diagnosis, infectious/inflammatory, nutritional, traumatic, cardiovascular, toxicosis, degenerative, deposition, reproductive and neoplastic. An additional category of environmental/mechanical plant failure was added for our study. Except for *no pathologic diagnosis*, it was possible for a single animal to have more than one diagnosis (e.g., emaciation with concurrent scuticociliate infection). Proportions are of total diagnoses made, not of total animals affected. As in Garner (2013), some disease processes were the cause of death, whereas others were background or non-specific findings. The results indicate the prevalence of different diseases within the population over the study period.

Table 1. The ten most frequent elasmobranch species sent for histopathological review, from Merlin Entertainments Group's Sea Life attractions to International Zoo Veterinary Group Pathology, during May 2003 to June 2013.

Scientific name	Common name	Sea Life cases	Percentage of submissions
Carcharhinus melanopterus (Quoy and Gaimard, 1824)	Blacktip reef shark	68	10.8%
Rhinoptera bonasus (Mitchill, 1815)	Cownose ray	60	9.5%
Raja clavata (Linnaeus, 1758)	Thornback ray	46	7.3%
Sphyrna tiburo (Linnaeus, 1758)	Bonnethead shark	42	6.6%
Scyliorhinus canicula (Linnaeus, 1758)	Lesser-spotted catshark	38	6.0%
Stegostoma fasciatum (Hermann, 1783)	Zebra shark	34	5.4%
Scyliorhinus stellaris (Linnaeus, 1758)	Nursehound	32	5.1%
Mustelus asterias (Cloquet, 1821)	Starry smooth-hound	29	4.6%
Raja undulata (Lacepède, 1802)	Undulate ray	23	3.6%
Heterodontus portusjacksoni (Meyer, 1793)	Port Jackson shark	21	3.3%

SPECIES DISTRIBUTION

Overall, at least 72 different species were included in the submissions, although 10 species stood out as most commonly submitted, constituting 62.2% of the total, which have been summarized in Table 1.

DISEASE PROCESSES

Of the categorized disease processes for all submissions between November 2003 and June 2013, infectious/inflammatory and nutritional diseases were by far the most common (Figure 1), together accounting for 73.6% of all diagnoses.

Presumptive nutritional disease was common. Elasmobranchs lack coelomic cavitary adipose, instead concentrating primary stores of fat in the liver. Nutritional disease was thus diagnosed when profound depletion of the normally abundant intra-hepatic fat stores was noted, indicating a significant period of negative energy balance. In some cases, concurrent chronic disease was identified, which contributed to emaciation, whilst in others, an acute process, such as bacterial sepsis, was considered too rapid to have caused the depletion. In these cases, nutritional compromise and debilitation impairing innate immunity to infection was concluded. A further cohort of fishes merely had lipid depletion in the absence of other findings, and nutritional stress may have been a primary underlying factor. Uncommonly, problems with food quality were implicated, rather than absolute intake. This applied to a cluster of ray mortalities, in which microbiological testing of the frozen cooked mussels used as a dietary item identified excessive total viable bacterial counts, consistent with food spoilage. Rare cases of thyroid hyperplasia indicated iodine deficiency and the need for a continuing focus on appropriate supplementation. High environmental nitrate concentrations may also interfere with thyroid use of available iodine, and lead to thyroid hyperplasia.

Trauma was identified infrequently as a primary issue, and included bites from other animals, animals that jumped out of tanks, and rare cases where prolapsed segments of caudal intestinal tract or liver were bitten off by other sharks in the

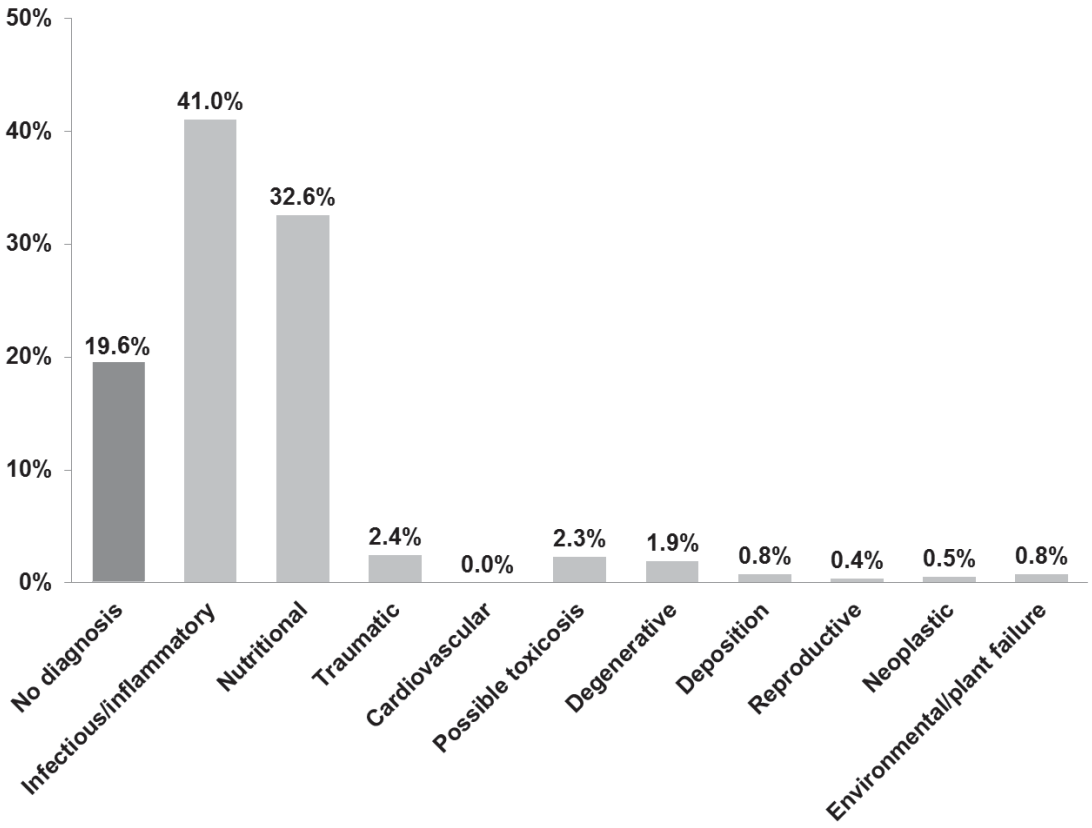

Figure 1. Histopathologic findings classified into major diagnostic categories (expressed as a percentage of total diagnoses made) for 632 elasmobranch fishes sent to International Zoo Veterinary Group Pathology from Merlin Entertainments Group's Sea Life attractions during May 2003 to June 2013.

exhibit. Species affected by prolapses included *Carcharhinus melanopterus* (Quoy and Gaimard, 1824), smoothhounds, *Mustelus* spp. (Linck, 1790), and sand tiger sharks, *Carcharias taurus* (Rafinesque, 1810), and various ray species suffered bite wounds. Skin discolorations, abrasions, ulcers and suspected bite wounds were reported more frequently by aquarists on submission forms than could be demonstrated histologically. In the future, improved sampling of skin lesions to identify contributory factors (such as cutaneous protozoal or fungal infections) may be helpful.

No cases were assigned to the cardiovascular category of Garner (2013), most of which he attributed to shock. It is likely that such cases are included in the infectious/inflammatory category in our study, or in the organ-based analysis, as myocarditis or vasculitis.

Aquarists suspected toxicoses more frequently than could be demonstrated by histologic changes or confirmed by other features in the history. There were a few cases, typically presenting with acute severe gill pathology, where there were strong circumstantial grounds to suspect toxicosis. These cases included death in a catshark, *Scyliorhinus* spp. (Blainville, 1826), preceded by an ammonia spike, as well as deaths in thornback rays, *Raja clavata* (Linnaeus, 1758). High zinc levels in artificial seawater in a newly refurbished exhibit caused a cluster of deaths in *C. melanopterus*. Excess zinc was measured directly in the exhibit water, and in laboratory test solutions of the salt in reverse osmosis water. Other examples may have been overlooked, since sub-acute reparative/regenerative changes, such as lamellar hyperplasia or fusion, which were noted in some cases, are not specific for toxin exposure. Inflammatory lesions, or other non-specific changes (e.g., variations in cellularity in the epigonal hematopoietic organs), may have been caused initially by toxicosis. However, more detailed historical and toxicological information was needed to make a definite diagnosis, and was not available.

Degenerative pathology was rare, including suspected renal dysplasia in a *R. clavata* and marked arteriosclerosis in an aged *C. melanopterus*. A handful of cases with chronic renal fibrosis were identified, for which the inciting cause was unknown. Deposition diseases were uncommon, limited to occasional examples of metastatic mineralization of unknown cause, renal mineralization and calcinosis circumscripta in rays. An enterolith resulted in intestinal obstruction and death after surgery on a *C. taurus.*

Disease specifically associated with reproduction was rare, but one *C. melanopterus* died acutely, and was found to have ingested its own fetus, suggesting that the death may have been an acute complication of parturition. Fetal retention was suggested by the submitting collection to be contributory to death in another *C. melanopterus*, but there was no histologic evidence to support this claim.

Neoplasia was unusual. Examples included undifferentiated soft tissue sarcoma of the skin in a cownose ray, *Rhinoptera bonasus* (Mitchill, 1815), large intestinal lymphosarcoma in a smoothhound, *Mustelus mustelus* (Linnaeus, 1758). Round cell tumor of the spleen of an undulate ray, *Raja undulata* (Lacepède, 1802) and papilloma/carcinoma-in-situ of the skin in a *R. clavata*.

Occasionally, known mechanical plant/environmental failure was closely associated with subsequent deaths. Examples included sharp changes in water temperature in two cases, and failure of an oxygen metering and management system in another. Several cases had factors listed in their clinical histories that were potentially significant, but for which there was no histologic evidence to either confirm, or refute, their involvement. These included stray voltage events and a suspected supersaturation event.

CASES FOR WHICH NO DIAGNOSIS WAS MADE

The percentage of submissions in which no diagnosis was made was 19.6% (annual mean ± S.E. = 19.6% ± 3.4%), which was considered unacceptably high (compared with only 4.2% in Garner, 2013). It also compared unfavorably with a previous syngnathid disease survey by IZVG for Sea Life (Stidworthy, unpublished results).

Factors contributing to reduced diagnostic success rates include pathologist inexperience and limited access to reference material and literature, aquarist inexperience, inappropriate sample selection and inadequate sample quality. Unfamiliar elasmobranch anatomy led to inadequate gill sampling and epigonal organs were often mistaken for kidneys. Inexperienced aquarists were misled by the normal pallor of the lipid-laden liver, and an inappropriate focus on the liver and gallbladder was common. The brain, spleen and pancreas were often

overlooked. Sampling of skin abnormalities was often poor, or absent, despite a clinical history of lesions. Tropical species autolyze quickly after unexpected death, but ample volumes of fixative and suitable tissue size help to limit post-mortem artefact. Except for very small neonates/juveniles submission of whole fixed elasmobranchs is undesirable, as fixation of internal organs and gills is inadequate for high quality histology.

Overall, an improving trend in the "no pathologic diagnosis" rate was apparent (Figure 2). By the first half of 2013, this rate had dropped to 7.8%. Probable causes for this improvement include increased pathologist experience and better education of aquarists in comprehensive sampling techniques. Nevertheless, additional training for staff performing post-mortem examinations is desirable.

ETIOLOGIES OF INFECTIOUS/ INFLAMMATORY DISEASE

The contribution of diagnosed etiologies for infectious/inflammatory disease processes is shown in Figure 3.

Bacterial infections were most commonly associated with evidence of generalized sepsis (e.g., splenitis), dermatitis or branchitis. Cultures were rarely available to make a specific etiologic diagnosis, but when Gram stains were undertaken most organisms were Gram-negative. Two outbreaks of presumptive mycobacterial infection were identified. Five lesser-spotted dogfish, *Scyliorhinus canicula* (Linnaeus, 1758), presented concurrently with granulomatous meningitis, within which acid-fast organisms, presumed *Mycobacterium* sp., were confirmed with Ziehl Neelsen stains. An additional case of dual mycobacterial and fungal infection was identified in a Port Jackson shark, *Heterodontus portusjacksoni* (Meyer, 1793). No mycobacterial cultures were undertaken in either case, and the identity of the organisms remains unknown. Epitheliocystis-like (presumptive Order Chlamydiales) organisms were rare, but infected groups of *C. melanopterus* associated with intralesional organisms presented with variable gill hyperplasia.

Most of the nematode infestations identified were presumptive dracunculoids. Nodules of larvae were particularly common in the gills of certain ray species, including *R. bonasus*, but were also identified within viscera, such as the kidney. Simi-

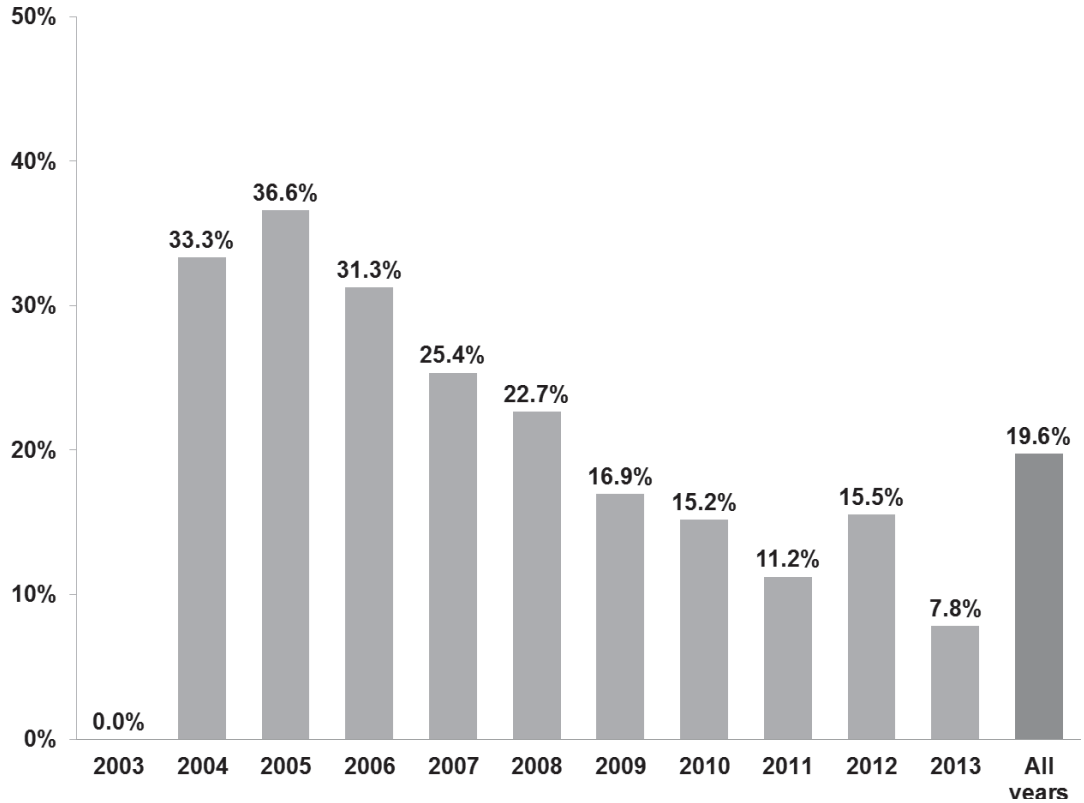

Figure 2. Histopathologic cases classified as "no pathologic diagnosis" (expressed as a percentage of total diagnoses made) ranked by year for 632 elasmobranch fishes sent to International Zoo Veterinary Group Pathology from Merlin Entertainments Group's Sea Life attractions during May 2003 to June 2013.

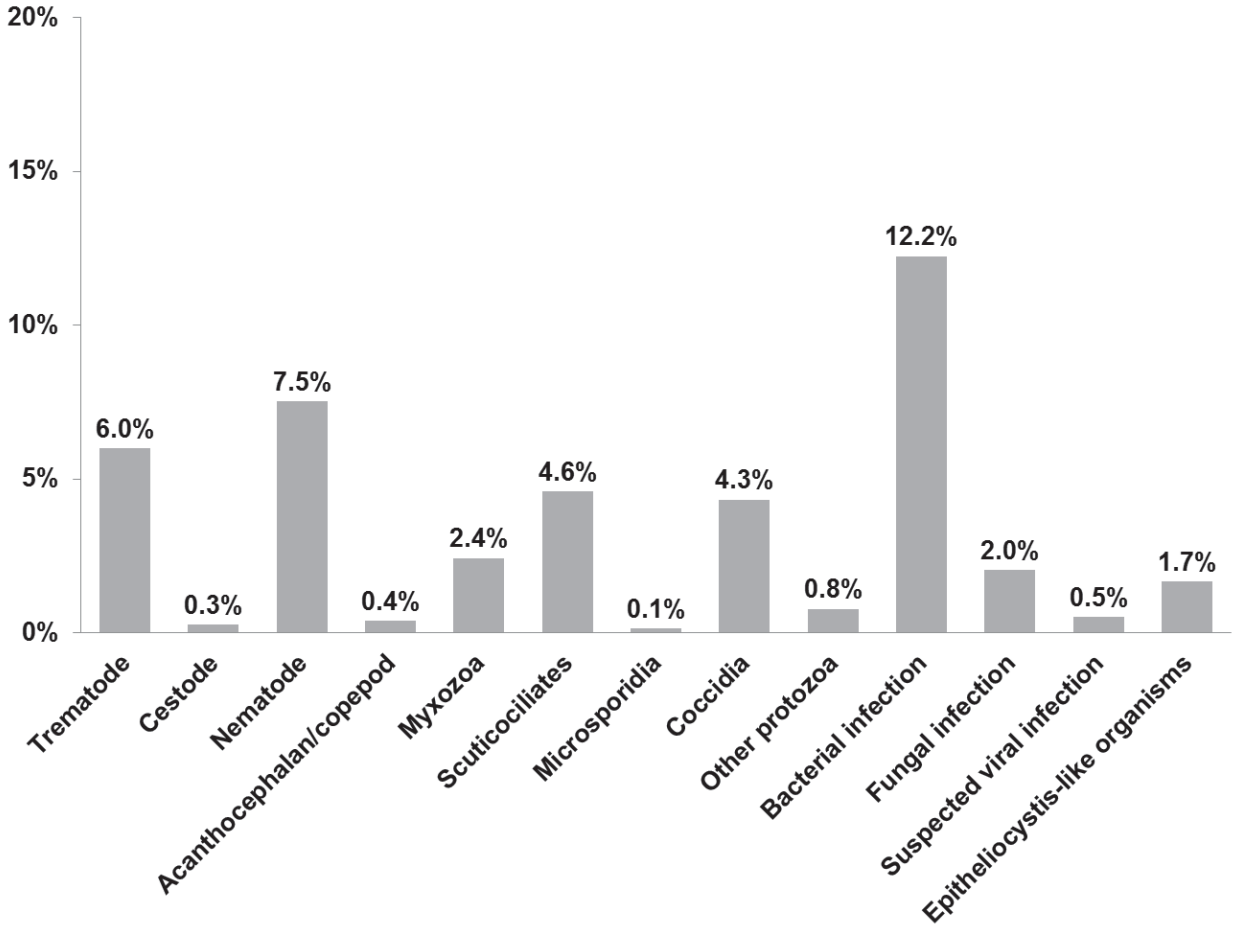

Figure 3. Infectious and parasitic etiologies (expressed as a percentage of total diagnoses made) for 632 elasmobranch fishes sent to International Zoo Veterinary Group Pathology from Merlin Entertainments Group's Sea Life attractions during May 2003 to June 2013.

lar parasites were seen occasionally in sharks, and migrating larvae (i.e., visceral larva migrans) were found in cases of meningoencephalitis in sharks.

Monogenean gill flukes dominated trematode infections, although there were occasional examples of enteric or hepatic digenean trematodes. Gill flukes were contributory to death in some cases.

Scuticociliate infections (*Philasterides dicentrarchi,* Order Scuticociliatida, Subclass Scuticociliata, Lynn and Small, 2000) were first recognized and characterized in detail during two outbreaks in *S. fasciatum*, *H. portusjacksoni*, and Japanese bullhead sharks, *Heterodontus japonicus* (Maclay and Macleay, 1884), in 2010; with further description in Stidworthy et al. (2014). The current review found prior cases of invasive disease by probable scuticociliates, with a wider range of elasmobranch species likely to be susceptible (Figure 4). Further cases with suspicious pathology, but no intralesional protozoa, were found, and cases may have been overlooked in the past, or suspected to be of bacterial origin.

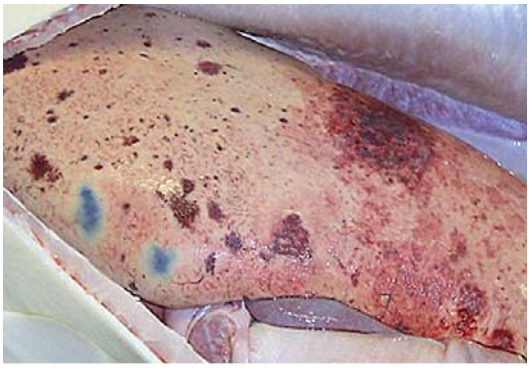

Figure 4. Scuticociliatosis infection in the liver of a Port Jackson shark, *Heterodontus portusjacksoni* (Meyer, 1793), showing severe disseminated multifocal necrohemorrhagic hepatitis. The underlying hepatic pallor is a normal feature in sharks, which have abundant physiologic hepatic fat deposits.

Diagnoses should ideally be confirmed by PCR-testing of unfixed or ethanol-fixed tissues; PCR is much less sensitive on formalin-fixed tissues.

The majority of coccidial infections were due to *Eimeria southwelli* (Halawani, 1930), colonizing the coelomic cavity of *R. bonasus*. Lymphocytic and hyperplastic coelomitis was frequently found in these fish, and occasionally in flapnose rays, *Rhinoptera javanica* (Müller and Henle, 1841), and was often associated with emaciation and depletion of hepatic lipid. Confirmation of the diagnosis was assisted if unfixed coelomic fluid was submitted together with tissues for histology. The characteristic free-floating banana-shaped oocysts were easily seen by direct microscopy, whereas parasites were harder to see in the mesothelium itself. The only other coccidial infection recognized on repeated occasions was gastric coccidiosis of *C. melanopterus*, where coccidial oocysts and gametocytes colonized the stomach mucosal epithelium (Figure 5). This observation appears to be novel, and not previously described in the literature (Duszynski, personal communication). Other cases of coccidiosis were seen in 2011 and 2012, and were considered incidental, associated with minimal lymphocytic gastritis. Myxozoan infections were uncommon and incidental, but infections of the gallbladder were noted in *R. bonasus*, spotted eagle rays, *Aetobatus narinari* (Euphrasen, 1790), and an unidentified ray species, as well as in a *C. melanopterus* and a common guitarfish, *Rhinobatos rhinobatos* (Linnaeus, 1758). Muscle infections were identified in bonnethead sharks, *Sphyrna tiburo* (Linnaeus, 1758), and blacknose sharks, *Carcharhinus acronotus* (Poey, 1860), and renal infection in a blue-spotted stingray, *Dasyatis kuhlii* (Müller and Henle, 1841). Other protozoa were rarely seen, but included a solitary case of amoebic gill disease and a few other cases in which the identity of the organisms was unclear.

Fungal infections were uncommon, and definitive diagnosis of the organism involved was rarely possible. *Fusarium solani* was cultured from nursehound sharks, *Scyliorhinus stellaris* (Linnaeus, 1758), where invasive necrotizing fungal infections were seen penetrating from the skin into cartilage, particularly in the head. Similar lesions were seen in *S. canicula* and a *C. acronotus*.

Viral infections were rare. Dermatitis/epidermal hyperplasia of viral (herpesvirus) origin was suspected in *C. taurus* and *C. melanopterus* on clinical and histologic grounds, but was never confirmed by electron microscopy or virus isolation/PCR testing. A case of suspected viral encephali-

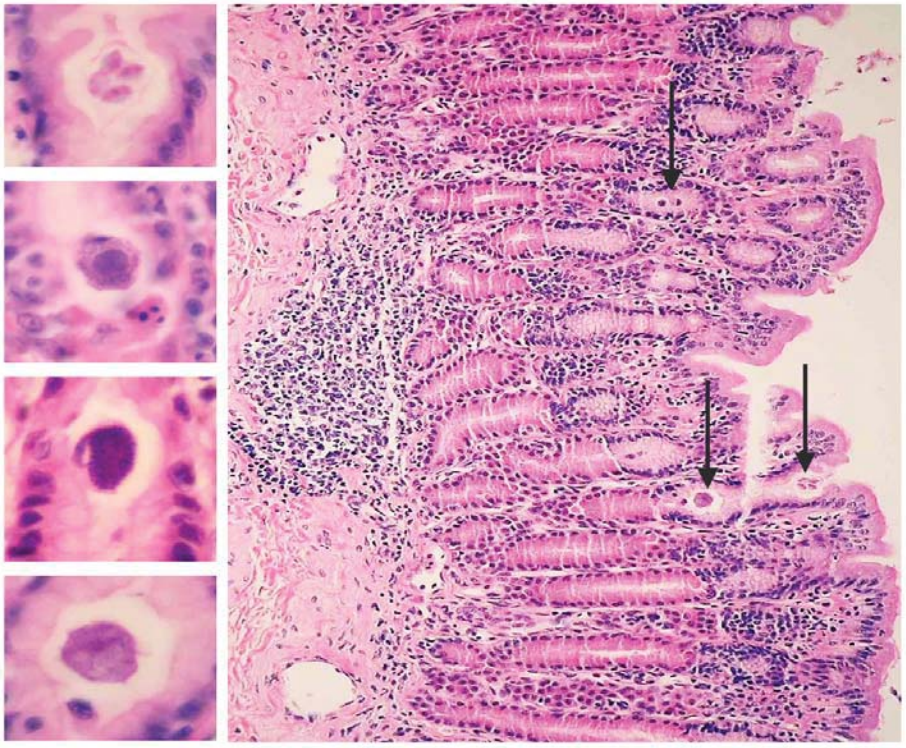

Figure 5. Gastric coccidiosis in the stomach of a blacktip reef shark, *Carcharhinus melanopterus* (Quoy and Gaimard, 1824). Note mild lymphocytic gastritis, with intra-epithelial protozoa, consistent with coccidia (arrows). Inset from top to bottom: oocyst and presumptive gametocytic forms within mucosal epithelial folds.

tis with suspicious intranuclear (herpesvirus) inclusions was identified in a single *C. melanopterus*. To date, no definitive, non-histologic, laboratory confirmation of a viral infection has been made.

LESION DISTRIBUTION BY ORGAN

The distribution of lesions by organ system (Figure 6) highlights the importance of gill pathology, and thus of adequate gill sampling to identify it. Furthermore, the skin and brain regularly showed lesions; however, sampling deficiencies were common. This shortcoming suggests that diseases may have been missed due to sampling error. Non-specific coelomic inflammation was also common. In *R. bonasus*, the association with *E. southwelli* is well established, but in these and other species, specific diagnosis may be enhanced by the (timely) submission of fresh coelomic fluid samples. The wide spectrum of changes across organ systems highlights the importance of collecting a complete tissue set, in order to maximize chances of diagnosis. Underrepresentation of pathology in some organs (e.g., kidney, spleen, pancreas, gonads, epigonal organ and endocrine organs) may reflect inconsistencies in sampling.

DISCUSSION

Comprehensive retrospective studies of disease in elasmobranchs maintained under human care are few, and the recently published article by Garner (2013) is the most significant. The case distribution in the Sea Life series differs in range and emphasis from that reported by Garner. While a preponderance of *R. bonasus* and *S. tiburo* also features in his report, *C. melanopterus*, *S. fasciatum*, *H. portusjacksoni* and various rays form a higher percentage of Sea Life submissions. There are broad similarities in the diagnoses between the two datasets. Infection/inflammation and nutritional disease are the two most important diagnostic categories in both studies. A relative prominence of morphologic diagnoses of branchitis, dermatitis and meningoencephalitis,

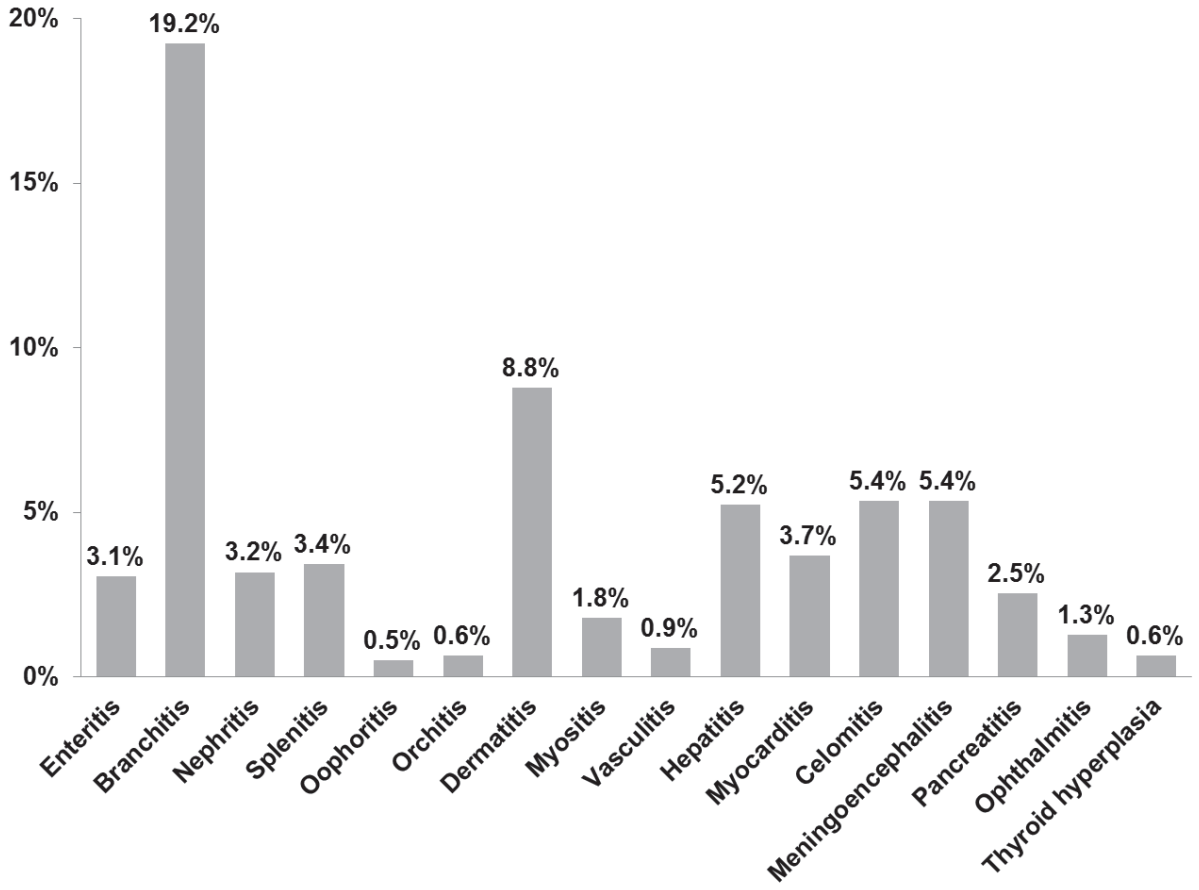

Figure 6. Main organ systems in which histopathologic lesions were identified (expressed as a percentage of total diagnoses made) for 632 elasmobranch fishes sent to International Zoo Veterinary Group Pathology from Merlin Entertainments Group's Sea Life attractions during May 2003 to June 2013.

for which the cause was unknown, was apparent in both populations.

Infection/inflammation accounted for 41.0% of Sea Life diagnoses and 33.5% of those cases examined by Garner (2013), followed by nutritional (mainly emaciation), at 32.6% and 11.9%, respectively. The proportion of trauma cases in our study (2.4%) was lower than that identified by Garner (11.3%). This difference may be because, in our survey, trauma cases were typically diagnosed at the aquarium level and relevant tissues were not submitted for histologic confirmation. In addition, the exact subclassifications within the broad diagnostic categories are likely to vary, due to differences in the coding of diagnoses in different laboratory systems. For example, Garner included "stress/maladaptation" as a traumatic diagnosis, whereas cases with emaciation due to general factors, such as maladaptation were considered nutritional in our survey. We suspect the discrepancy in cardiovascular disease (0% in our study, but 5.5% in Garner) was due to differences in interpretation, rather than a genuine causal difference. Definitive, or strongly-suspected, toxic events with histologic confirmation were relatively infrequent in both case series (2.4% in the Sea Life series, or 3.2% if known environmental/plant failures were included, and 3.7% in Garner, 2013). Gas bubble disease was confirmed histologically in the review by Garner, but not in the Sea Life group, although suspected.

The remaining categories occurred at low levels in both studies: degenerative (Sea Life 1.9%; Garner 2.9%), deposition (Sea Life 0.8%; Garner 2.7%), reproductive (Sea Life 0.4%; Garner 2.4%), and neoplastic (Sea Life 0.5%; Garner 0.4%). Some of the variance likely reflects bias in pathologist classifications, thus should be interpreted conservatively. An enterolith in a *C. taurus* was an unusual solitary occurrence in the Sea Life series and the spectrum of neoplasia was different. Such disease processes appear to be occurring at low levels in global aquarium elasmobranch populations. Focus on more significant factors, particularly infection and nutrition, should be the major priority for husbandry improvement, as many of these lesser processes are of uncertain cause (e.g., degenerative disease) or hard to control (e.g., spontaneous neoplasia).

Garner (2013) found that bacterial infections accounted for 15.0% of diagnoses, compared with 12.2% of Sea Life cases (13.9% if epitheliocystis-like infections are included). In both studies, it was rare for specific bacteria to be identified and many infections were suspected to be opportunistic. Interestingly, hammerhead sharks (*Sphyrna* spp.) were overrepresented amongst epitheliocystis-like cases in the Garner series, whereas *C. melanopterus* were overrepresented amongst Sea Life cases. There were no cases of mycobacteriosis in the Garner series, whereas two outbreaks were identified in the Sea Life series. Cases of mycobacteriosis in the elasmobranch literature remain rare, although several cases have recently come to light (Anderson et al., 2012; Janse and Kik, 2012; Clarke et al., 2013).

A slightly higher proportion of fungal infections was identified in the Sea Life cohort (2.0%, compared to 0.6% in the Garner series), but definitive cultures to identify the organisms were rarely performed in either study. However, severe necrotizing lesions due to fusariosis were confirmed in both groups (*S. tiburo* in the Garner series, *S. stellaris* in the Sea Life series).

Viral, or suspected viral infections occurred at low levels in the Garner study (1.0%), and included papillomatosis, herpesvirus and adenovirus. Suspected viral infections in Sea Life cases accounted for only 0.5% of cases, all of which were histologically suspected herpesvirus infections.

The pooled total of parasitic diagnoses (26.4%) for Sea Life cases is notably higher than the 9.0% quoted by Garner, suggesting a higher detection rate and/or overall parasite burden in the Sea Life animals. Differences may relate to parasite treatment strategies on either side of the Atlantic. However, when proportions of different parasites are considered, there are broad similarities and a few notable differences. Respective proportions are: nematodes (Sea Life 28.5%; Garner 26.0%), ciliates (Sea Life 17.4%; Garner 23.0%), trematodes (Sea Life 22.7%; Garner 20.0%), coccidiosis (Sea Life 16.4%; Garner 6.0%), myxozoanosis (Sea Life 9.2%; Garner 5.0%), other protozoa (Sea Life 2.9%; Garner 5.0%) and cestodes (Sea Life 0.9%; Garner 1.0%). The elevated rate of ciliate infection in the Garner series is attributable to a cluster of cutaneous ciliatosis cases in dusky smoothhounds, *Mustelus canis* (Mitchill, 1815), whereas cutaneous ciliate infections were found only occasionally in the Sea Life series. This difference may reflect patterns of skin sampling for histology, as discussed above. Systemic scuticociliatosis, with the triad of gill, liver and brain lesions, was the other main presentation in both groups. Visceral coccidiosis (*E. southwelli* infec-

tion) was the dominant coccidial diagnosis in both series, but the Sea Life group had additional examples of gastric coccidiosis in *C. melanopterus*. Myxozoan infections were mainly in the gallbladder in both groups, but muscle and renal infections were occasionally seen in the Sea Life series, and renal and meningeal infections were recognized in the Garner series.

Definitive diagnosis of toxic etiologies appears to have been more common in the Garner series, including iatrogenic fenbendazole and chloramine toxicities. Environmental toxins were suspected, but not always confirmed in the Sea Life group, and enhanced data collection and sharing would benefit both veterinary pathologists and aquarists during these toxicity investigations, enabling more confident diagnoses.

FUTURE PROSPECTS

In the future, aquarist education and training should focus on normal elasmobranch anatomy, to ensure reliable organ recognition, and on the consistent application of standardized systematic necropsy techniques across all sites. Furthermore, such training should be reviewed and reinforced regularly to allow for staff turnover, which is common in aquarist positions. Comprehensive sampling and adequate fixation will ensure that pathologists have the best chance of identifying significant lesions. Targets for potential enhancement include key organs that are frequently overlooked, such as the gills, skin, kidney and brain. Increased use of direct microscopy and cytology is likely to assist rapid diagnosis (e.g., collection and examination of coelomic fluid from *R. bonasus* for *E. southwelli* and identification of gill flukes in all species).

Our current knowledge of microbial disease in elasmobranchs remains limited in comparison with teleosts, although recent years have seen several new case reports, building on those summarized previously (Bertone et al., 1996; Briones et al., 1998; Stoskopf, 2010; Camus et al., 2013). This chapter, and the review by Garner (2013), demonstrate that inflammatory disease of presumed microbial origin is important in aquarium elasmobranchs. However, in many cases in both studies, it was not possible to be confident of the specific bacterial agent responsible. The most important reason for this was that appropriate fresh tissue samples or swabs were not collected by aquarists for submission with the histology samples, so that when bacteria were seen in histological sections, microbiology results could not be obtained for correlation. Previous studies have shown that neither blood, nor tissues, of apparently healthy elasmobranchs, are bacteriologically sterile, further complicating interpretation (Grimes et al., 1984; Borucinska and Frasca, 2002; Mylniczenko et al., 2007; Tao et al., 2014). Only consistent necropsy collection and submission of superficial swabs (e.g., gills and skin) and normal and diseased internal organs (e.g., liver, spleen, kidney, heart blood, brain) will facilitate a good understanding of the post-mortem bacterial flora of healthy and diseased elasmobranchs. Collection of such samples for microbiology should be part of the routine necropsy protocol in all cases, and maintenance of frozen or RNALater™ banks of the same tissues will ensure that material is available for the application of rapidly-developing molecular techniques, such as qPCR and deep sequencing.

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Chapter 29

Pharmacology of elasmobranchs: updates and techniques

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Abstract: There is little information on pharmacotherapeutics in elasmobranchs, with much of veterinary clinical practice relying on extrapolation from other species. While this action is often our only recourse, it can be augmented with knowledge of elasmobranch anatomy and physiology, environmental influences and the properties of the drugs themselves. The experiences of veterinary and husbandry colleagues adds to the databank of therapeutics that have been used without negative consequence, and that, in some cases, have had desired effects. However, actual biologic drug levels and safety studies are lacking in most cases. Despite these shortcomings, a number of drugs have been successfully used to treat elasmobranch maladies.

INTRODUCTION

Pharmacotherapy is the study of the therapeutic uses and effects of drugs on a living organism. Pharmacodynamics refers to what a drug does to a body, while pharmacokinetics shows how the body processes drugs, or what the body does to the drug.

Although elasmobranch medicine continues to grow, there is little pharmacokinetic or pharmacodynamic information available for drugs and supplements used with these animals. This document builds on a chapter within the *Elasmobranch Husbandry Manual* (Stamper et al., 2004) and offers an enhanced *Elasmobranch formulary* (available as an electronic download from the online resources for this manual; www1). Readers are directed to Stamper et al. (2004) for details on basic pharmacology, physiology and drug information. Here, we focus on methods to administer medications and expand on the available literature. Anesthesia will not be discussed. Instead readers are directed to a recent publication on elasmobranch immobilization and restraint by Mylniczenko et al. (2014).

Disclaimer

There are few reports of prospective pharmacokinetic studies conducted in elasmobranchs. Most of the drugs and dosages reported here are contributions from the collective experiences of specialists in elasmobranch medicine and do not reflect safety or efficacy trials. Using any of the listed pharmacotherapeutics at the listed dosages is solely at the discretion of the attending clinician.

RELEVANT LITERATURE

Elasmobranchs are not typically viewed as laboratory research animals, yet most of the

classic pharmacokinetic literature is available only because of this venue. Early research on pharmacokinetic modeling was established using phenol red in the piked dogfish, *Squalus acanthias* (Linnaeus,1758) (Bungay et al., 1976), methotrexate in the Atlantic stingray, *Dasyatis sabina* (Lesueur, 1824) (Zaharko et al., 1972), and chlorodiphenyl ether in the winter skate, *Leucoraja ocellata* (Mitchill, 1815) (Chui et al., 1986). Detailed experiments were conducted on a variety of substances by Fenstermacher et al. (1972) in *S. acanthias,* and Adamson and Guarino (1972), Guarino and Anderson (1976) and Guarino et al. (1977), on multiple elasmobranch species. These are seminal works in drug distribution and modeling, and interested readers and future pharmacokineticists are encouraged to peruse these papers.

Additional studies on pharmacokinetics are noteworthy. Gorbi et al. (2004) found that metabolizing enzymes in the lesser-spotted dogfish, *Scyliorhinus canicula* (Linnaeus, 1758), had a lower efficiency than those of teleosts, making them more susceptible to oxidative damage by toxins. Several papers highlight the toxicity of metals in marine elasmobranchs. Sublethal levels of lead (20 µM and 100 µM) in *S. acanthias* resulted in notable respiratory disturbance, although the animals were able to maintain homeostasis (Eyckmans et al., 2013). *S. acanthias* exposed to silver had a more dramatic reaction, including death due to respiratory and osmoregulatory failure (De Boeck et al., 2001), than teleosts exposed to the same concentration. At lower concentrations of silver the effects were less severe and only osmoregulatory toxicity was observed (due to specific effects on NaCl excretion mechanisms). In a more practical study, *S. acanthias* and longnose skates, *Raja rhina* (Jordan and Gilbert, 1880), were exposed to waterborne silver levels at 14.5 mg/L for 21 days in 30 g/L seawater. Silver levels increased 2 - 20 times in most tissues, with the greatest concentrations occurring in the livers of teleosts and the gills of elasmobranchs. In elasmobranchs, the liver accumulated silver 5 - 15 times faster than in the teleosts.

Zubrod and Rall (1959) identified that the liquid in the space around the brain cavity of *S. acanthias* is not cerebrospinal fluid, but rather ventricular fluid external to the brain. Additionally, the meningeal morphology was suggestive of the absence of a blood-brain barrier, unlike higher vertebrates. Further research indicated that there actually is a blood-brain interface, but that the ultrastructure shows fenestrations (Bundgaard and Cserr, 1981). In addition, the presence of a functional glial membrane acts to provide some impermeability and homeostasis.

Oppelt et al. (1966) conducted a study that detailed the composition of electrolytes in ventricular fluid, when compared to blood, and further evaluated fluid production rates in the brain case in the presence of different drugs.

DRUGS, DOSES AND DELIVERY

Drug choice

How does one choose the right drug or combination of therapeutics? Ideally, a definitive diagnosis would be available, based on examination and diagnostics. The reality, however, is that nonspecific clinical signs and a potentially unapproachable animal often influence and retard a clinician's capacity to determine a definitive diagnosis. In many elasmobranch cases the most likely differentials for a medical condition will direct the clinician to speculate at the organ system(s) involved, which will then guide pharmacologic choices. If there is data available for the species (or the closest taxonomic equivalent), the clinician will likely use that information to further guide treatment choices.

Drug dose

Once the clinician decides what drug(s) may be best for a treatment, a new set of challenges awaits. The dose, frequency and duration of treatment must be decided, as well as what route of delivery will be most suitable. In general, there is little published information on pharmacokinetic or pharmacodynamic parameters of drugs in non-domestic species. Drug absorption can vary within a species, so extrapolation between species can pose even greater room for error. Factors such as core body temperature and variations in plasma protein binding can additionally influence drug metabolism (see Stamper et al. (2004) for more detail).

Despite the risks associated with having to extrapolate between species and treat by "trial and error", this approach is typically how elasmobranchs treatments are determined. Clinicians will typically review available information (published and not) on drugs or supplements that have been used in elasmobranchs without negative consequences. If information does not exist for elasmobranchs, they will often turn to available information and

experiences with teleosts, reptiles or amphibians, as they are potentially more metabolically similar to elasmobranchs than mammals or birds. This process is the most typical method of extrapolation, but may result in linear increases in the amount of drug used as the body weight of the animal increases, which could lead to overdosing larger animals and under-dosing smaller animals.

Another method for extrapolating doses involves metabolic scaling, whereby the dosage is linked to a physiologic function or anatomic feature, such as basal metabolic rate or body-surface area. This approach is a ratio-based method of calculating doses, not based on body weight, and assumes that any differences in species pharmacokinetics and pharmacodynamics are not clinically relevant. This method has been used in zoological medicine, and as such, simplifies the process by placing animals in one of five Hainsworth energy groups (i.e., passerine birds, non-passerine birds, placental mammals, marsupial mammals or reptiles), with each group having predetermined K-values used to calculate metabolic rates for the selected species. Though this method is relatively simple, and a computer program is available for dosage calculations, it has not been well validated and several articles have illustrated failures with this method of extrapolation.

A third approach for determining doses of drugs is allometric scaling. With this method, pharmacokinetic parameters are measured in multiple species and the data plotted against weight to derive an allometric equation, which can be used to estimate the pharmacokinetic parameter in an unknown species. This method assumes that drug differences are clinically negligible between species, and that drug pharmacokinetics has a nonlinear (allometric) relationship to body weight. It also assumes that pharmacokinetic parameters, as well as dosage, are allometrically scalable. Although there are advantages to using allometric scaling, compared to linear or metabolic scaling, it is not widely used in zoological and aquatic medicine (Hunter and Isaza, 2008). Riviere et al. (1997) reports that 75% of drugs examined are not scalable across multiple species. In elasmobranch medicine, due to the limited numbers of available animals, limitations on handling for adequate pharmacokinetic samples, and in many cases, limited financial resources, “trial and error” still remains the primary method by which the dose, duration and frequency of administration of a drug is determined.

Drug delivery

How a drug can be delivered or administered most effectively is often a major factor driving decisions about chosen therapeutics.

Oral

If a ‘hands-off’ approach is chosen, oral administration via food is the simplest technique for drug delivery if an animal is still actively feeding. There are some special considerations for oral administration via food: some animals may take food but not consume it (rays are notorious for this); some species do not eat routinely or frequently; and more critically, orally administered medications or supplements may not be adequately absorbed (or, conversely, too well absorbed). One of the authors (NDM) has routinely administered fenbendazole to elasmobranchs in a gelatin diet, via voluntary feeding behavior, over several years, with no side effects, yet four animals perished after the same dosage was administered via gavage (Mylniczenko, personal experience). No literature is available on the pharmacokinetics or pharmacodynamics of orally administered fenbendazole in elasmobranchs. In bonnethead sharks, *Sphyrna tiburo* (Linnaeus, 1758), some sedatives and anesthetics (chloral hydrate, haloperidol, midazolam) that were effective when administered intravenously (IV), were not effective, even at doses up to five times greater, when administered orally (PO) in food, suggesting that absorption was profoundly compromised (Clauss, personal experience).

Immersion

A reliable and potentially passive method of drug administration, without concerns about animal compliance, involves a bath or immersion treatment. This technique can be carried out in the primary system (i.e., whole tank treatment) or the animal(s) may be temporarily moved to a smaller container or system. Immersion or bath treatments may be effective in some cases (e.g., praziquantel is ideally administered this way for treatment of monogeneans), but it is unlikely to be effective if systemic absorption is necessary, as marine elasmobranchs drink very little water and gill absorption is not likely as they have tight and relatively impermeable gill epithelia (Ballantyne and Fraser, 2013).

Primary system treatments have some disadvantages, including: exposure of non-target animals; high volumes, and thus large amounts of drug; higher cost; regulations governing discharge of the drug via effluent; and potential effects on system bio-filters.

In many clinical cases, a more direct 'hands-on' approach may be chosen for drug administration, as this better ensures the effectiveness of delivery and therefore the efficacy of the treatment. These approaches also provide an opportunity to better examine the animal up close, but do imply animal capture and restraint. Overall, efficient and effective handling of elasmobranchs is a necessary clinical tool and husbandry teams that condition their animals to restraint and handling greatly facilitate exams and treatments.

Gavage

Delivery of whole food items using tongs, or gavage administration, can be used to deliver oral medications, supplements or balanced diets. These routes may be chosen if an oral formulation is the only available option for a medication, but it may also be chosen if an animal is not eating voluntarily and needs supplemental nutrition as well. There are various types of tubes or devices that may be used for gavage administration, such as red rubber catheters, nasogastric tubes, reinforced vinyl tubing, and even metal gavage/feeding tubes for neonates or small species of elasmobranchs (Figure 1). In some situations, a plunger type feeding system may be used for the delivery of solid food pieces.

Figure 1. Gavage feeding of a blacktip reef shark, *Carcharhinus melanopterus* (Quoy & Gaimard, 1824).

Injection

Injectable medications are likely the most reliable means of achieving systemic therapeutic drug levels in elasmobranchs. Injections can be administered intramuscularly (IM), intravenously (IV) or intracoelomically (ICe), with IM being preferred.

For IM injections in sharks and elongated batoids (e.g., sawfishes, guitarfishes), the injection site is just below the first ridge, off midline, between the 1st and 2nd dorsal fins (Figure 2), where the fibrous saddle is not too thick. With larger animals, longer needles (e.g., 7.5 - 9.0 cm for a 7 m total length animal) should be used. Elasmobranchs possess both red muscle (myotomal) and white muscle ("slow twitch"). Theoretically, red muscle would be the ideal injection target. However, red muscle accounts for only a small percentage of the total body mass and there is considerable interspecific variability in its distribution. Since white muscle is predominant, it is the default target for injection and any clinical judgments have been based on absorption into this muscle type (Figure 3).

IM injections can be administered using manual restraint by hand (with or without anesthesia). IM infections can also be given using a modified pole syringe made from a length of PVC pipe with a plastic needle cap and dart (Dan-Inject of North America, Knoxville, Tennessee 37931, USA) taped to the side of one end. The soft tape keeps the syringe in place, but allows it to break away if necessary. Alternatively, a power-charged "jab

Figure 2. Intramuscular (IM) injection in the dorsal saddle of a largetooth sawfish, *Pristis microdon* (Latham, 1794).

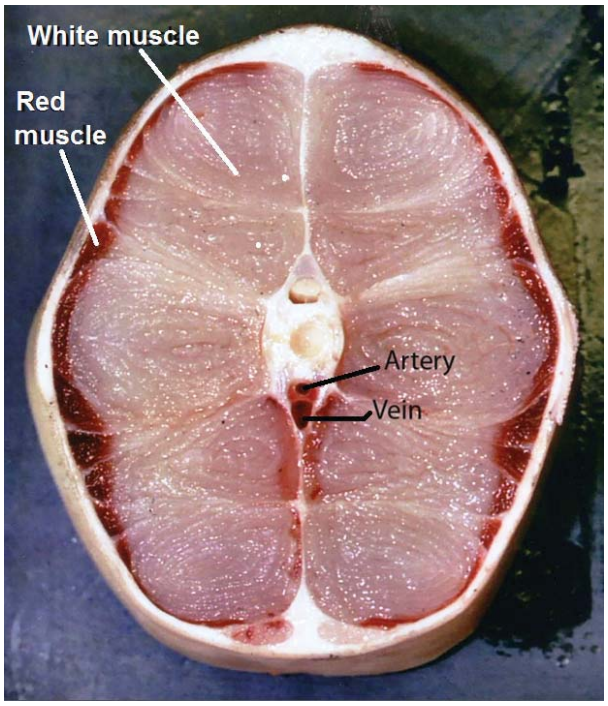

Figure 3. Cross section of the tail of a whitetip reef shark, *Triaenodon obesus* (Rüppell, 1837), showing white muscle, red muscle, the caudal artery and the caudal vein.

stick" can be fashioned from a lightweight pole and Cap-Chur equipment (Palmer Cap-Chur Inc, Powder Springs, GA 30127, USA), modified to accommodate Dan-inject darts. A spear gun modified to hold these darts can also be used to administer IM medications (Runnells, personal communication).

There are a number of challenges to the use of drugs delivered with injection darts:

1. The thickness of elasmobranch skin and the abrasiveness of the denticles can rapidly dull needles;
2. Fish muscle is not elastic and does not retain fluid like mammalian muscle, so drug leakage can occur at the puncture site;
3. Large animals require either large volumes (multiple darts) or concentrated drugs;
4. Injection darts can misfire at depth; and
5. Inaccurate darting can result in injection site variability and failed attempts to inject.

Drug leakage from an injection site is a significant problem for both teleosts and elasmobranchs. In a brief trial performed using Nile tilapia, *Oreochromis niloticus* (Linnaeus, 1758), small volumes of 'drug' (0.9% NaCl mixed with food dye) leaked out while the animal was sedated. Once recovered, if the animal made any muscular exertion, additional fluid leaked out up to several minutes later (Figure 4). The same challenge is faced when injecting elasmobranchs. Long needles, tracked deeply into the muscle, can help reduce drug leakage, as can sealing the injection site with tissue glue or bone wax (CP Medical Inc., Portland, OR, USA) (Figure 5). With remote injection systems, a barbed needle allows *in situ* blockage of the leak and, therefore, increased absorption time. However, retrieval of the barbed needle can be a challenge.

Some drugs, like enrofloxacin, can cause reactions at the injection site when administered IM in lower vertebrates. Consideration should be given to

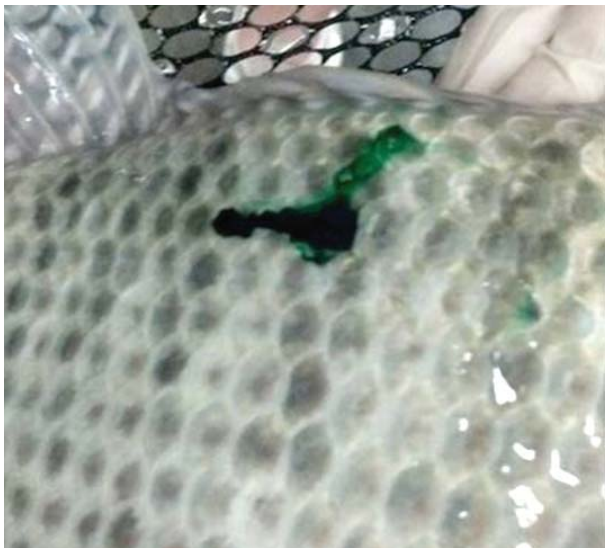

Figure 4. An example of leakage (dark green dye), from an injection site after injection, gentle massage and withdrawal of pressure.

Figure 5. Sealing an injection site on a whale shark, *Rhincodon typus* (Smith, 1828), using bone wax, to prevent leakage of drug post injection.

diluting enrofloxacin, and similar drugs, or giving them by routes other than IM.

IV administration is occasionally desired, but anatomy must be kept in mind if using caudal vessels (Figure 3). When using the tail, arterial administration is possible but is contraindicated with some drugs. Typical locations for vascular access in sharks include the caudal vessels (Figure 6), the dorsal sinus (Figure 7), dorsal cutaneous veins, and the dorsal and ventral precaudal pit or cutaneous veins. Intrasinus administration of medications and fluids is a convenient and preferred location when compared to most other vascular access points. In rays, the caudal vessels (Figure 8), and vessels within the wings (Figure 9) are standard locations for the collection of blood. The wing vessels can be used for administration of medications or fluids if an IV therapeutic is warranted. Detailed instructions on locating these vessels are described elsewhere (Mylniczenko et al., 2014).

Figure 6. Ventral caudal vessel blood sampling in a blacktip shark, *Carcharhinus limbatus* (Müller & Henle, 1839).

Figure 7. Dorsal sinus blood sampling in a blacknose shark, *Carcharhinus acronotus* (Poey, 1860).

ICe administration of medications may occur via needle trans-cutaneously. This mode of delivery is accomplished by inserting a needle at an acute angle, directed toward the head of the animal, near the pelvic girdle on the right side of the abdomen. Ultrasonography may aid the process. ICe medications and fluids may also be delivered via flexible tubing advanced into the abdominal pore. ICe delivery requires that drugs are absorbable through the serosal membranes of the coelomic cavity organs. When delivering ICe medications, particularly those of low pH, they must be administered with fluids.

Behavioral conditioning and training can be a valuable tool to aid the administration of medications. When animals are behaviorally conditioned to swim into smaller spaces, or even

Figure 8. Ventral caudal vessel blood sampling in a southern stingray, *Dasyatis americana* (Hildebrand & Schroeder, 1928).

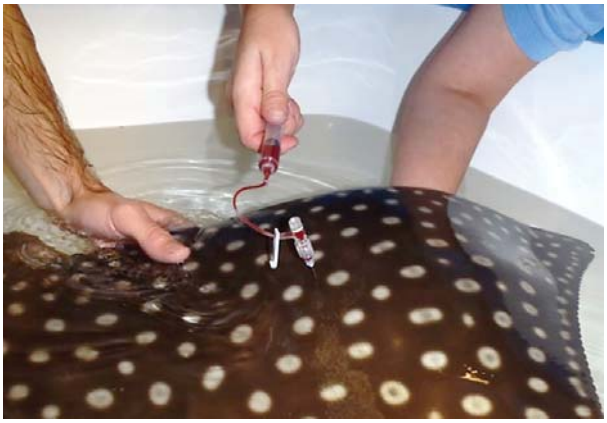

Figure 9. Wing vessel blood sampling in a spotted eagle ray, *Aetobatus narinari* (Euphrasen, 1790).

into a net or stretcher, shifting them to treatment containers is much easier. It is even possible to condition animals to station and directly accept injections or other treatments (e.g., oral medications).

Other forms of drug delivery

Other routes of drug administration include topical, intrathecal or subconjuctival, gill flush or irrigation, and percloacal-colonic via suppository or lavage (Figure 10).

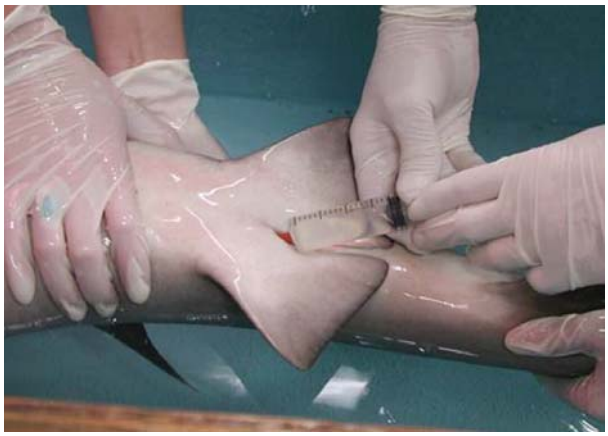

Figure 10. Colonic administration of saline for fecal collection; the same process can be used for administration of medication.

Topical administration of some medications can be accomplished by the immersion method, but in a more traditional sense it is the direct application of a therapeutic to the affected area. Topical administration is a highly effective treatment strategy, particularly for traumatic wounds. Treatments can involve solutions, sprays, ointments, creams or gels. Additionally, these products can be mixed with, or covered with, a variety of waterproofing products. In general, the authors recommend against the use of topical iodinated products or alcohol. Table 1 presents a list of topical products that the authors have found to be useful.

Antibiotic gels can be purchased or compounded at pharmacies. These products change phase (liquid to solid) when placed within a wound. Plaster of Paris absorbable beads (or non-absorbable methyl methacrolate beads) can be impregnated with antibiotics and placed within infected wounds. Cellular wound enhancing products can also be used, which encourage growth of fibroblasts, endothelial cells, and epidermal cells. For example, becaplermin (Regranex®, Smith & Nephew, Inc., Andover, Massachusetts 01810, USA) is a recombinant platelet-derived growth factor, which facilitates endothelial proliferation and migration, and mediates angiogenesis. Other products, like an extracellular matrix such as BioSisT® (Smiths Medical, Dublin, Ohio, H 43017, USA) or MatriStem® (A-Cell®, Columbia, Maryland, 21046, USA), tacked into wounds, can facilitate

Table 1. Topical medications successfully used on elasmobranchs.

Type	Product	Manufacturer
Solution	Tricide	Molecular Therapeutics, LLC. Athens, Georgia 30602, USA
	Opthalmic solutions as topical wound agents	Various manufacturers
	Saline; dilute disinfectants (chlorhexidine)	Various manufacturers
Ointments, gels and cream	Silver sulfadiazine	The Kendall Co., Mansfield, Massachusetts 02048, USA
	Doxirobe	Zoetis, Florham Park, New Jersey 07932, USA
	Clindamycin gel	Taylor's Pharmacy, Winter Park, Florida 32789, USA
	Collasate silver	PRN Pharmaceuticals, Pensacola, Florida 32514, USA
Spray on	Aluspray	Neogen Corporation, Lexington, Kentucky 40511, USA
	Durafilm (Gentocin, betamethasone)	Bayer, Pittsburgh, Pennsylvania 15205-9741, USA
Waterproof barriers and protectants	Ilex	Medcon Biolab Technologies, Inc., Grafton, Massachusetts 01519-0196, USA
	Orabase	Colgate Oral Pharmaceuticals, Inc., http://www.colgate.com
	Fixodent	Procter and Gamble, Inc. http://us.pg.com
	New Skin	Prestige Brands, Tarrytown, New York 10591, USA
	Misoprostol 0.0024% and Phenytoin 2% powder	Taylor's Pharmacy, Winter Park, Florida 32789, USA
Wound healing promoters	Becaplermin (Regranex)	Smith and Nephew, Inc., http://www.smith-nephew.com
	Acemannan (Carravet)	Carravet, Palmetto, Georgia 30268, USA
Osmotics	Medihoney	Derma Sciences, Princeton, New Jersey 08540, USA
	Maltodextrin powder	DeRoyal Medical, Inc. http://www.deroyal.com
Hemostatics	Bleed X Vet	Bleed-X, 7667 Cahill Road, #100, Edina, Minnesota 55439, USA
	ActCel hemostatic gauze	Coreva Health Science, LLC; Westlake Village, California 91362, USA
Extracellular matrices	A-Cell	ACell Inc., Columbia, Maryland 21046, USA
	BioSisT	Smiths Medical, Dublin, Ohio 43017, USA

epithelialization of large open tissue and have been used successfully in aquatic animals (Mylniczenko and Travis, 2005; Clauss, personal experience).

If topical delivery alone is not sufficient, then bandaging can be employed to augment treatment, although it can be complicated to bandage aquatic animals. A variety of materials can be affixed to the affected area with suture. This kind of bandaging provides a physical protective cover, as well as allowing increased contact time with wound-healing enhancement agents that can be applied underneath. Fontenot and Neiffer (2004) provide numerous ideas for wound management and although they focus on teleosts, the techniques can easily be applied to elasmobranchs.

Intrathecal or subconjuctival injections of drugs are usually a volume-limiting focal treatment in, or around, the eye. This form of treatment is useful for ocular abrasions, gas bubble accumulation or similarly focused lesions.

Gill flushes can be performed for the administration of anesthetic drugs or other compounds. One institution used praziquantel gill flushes to treat monogeneans (Croft, personal communication). In some cases, where an elasmobranch patient has become apneic, doxapram can be suffused over the gills.

An underused, but viable, access point for drug administration is percloacal. Colonic administration of a suppository or lavage in highly absorbent mucosa holds great promise as a treatment mode. However, no literature or anecdotal information is available on percloacal medication. One of the authors (NDM) has used this mode of treatment for the administration of sucralfate (Mylniczenko, personal communication). Similarly, intrauterine access is possible percloacally. If this techniqe is employed, consider possible topical effects on the mucosa or trophonemata. One of the authors has used weekly, and then bimonthly, intrauterine flushes with tricide solution (Tris/EDTA, Rood and Riddle Veterinary Pharmacy, Lexington, Kentucky, 40511, USA) and enrofloxacin after a dystocia trauma, resulting in adhesions with good success (Clauss, personal communication).

ANTIBIOTICS, ANALGESICS AND SUPPORTIVE CARE

Much of the published information on supportive care in elasmobranchs can be found within recent published medical case reports. While information on supportive care does not represent the focus of these studies, data on drugs and dosages is frequently reported (refer *Elasmobranch formulary*; www1).

Antibiotics

In one prospective study, florfenicol levels after IM injection were assessed in blood and cerebrospinal fluid (CSF) of whitespotted bamboosharks, *Chiloscyllium plagiosum* (Bennett, 1830). This was a landmark paper, identifying that the drug accumulated in the CSF and that its effects lasted minimally 102 hours in the blood and 72 hours in the CSF (Zimmerman et al., 2006). Comparatively, cefovecin, another long-acting drug, was administered to adult *C. plagiosum* with a single dose at 8 mg/kg subcutaneous (SC). The terminal half-life of the cefovecin was 2.02 ± 4.62 hours, suggesting an elimination time within 24 hours, much lower than the expected seven days (Steeil et al., 2014). Trovafloxacin was trialed orally (PO) in the little skate, *Raja erinacea* (Mitchill, 1825), at 10 mg/kg and 100 mg/kg, with repeat dosing every 24 hours for 3 doses. Therapeutic levels were attained and drug levels continued to rise after 144 hours (Willens et al., 1999). When joints were histologically assessed, no arthropathies in adult animals were observed. With the increased use of long acting drugs, many necrotic or granulomatous reactions are being seen.

Concern has arisen over the use of sulfa drugs in some species of elasmobranchs. In one case, juvenile *S. tiburo* were administered sulfamethoxazole and trimethoprim as an immersion bath, at a standard fish dose of 25 mg/L, with 95% mortality within 16 hours from the start of the treatment (Young and Bernal, personal communication). At two separate facilities cownose rays, *Rhinoptera bonasus* (Mitchill, 1815), were treated for coccidia using sulfadimethoxine at 50 mg/kg ICe every 24 hours for 7 days, followed by a week of no treatment, then a repeat treatment for an additional 7 days. During the second set of treatments, the animals exhibited severe ulcerative wingtip dermatitis with varying degrees of severity, the most severe reaction involved exposed muscle. The animals became anorectic and succumbed thereafter. Histopathology did not reveal a definitive cause, but the timeline and appearance of the lesions were consistent with a reaction to the drug (Clarke, personal communication).

Analgesics

Elasmobranchs have opioid receptors and other indicators of pain perception (i.e., DNA sequences consistent with pain perception, the presence of cyclooxygenase). However, some axonal layers are not present in the spinal cords of certain elasmobranch species, suggestive that they may not 'recognize' pain (Davis et al., 2006). Clinically, it is understood that elasmobranchs often respond negatively to noxious stimuli (like injections). Therefore, it is important to err on the side of caution and assume analgesia is important. On a similar note, there is subjective evidence that corticosteroids have positive effects during, or following, stressful events such as handling (with or without anesthesia), trauma or transport. Corticosteroids may have both analgesic and anti-inflammatory effects (Clauss, personal experience).

Supportive care

Supportive care is a complicated endeavor that weighs the benefits of outcome (nutritional supplementation, medication administration, wound care management) versus the risks implied by the 'support' (stress, handling and trauma). The goals of supportive care are primarily to provide the animal with physiological and biological assistance that they cannot provide to themselves under their present environmental circumstances. One example of supportive care is the direct manipulation of the environment—e.g., altering the salinity to manage hydration or electrolyte loss. Hydration can also be managed by the administration of freshwater, orally via gavage, by IV (7 - 10 mL/kg), or ICe administration of elasmobranch-tailored fluids (Table 2).

Nutritional support

Metabolic needs of elasmobranchs increase, and food intake and the possibility of nutritional support should be an utmost concern, during illness, healing or immediately following transport. Frequency of assisted feeding and items fed is dependent on the needs of the animal and the capacities of the animal care staff. Feeding whole prey items to the elasmobranch is sufficient and preferred if digestion is functioning properly. This approach minimizes the opportunity for regurgitation and is more natural. Using long, blunt forceps, food items can be inserted into the stomach of the animal. If squid (order: Teuthida) are used as food, removing the 'pen' is recommended as poor digestion can cause them to accumulate and impact the digestive tract. Solid pieces of food can be delivered into the stomach via a food-filled tube and plunger. Alternatively, food gruel can be blended and administered via a gavage tube; thicker gruels are preferred, but require a larger bore tube. The gruel may consist of normal diet pulverized or blended to a semi-liquid consistency, or it may be augmented with powdered or gel diets. When using premade gavage formulations avoid complex carbohydrates, as they are not easily digestible.

Frequency of assisted feeding will depend on animal body condition, liver health and tolerance of the animal to manual handling and/or anesthesia. An animal will voluntarily commence eating, while on a regime of assisted-feeding, once its condition starts to improve. If necessary, daily or twice daily gavaging can be tolerated for weeks to months, depending on the species in question. It is anticipated that within this time any nutritional crisis will have been averted, but it should be noted that animals tolerate handling stress well and nutritional support should take precedence over concerns of 'stressing' animals, unless there are profound contraindications to repreated restraint and handling.

ANTIPARASITICS

Praziquantel

Praziquantel has gained a lot of attention in recent years as many aquaria use the drug to manage parasitic flatworms (class: Monogenea) in mixed species exhibits. Praziquantel is less aggressive than many alternatives, but is not always completely effective against tenacious monogeneans. Spotted eagle rays, *Aetobatus narinari* (Euphrasen, 1790), are an elasmobranch species of particular interest, as they are frequently infested with difficult to eradicate species-specific monogeneans. Praziquantel is a favored treatment as it paralyzes the adult parasite and facilitates their removal from *A. narinari*. Praziquantel treatments largely involve immersion at varying ranges (refer to *Elasmobranch formulary*; www1). One author (NDM) recommends a treatment protocol of alternating praziquantel immersion and trichlorfon immersion as follows: dose 4 - 6 mg/L praziquantel for three days, wait 5 - 7 days, then dose 0.25 mg/L trichlorfon for six hours, then wait an additional seven days. Repeat the treatment regime for a total duration of one month. For this treatment protocol (particularly when treating *A. narinari*) animals should be pre-medicated with atropine prior to trichlorfon dosing, as severe consequences (e.g., death) have been observed with this drug (refer to *Elasmobranch formulary*; www1).

Table 2. Examples of fluid formulations used for elasmobranch hydration.

Fluid recipe 1
Extract fluids from 1 L bag of 0.9% NaCl and mix with 40 mEq (mg) sodium acetate. Reinject through 0.2 micron syringe filter **or** 1 mEq/kg diluted 1:10 with standard fluids.

Fluid recipe 2: Shark Ringer's Solution
Measured in g/L pure water (or 0.9% NaCl)

Sodium chloride (NaCl)	16.35 g/L
Potassium chloride (KCl)	0.45 g/L
Calcium chloride ($CaCl_2$-$2H_2O$)	0.74 g/L
Magnesium chloride ($MgCl_2$-$6H_2O$)	0.61 g/L
Sodium sulfate (Na_2SO_4)	0.07 g/L
Sodium monophosphate (NaH_2PO_4)	0.12 g/L
Sodium bicarbonate ($NaHCO_3$)	0.67 g/L
Urea	21.00 g/L
Trimethylamine oxide (TMAO-$2H_2O$)	7.99 g/L
Glucose	0.90 g/L

A filter sterilized stock solution can be mixed ahead of time, minus the urea and TMAO. These ingredients must be in solution and filter sterilized, and should be mixed prior to administering. Discard after 24 h.

Fluid recipe 3: Shark Ringer's Solution
Hanks Balanced Salt Solution (1000 mL)
Add 8 grams of NaCl crystals
Add 21 grams of urea

These ingredients must be in solution and filter sterilized, and should be mixed prior to administering. Discard after 24 h.

Fluid recipe 4: Shark Ringer's Solution
Measured in g/L pure water (or 0.9% NaCl)

NaCl	9.00 g/L
Urea	22.00 g/L

In one study, *A. narinari* were given 10 - 40 mg/kg praziquantel PO with no effect, while treatments by immersion at 20 mg/L for 45 - 90 min showed good results. One of the authors (NDM) observed dose responsive effects in *A. narinari*, manifested as agitation and increased respiration, when they were given an immersion treatment of praziquantel at the higher end of the treatment range (20 mg/L). Observed responses were serious enough to compel staff to remove animals from the treatment prematurely. At the same institution, praziquantel was administered at 100 mg/kg PO (derived from Vaughan and Bye, 2012), via gavage, with significant decreases in monogenean levels when compared to results from immersion treatments (Mylniczenko et al., 2015).

Vaughan and Bye (2012) dosed a group of Japanese meagre, *Argyrosomus japonicus* (Temminck & Schlegel, 1843), with 20 mg/L praziquantel for 12 h, and another group of *A. japonicus* with an oral gavage of 150 mg/kg praziquantel tablets. The teleosts given the gavage treatment exhibited a superior recovery. The researchers posited that first pass metabolism was efficient and rapid, resulting in the

observed success, but also noted that the parasites feed on blood and get direct exposure to the drug. Immersion treatments may not always be successful when parasitic organisms 'hide' or are embedded in tissues and have no direct contact with the drug. In short-tail stingrays, *Dasyatis brevicaudata* (Hutton, 1875), a 150 mg/kg PO dose was found to be highly effective against hexabothrid monogeneans (Cole, personal communication). Although toxic levels are not known, no negative side effects were observed.

Praziquantel, trichlorfon and copper sulfate have been used to treat a monogenean, *Benedeniella posterocolpa*, in *R. bonasus* (Thoney, 1990). Praziquantel was very effective at 20 mg/L, while trichlorfon was found to be less effective, even with two treatments. *In vitro* praziquantel killed the flatworms and prevented egg deposition, while *in vitro* copper sulfate had little effect on the parasites.

Alternative parasite treatments

In the upper extreme for copper exposure, one study noted that *S. canicula* had median lethal concentrations at 24 h and 48 h when exposed to 16 mg/L and 4 mg/L copper, respectively. It was further observed that copper was 15 - 20 times more toxic than zinc (toxic at 180 and 80 mg/L at 24 h and 48 h, respectively). In a separate study using *S. acanthias*, copper exerted a toxic effect on Na+/K+-ATPase activities, as well as urea retention capacities, with a 96 h LC50 at 800–1000 μg/L. Despite the general concern of using copper with elasmobranchs, multiple clinicians have used copper, some with great success and others with adverse effects (refer to *Elasmobranch formulary*; www1).

Serial treatment with levamisole at 10 mg/kg IM cleared *Huffmanela* sp. egg tracks from a sandbar shark, *Carcharhinus plumbeus* (Nardo, 1827), within 21 days. No recurrences of the parasite, or apparent complications, were observed (MacLean et al., 2006).

Sea louse, *Lepeophtheirus acutus*, infestations in a variety of species have been managed using both trichlorfon and diflubenzuron (Kik et al., 2011). The efficacy of diflubenzuron is dependent on the development stage of the parasites and their capacity to build chitin. It will not be effective on attached adults and will only work on organisms during chitin formation phases.

The piscicolid marine leech, *Branchellion torpedinis*, is a notorious challenge, as they can conceal themselves on elasmobranchs and they are highly resistant to most treatments. Trichlorfon appears to be the only effective technique for eradication, and has been used on *R. bonasus*, *A. narinari*, *Pristis* spp., *S. fasciatum,* southern stingrays, *Dasyatis americana* (Hildebrand & Schroeder, 1928), and other elasmobranchs and teleosts as a full system treatment (Clauss, personal experience).

As an adjunct to using chemotherapeutic agents to manage external parasites, a treatment option to consider is the concurrent use of natural biological control agents—e.g., the bluestreak cleaner wrasse, *Labroides dimidiatus* (Valenciennes, 1839) (Janse and Borgsteede, 2003). Other animals that can be considered include butterflyfishes (family: Chaetodontidae) and nudibranchs (class: Gastropoda; clade: Nudipleura). One of the authors successfully eradicated *Neobenedenia* sp. from ribbontail stingrays *Taeniura lymma* (Forsskål, 1775), and blue-spotted stingrays, *Neotrygon kuhlii* (Müller and Henle, 1841), using *Labroides* spp. and pulsatile treatments of praziquantel with diflubenzuron.

OTHER TREATMENTS

Goiter continues to be a problem in aquarium elasmobranchs, despite an understanding of the precursors. Outside of nutritional deficiencies, two environmental conditions have been shown to predispose animals to this condition: high concentrations of nitrates and ozone treatment of system water (Morris et al., 2011). Current recommendation for iodine supplementation of the water is 0.01 - 0.02 mg/L (in the absence of ozone appluication) and for dietary supplementation is 10 - 30 mg/kg/week dry diet.

In one case, lesser devil rays, *Mobula hypostoma* (Bancroft, 1831), accidently received iodine-soaked krill at every feed, versus the intended once daily supplementation, with the krill soaking for an entire day in the iodine solution. The animals did not show overt effects from the overdose, however, their serum changed to a color consistent with the color of iodine. Once the iodine was removed from the diet, serum color progressively returned to normal (Fontenot, personal communication).

SIDE EFFECTS

There is the potential for side effects with nearly all medications or supplements, even in well-studied animal models. With so little information regarding pharmacokinetics or pharmacodynamics in elasmobranchs, one must be aware of the potential risk of side effects, including individual hypersensitivity. These effects can range from a focal reaction to anaphylaxis and, in some cases, death. Most of the time, we assume those risks, and also assume that the benefit of the treatment will outweigh potential negative consequences. Elasmobranchs are a notoriously 'delicate' or 'sensitive' group of animals, however, one must separate out other proximate causes as contributory when evaluating negative reactions. Overdosage is always a possibility, but without knowledge of actual effective dosages, it is hard to predict when a drug may be toxic, or at the least, not appropriate for the species.

There are many examples of adverse effects of pharmacotherapeutics applied to elasmobranchs (refer to *Elasmobranch formulary*; www1). Some of these examples include: (1) osmolality shifts and sudden death in stenohaline species when placed in a different salinity; (2) reaction or focal irritation to topical medication (Figure 11); (3) reaction or focal irritation to IV medication—e.g., chloral hydrate in *S. tiburo* and haloperidol lactate in *S. tiburo* (Figure 12); (4) injection site abscess reaction to IM administration of enrofloxacin in *R. bonasus*, also observed in *D. americana* (Thompson, personal communication); (5) over-supplementation of iodine-soaked krill resulting in a change of serum color post-exposure in an otherwise clinically normal *M. Hypostoma* (Fontenot, personal communication); and (6) over-supplementation of IM iron in an *R. bonasus* resulted in elevated blood iron levels, and, although no immediate negative effects were noted, long term effects remain unknown (hemosiderosis and hemochromatosis are undocumented in elasmobranchs).

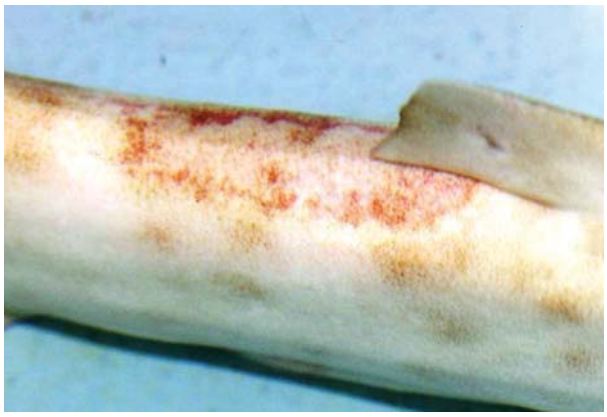

Figure 11. Dermatitis, secondary to light scrubbing with alcohol, in a zebra shark, *Stegostoma fasciatum* (Hermann, 1783).

Figure 12. Focal reaction, seven days post treatment, to the administration of intravenous (IV) haloperidol lactate in a bonnethead shark, *Sphyrna tiburo* (Linnaeus, 1758).

CONCLUSIONS

Elasmobranchs, though still somewhat enigmatic, continue to gain popularity in aquaria as charismatic educational display and research animals. With advancements in husbandry and health care, facilities are becoming more adept at maintaining these animals. Increased expectations for a high quality of medical care has necessitated that more veterinarians become involved in the clinical management of elasmobranchs. Correspondingly, the scope of managing elasmobranch cases with modern veterinary technology and medicines has increased. Consistent with other exotic wildlife and zoo species, relatively few pharmacological studies have been carried out with elasmobranchs. This chapter provides significant information on drugs, supplements and chemicals that have been administered to elasmobranchs, and highlights the resulting successes and challenges.

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INTERNET RESOURCES

www1 www.elasmobranchhusbandry.org

The Elasmobranch Husbandry Manual II: Recent Advances in the Care of Sharks, Rays and their Relatives, pages 303-324.

Chapter 30

Diagnostic imaging of elasmobranchs: updates and case examples

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Abstract: Diagnostic imaging of elasmobranchs allows veterinarians to better evaluate internal structures during routine health and reproductive examinations, and allows for a more thorough evaluation of underlying disease processes. Radiography is helpful in examining skeletal anatomy, while ultrasonography is more useful in evaluating soft tissue organs. Endoscopy, computerized tomography and magnetic resonance imaging are more advanced tools that can be used in cases where additional information is required to make a diagnosis or to evaluate progression of a disease process.

INTRODUCTION

This chapter is an addendum to Stetter (2004), offering updated literature and presenting select cases that have benefited from the use of different imaging techniques.

RADIOGRAPHY

The first 'go to' imaging modality for most circumstances is radiography. However, when working with elasmobranchs, it has limited capacity for a good return on investment of time and effort. The low density of internal structures (i.e., lack of bone and diffuse coelomic fat) result in a lack of contrast and limits the value of this imaging modality with elasmobranchs, when compared to other animal species. Radiography does, however, provide appreciable detail on cartilaginous structures, allowing for evaluation of fractures, proliferation, malformation or lysis. In cases where ultrasonography or more advanced imaging may not be available, the use of agents such as barium or iohexol can provide contrast and allow for the evaluation of the size and location of certain organs.

The strength of radiography as a tool during quarantine or for physical diagnostics when an animal comes into the institution is unquestionable, aiding evaluating for hooks or similar remnants from capture. Hook trauma may also be identifiable by evaluation of symmetry of jaw cartilaginous structures. In species like the sand tiger shark,

Carcharias taurus (Rafinesque, 1810), radiography is an instrumental tool for evaluating baseline spinal anatomy. Spinal anatomy can be tracked thereafter to evaluate changes that may be associated with scoliosis, a challenge more common in this species (Berzins et al., 1998; Berzins and Walsh, 2000). Portable digital radiography allows for convenient and rapid evaluation as well as the ability to modify imaging technique or position. Two views of a structure are standard for appropriate interpretation, however, size and shape of the animal may prohibit this option (e.g., dorso-ventrally compressed batoids). Additionally, some clinicians use radiography for evaluation of post-mortem specimens, both in finalizing a case, as well as for educational purposes (Boylan, personal communication).

Details on how to perform a radiographic exam are covered by Stetter (2004). While not specifically focused on elasmobranchs, Love and Lewbart (1997) present a document on radiography in fishes, which provides a good source for managing aquatic animals while using radiography equipment.

Contrast radiography

Contrast media can be used to improve the diagnostic quality of radiographs. This technique works by creating a situation where the contrast enters a structure and provides differing radiodensity, separate from surrounding tissues. Administration of contrast can occur intraluminally (oral, cloacal, colonic, intrauterine) or intravenously (intra-arterial, intrathecal).

There are two categories of contrast radiography: negative contrast and positive contrast. Negative contrast uses gasses, which are not radio-dense, such as air, carbon dioxide and nitrous oxide. Positive contrast involves the administration of radio-dense materials, such as barium sulfate or iodinated products. Barium is typically administered intraluminally and is not used for vascular application or if there is concern of gastrointestinal perforation. Iodinated compounds can be used vascularly. Positive contrast materials are typically developed for mammalian osmolarity, thus considerations need to be made for the physiologic responses of elasmobranchs to these products.

A contrast radiograph of an adult blacknose shark, *Carcharhinus acronotus* (Poey, 1860), reveals a large blood vessel starting at the first gill arch and running parallel to the mandible (Figure 13). It is posited that circle hooks often cause bleeding due to trauma to this vessel (Boylan, personal communication). From a research perspective, Alexander (1991) used contrast radiography in a variety of elasmobranchs (post-mortem) to successfully map whole-body major vessels.

When administered orally, the migration of contrast material at timed intervals can assist in the assessment of gut functionality, assuming there are baseline data to reference. Most elasmobranch species have rapid gut transit times of ~1 - 3 h, so that studies may be performed during the same anesthetic or handling event. If an elasmobranch is administered oral contrast by gavage, the material can be prematurely pushed into the distal gastrointestinal tract, shortening gut transit time even further.

In certain circumstances, a perforation can be identified via leakage of contrast into the coelomic cavity. When evaluating a suspected perforation, less tissue-reactive contrast material is preferable (e.g., iodinated compounds). Additionally a food dye can be gavaged, followed by collection of coelomic fluid to verify passage of dye across the gastrointestinal mucosa.

Select cases

A number of case histories where radiography was employed to aid diagnosticians have been detailed in Figures 1-13.

ULTRASONOGRAPHY

Where radiography is limited by elasmobranch anatomy, ultrasonography excels. While radiology can provide excellent images for evaluating skeletal anatomy, ultrasonography (real time, B mode) is most helpful in evaluating soft tissue structures. Probes have been used without gel, or protected in a plastic bag, with gel inside as a contact media, to minimize concerns about saltwater exposure and abrasions from denticles. The probe should always be orientated (i.e., the slit on the probe) to the front of the animal, or to the right, as a standard of practice. It is ideal to get both transverse and sagittal views of an organ. Transducer sizes will vary depending on the target organ and the depth of that organ in the body. If the clinician has ready access to an ultrasound unit, regular use during routine exams is strongly encouraged. Walsh et. al (1993) documented a detailed review of ultrasound in three shark species.

A stepwise approach to ultrasound assessment of an animal can include the following:

1. *Heart*: examination of heart contractility, rhythm and rate. Ventilatory rate (e.g, gill slit movement, spiracle movement or 'gilling rate') often matches cardiac rhythm, particularly in stingrays (order: Myliobatiformes). Some pericardial fluid is normal, notable mostly in the systolic phase. Echocardiograms have been performed in the brown smoothhound, *Mustelus henlei* (Gill, 1863); horn shark, *Heterodontus francisci* (Girard, 1855); swellshark, *Cephaloscyllium ventriosum* (Garman, 1880); shortfin mako, *Isurus oxyrinchus* (Rafinesque, 1810); and the blue shark, *Prionace glauca* (Linnaeus, 1758) (Chin Lai et al., 2004).

2. *Thyroid*: baseline evaluation of the thyroid, followed by tracking thyroid size over time. Some limited published data of thyroid evaluation is available (Crow et al., 1998). Anatomical location and appearance of the thyroid varies by species.

3. *Gastrointestinal tract*: ultrasonography of the esophagus, cardiac stomach, pyloric stomach, duodenum, spiral valve and the rectal gland. Note that there are four different types of spiral valves in elasmobranchs (Hamlett and Koob, 1999) and that the rectal gland is difficult to image.

4. *Liver, gallbladder, spleen, and pancreas:* the echotexture and size of the liver are excellent indicators of general nutritional status of elasmobranchs. If lipid stores become depleted, the echogenicity of the liver will decrease and appear similar in echogenicity to the spleen (Grant et al., 2012; Mylniczenko, 2012). The gallbladder is anechoic (black) compared to the liver. A recent paper established formal guidelines for assessing liver to coelom ratio, specifically using southern stingrays, *Dasyatis americana* (Hildebrand & Schroeder, 1928), under managed care, in comparison to recently acquired specimens (Grant et al., 2012). The authors measured the distance between the caudal margin of the liver and cartilaginous pelvic girdle, which provided some measurable data to evaluate the nutritional condition of the animals. Note that the pancreas is difficult to image, but possible with the correct probe and machine.

5. *Urogenital system (kidneys)*: the kidneys are located in the caudal coelom, bilaterally against the dorsal body wall and ventral to a prominent muscle body.

6. *Reproductive system*: the anatomy of reproductive systems is highly variable, necessitating species-specific familiarization of organ location and symmetry. Elasmobranchs use every mode of reproduction: oviparity, aplacental yolk sac viviparity, aplacental viviparity with uterine villi or trophonemata, aplacental viviparity with oophagy and (with or without) intrauterine cannibalism, and placental viviparity (Hamlett and Koob,1999). The epigonal organ can be identified, however, it becomes more difficult to identify in females with active ovaries, and easier to identify in reproductively active males. Pregnancy detection is a common use for ultrasonography. Some reproductive ultrasound techniques have been described for spotted wobbegong, *Orectolobus maculatus* (Bonnaterre, 1788) (Otway and Ellis, 2011); broadnose sevengill sharks, *Notorynchus cepedianus* (Péron, 1807) (Daly et al., 2007); nurse sharks, *Ginglymostoma cirratum* (Bonnaterre, 1788); lemon sharks, *Negaprion brevirostris* (Poey, 1868); bonnethead, *Sphyrna tiburo* (Linnaeus, 1758), (Carrier et al., 2003); and "various oviparous" elasmobranchs (Whittamore et al., 2010). The size of fetuses may vary, depending on timing of fertilization, oophagy or cannibalism. A review of ultrasound in pregnant ribbontail stingrays, *Taeniura lymma* (Forsskål, 1775) is detailed by Pereira et al. (this volume). Examination of egg cases with ultrasound allows for a rapid evaluation of viability. Establishing normal reproductive cycle parameters will lead to artificial insemination opportunities, which is of high interest to aquaria.

7. *Coelom*: there is normally a small amount of coelomic fluid present, but it is typically limited to the caudal end in a normal, dorsally recumbent animal. A transesophageal technique has been described (Stetter, 2004) and may have utility in larger animals; however, shark teeth readily damage the cable of the probe if caution is not exercised.

Select cases

A number of case histories where ultrasonography was employed to aid diagnosticians have been detailed in Figures 14 – 24.

A companion document to this chapter, Stingray Anatomy and Ultrasound, is available for download on the Elasmobranch Husbandry website (www1).

THERMOGRAPHY

Thermography is a diagnostic tool that can evaluate surface temperature change (as with inflammation) in mammalian species. However, in aquatic animals usefulness appears limited, either because

there is no significant heat response with inflammation, or because the poikilothermic nature of the animal assimilates environmental temperature, exclusive of any inflammatory change. A comparison of a dolphin, *Tursiops truncatus* (Montagu, 1821), and a cownose ray, *Rhinoptera bonasus* (Mitchill, 1815), following evidence of trauma in both cases, is provided (Figure 25). In the thermograph of the *R. bonasus* the silhouette of the animal cannot be identified, but the aquarist holding the animal can be readily seen.

ENDOSCOPY

Endoscopy using either flexible or rigid scopes can be used to evaluate intraluminal or coelomic structures, and can provide ports for biopsies when indicated. Flexible endoscopy is minimally invasive and can be used to access the oral cavity, esophagus, stomach and, occasionally, duodenum. Ulcers have been observed in the duodenal area in a shark, using an endoscope, by one of the authors. Cloacally, endoscopy can access the colon or the uterus in females. For examination of the colon the approach is on the right side of the animal and gentle pressure will allow access into the spiral valve. Uterine endoscopy can be accomplished by palpation of the cervix, or alternatively, passage of a soft rubber tube/catheter followed by the scope. Normal uterine anatomy shows complete coverage of the walls with soft pink trophonemata that flow easily and individually. Insufflation with elasmobranch Ringer solution might be necessary to aid uterine examinations. If there is histotroph in the uterus, visualization might be obscured as it can be an opaque fluid. In some species, yolk or remnants of ova might be present in the uterine cavity. These remnants can be naturally expelled by the animal in a normal state. In one article on reproductive assessment of *G. cirratum*, ultrasonography coupled with endoscopy was used to visualize embryos and stage gestation time (Carrier et al., 2003). While this technique provided excellent visualization of the embryos, there were some concerns over the loss of fetuses in the weeks following the procedure, potentially attributed to endoscopy or handling (Carrier et al., 2003).

Rigid endoscopy may be used for gill or oral examinations, and can be advanced into the abdominal (coelomic) pores to view the internal organs. Some concerns have been raised anecdotally about the functionality of the abdominal (coelomic) pore, and whether use of this structure as an entry port should raise safety concerns. However, no untoward side effects have been observed by these authors despite numerous uses of this port of entry. Biopsy of the liver is facilitated via the abdominal pore. The most common use of the rigid scope is for assessment of the coelom via a small incision. In this case, for most species of sharks, a paramedian approach just cranial to the vent is commonly used. The reader is referred to the article by Murray (2010) on endoscopy in sharks for more detailed information on this technique.

Endoscopic evaluation of the gills is simple. Elasmobranch gills have a thick membrane and prominent arches.

Select cases

A number of case histories where endoscopy was employed to aid diagnosticians have been detailed in Figures 26 - 39.

ADVANCED IMAGING

When other imaging modalities cannot provide adequate information, then computerized tomography (CT) and magnetic resonance imaging (MRI) can be used to evaluate more specific lesions or anatomic areas. The logistics and equipment needed to perform advanced imaging in sharks and rays are much more challenging than for terrestrial species. Generally, animals must be transported to external facilities, where advanced imaging can be performed. Considerations for the size and shape of the transport container, along with the ability to keep the animal well-oxygenated and anesthetized in water during the procedure, are all factors that should be considered and planned well in advance. It should be noted that water poses a risk to the imaging equipment. Movement of the water or animal ventilation/respiration can cause artifacts in the final image. For this reason, most advanced images are taken post-mortem. An MRI examination of a live whitetip reef shark, *Triaenodon obesus* (Rüppell, 1837), with chronic esophagitis, was successfully achieved by Dumonceaux et al. (2010; 2012). Results indicated an apparent foreign object lodged in the dorsal esophagus, which later was suspected to be scarring. CT techniques have been used on an Arabian carpetshark, *Chiloscyllium arabicum* (Gubanov, 1980) (Blackband and Stoskopf, 1990). Berzins et al. (1998), and Berzins and Walsh (2000), used MRI and CT to evaluate spinal deformities in *C. taurus*. In another study, MRI was employed to evaluate the brain of a whale shark, *Rhincodon typus* (Smith, 1828), great white shark, *Carcharodon car-*

charias (Linnaeus, 1758), basking shark, *Cetorhinus maximus* (Gunnerus, 1765) and *C. taurus* (Yopak and Frank, 2009). Advanced imaging can also be used in a research capacity, where specific anatomy is evaluated for assisting with shark age determination (Geraghty et al., 2012). The Digital Morphology Group, in conjunction with the University of Texas, hosts a website with multiple high-resolution CT images of various elasmobranch species (http://digimorph.org).

Select cases

A number of case histories where advanced imaging was employed to aid diagnosticians have been detailed in Figures 40 and 41.

CONCLUSION

While limitations exist with any imaging modality, most techniques may be adapted for use with elasmobranchs. Radiography and ultrasonography are important tools for regular examinations. Endoscopy is an advanced diagnostic that provides superior photographic image quality. CT and MRI, while challenging, are possible, and can provide diagnostic opportunities not afforded by the other techniques.

ACKNOWLEDGEMENTS

We would like to gratefully acknowledge the following people: Shane Boylan, South Carolina Aquarium; Charlene Burns, Disney's Animals, Science and Environment; Genevieve Dumonceaux, Palm Beach Zoo and Conservation Society; Alexa McDermott, Georgia Aquarium; Michael Murray, Monterey Bay Aquarium; and the veterinary and husbandry staff of the John G. Shedd Aquairum, Chicago, Illinois.

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Figure 1. An adult whitetip reef shark, *Triaenodon obesus* (Rüppell, 1837), in preparation for radiographic imaging.

PERSONAL COMMUNICATION

Boylan, S. Personal communication. South Carolina Aquarium, Charleston, South Carolina 29401, USA.

Murray, M. Personal communication. Monterey Bay Aquarium, Monterey, California 93940, USA.

INTERNET RESOURCES

www1 www.elasmobranchhusbandry.org

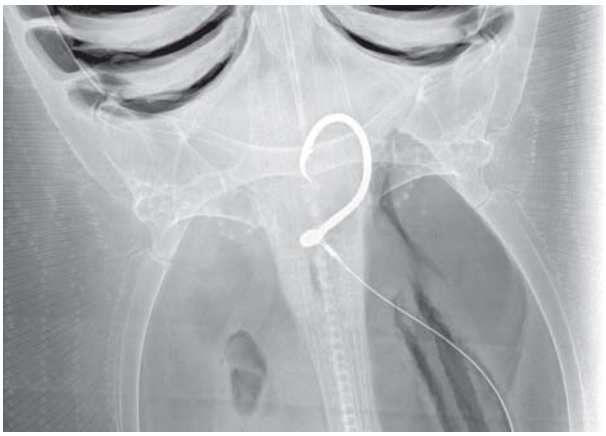


Figure 2. A radiograph of an adult female round ribbontail ray, *Taeniurops meyeni* (Müller & Henle, 1841), with a hook in its esophagus. Refer to Figure 30 for a corresponding endoscopy image.

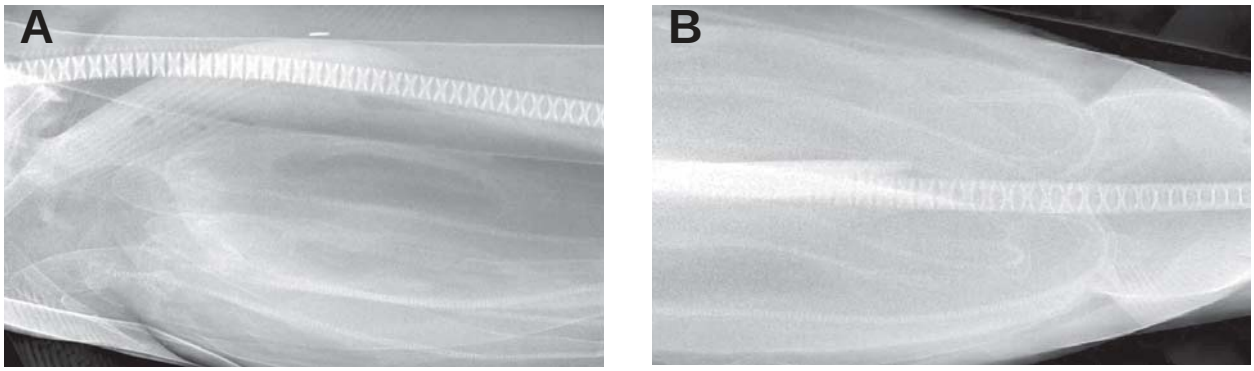


Figure 3. Radiographs (A-B) of a gravid adult female blacktip shark, *Carcharhinus limbatus* (Müller & Henle, 1839).

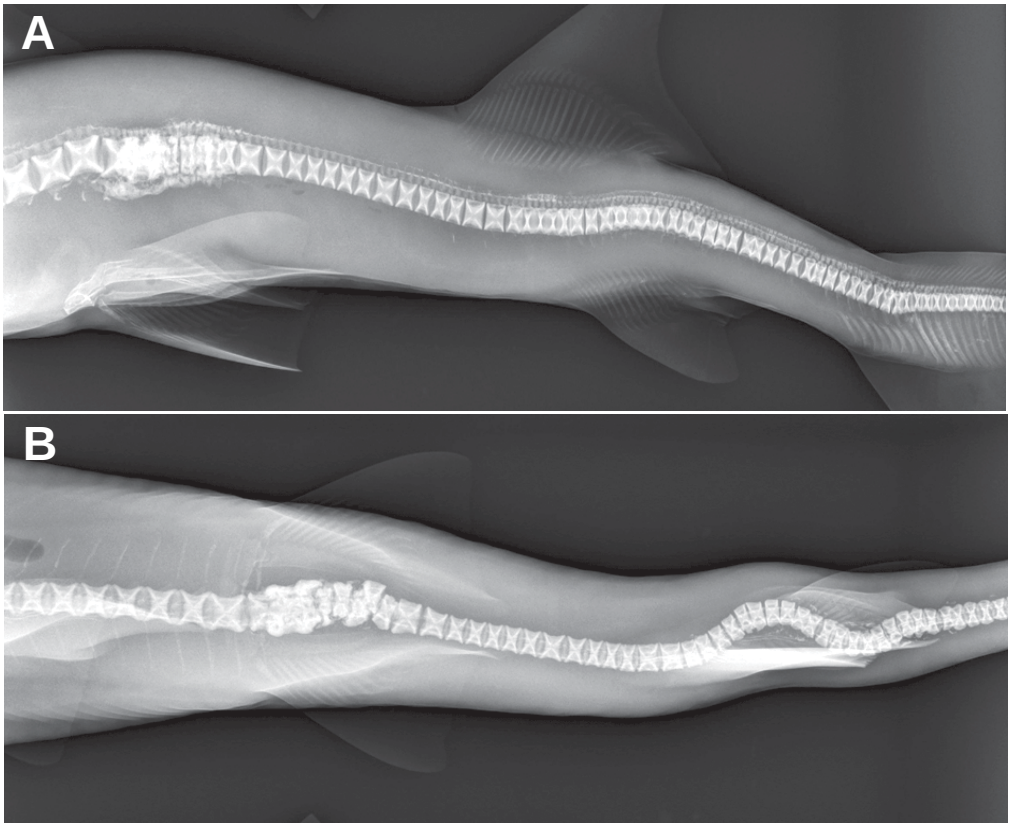


Figure 4. Radiographs (A-B) of an adult male leopard shark, *Triakis semifasciata* (Girard, 1855), with vertebral spondylosis.

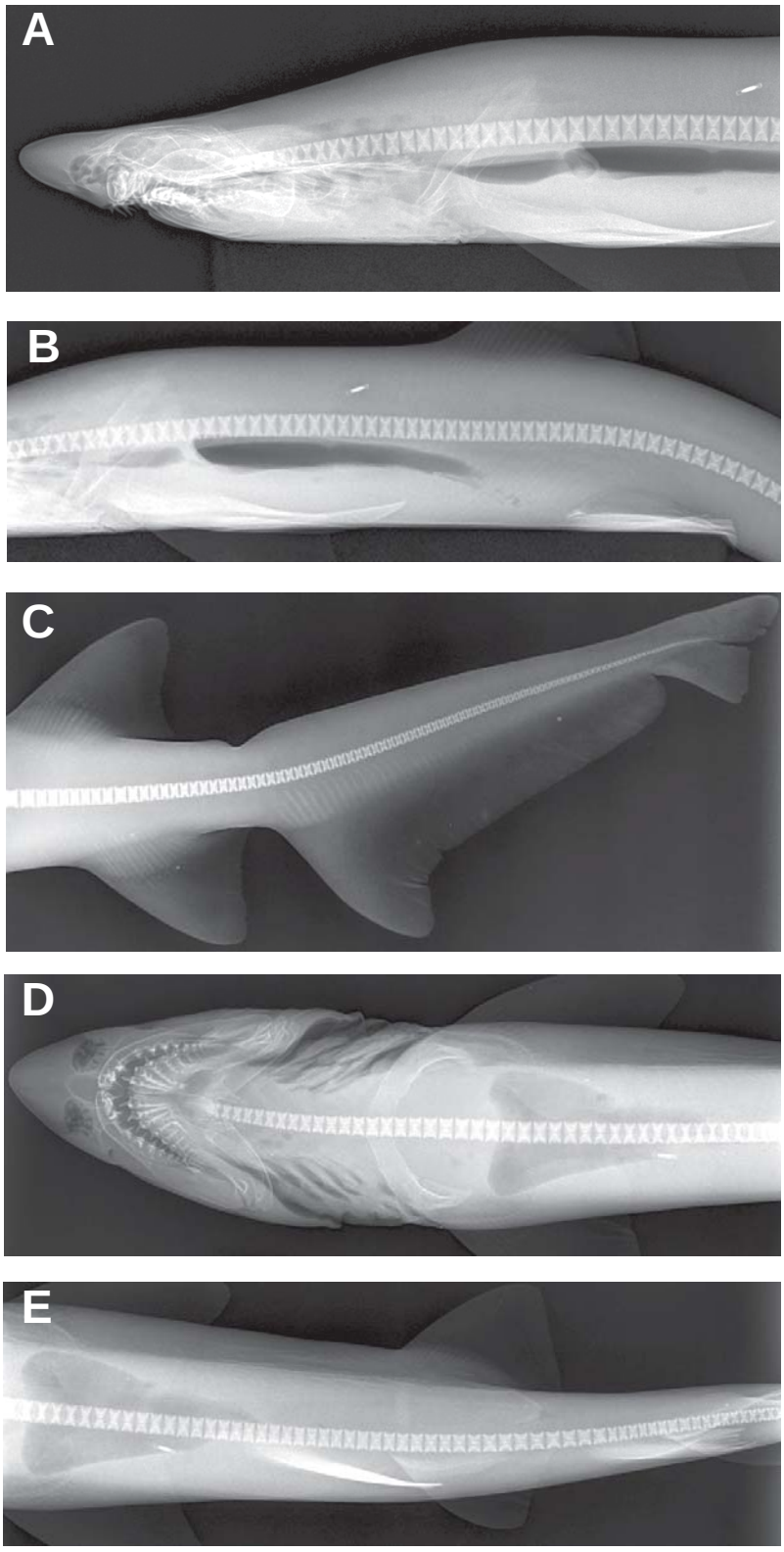


Figure 5. Standing lateral (A-C) and ventrodorsal (D-E) radiographic images of a sub-adult female sand tiger shark, *Carcharias taurus* (Rafinesque, 1810), post-mortem. The animal had a history of bobbing, an inability to regulate buoyancy, and decreased capacity to store gastric air (images courtesy of Boylan, personal communication).

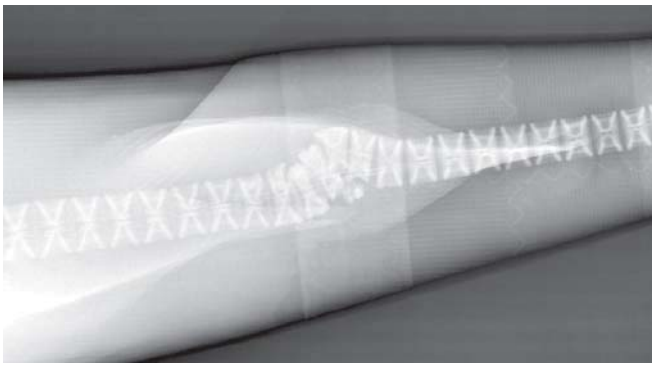


Figure 6. Radiograph of an adult sand tiger shark, *Carcharias taurus* (Rafinesque, 1810), with vertebral spondylosis.

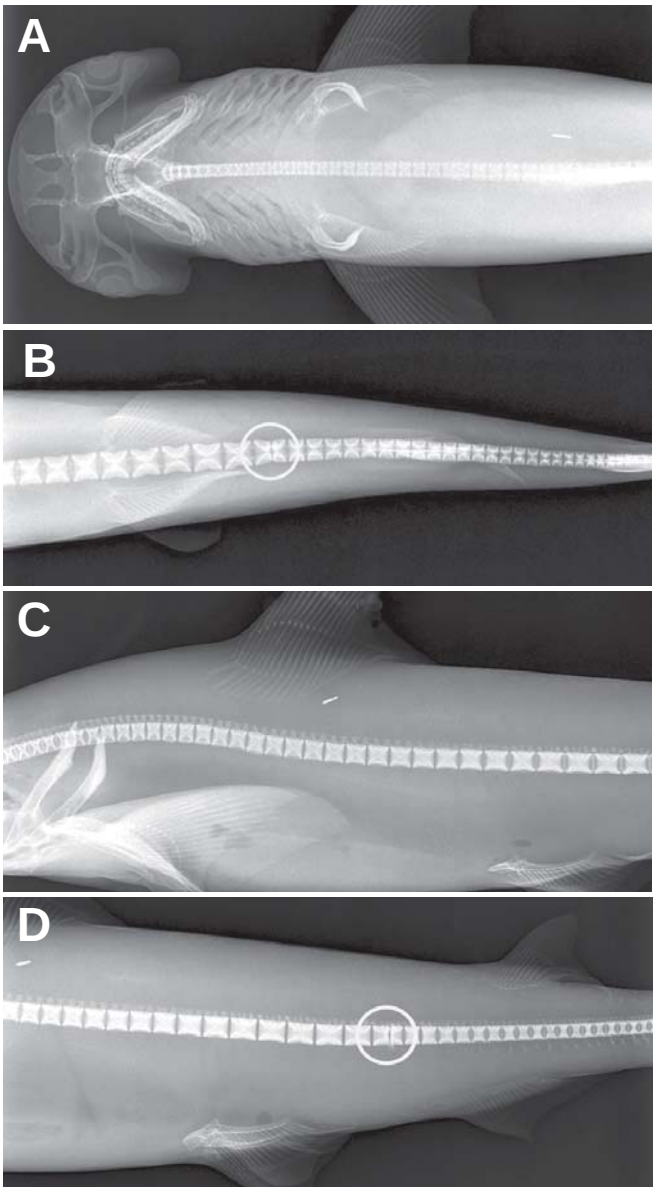


Figure 7. Ventrodorsal (A-B) and lateral (C-D) radiographic images of an adult bonnethead shark, *Sphyrna tiburo* (Linnaeus, 1758), following trauma. Note the fractured vertebrae (post-mortem radiograph images courtesy of Boylan, personal communication).

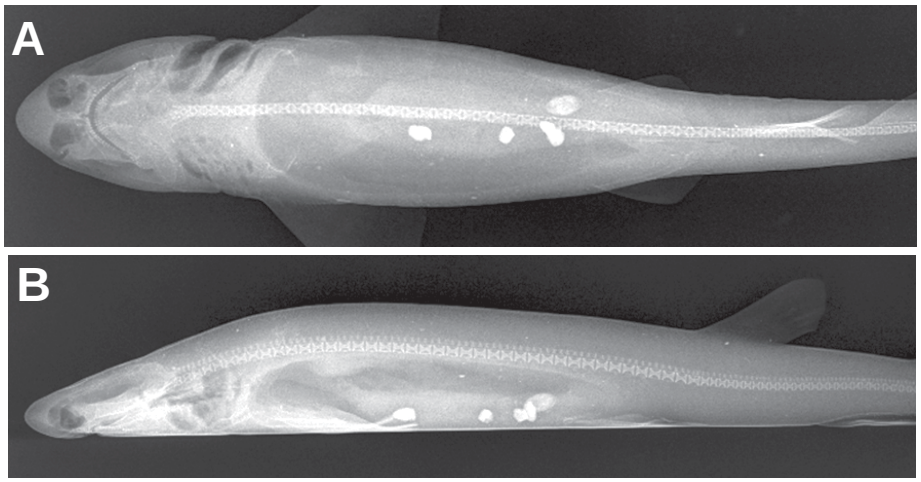


Figure 8. Normal case series radiographs (A-B) of an adult male chain catshark, *Scyliorhinus retifer* (Garman, 1881). Stones were noted in the gastrointestinal tract, but passed without event (images courtesy of Boylan, personal communication).

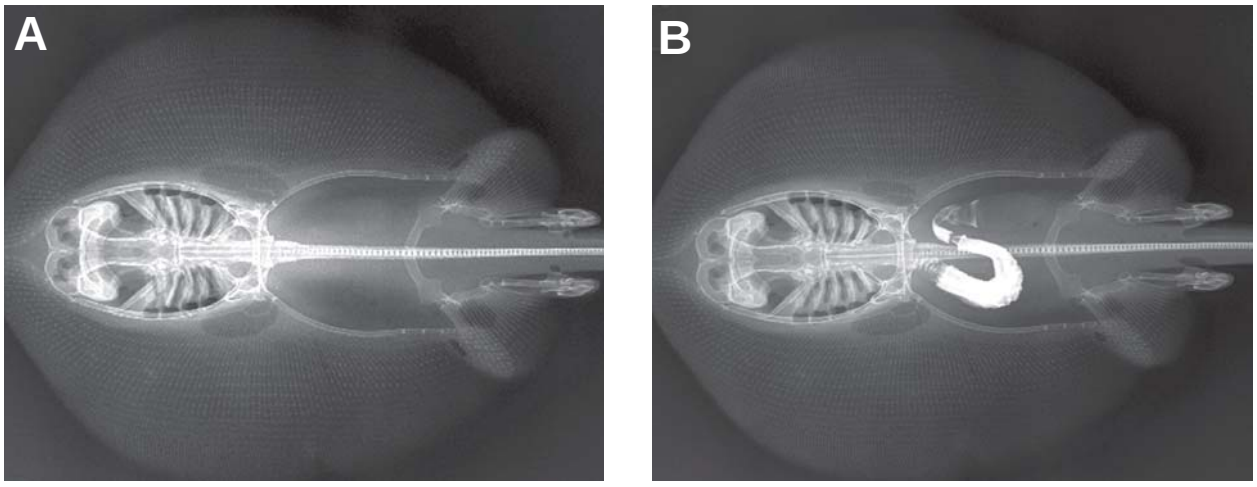


Figure 9. Radiographs of an adult male yellow stingray, *Urobatis jamaicensis* (Cuvier, 1816), with a history of inappetance and poor acclimatization. The images show before (A) and after (B) administration of a non-iodinated contrast material (diatrizoate meglumine and diatrizoate sodium, Gastroview MD, Guerbet LLC, Bloomington, Indiana, USA) (images courtesy of Boylan, personal communication).

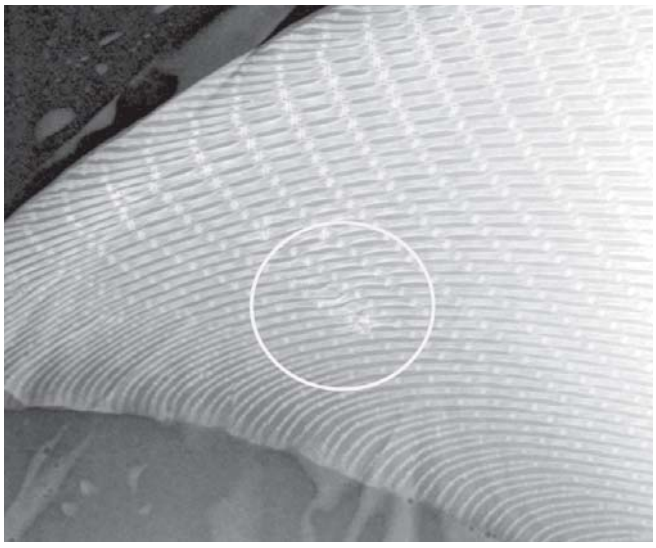


Figure 10. Radiograph of an adult female cownose ray, *Rhinoptera bonasus* (Mitchill, 1815), with a history of swimming in right-hand circles. The diagnosis was chronic degenerative joint disease and healed fractures, focused on the right wing.

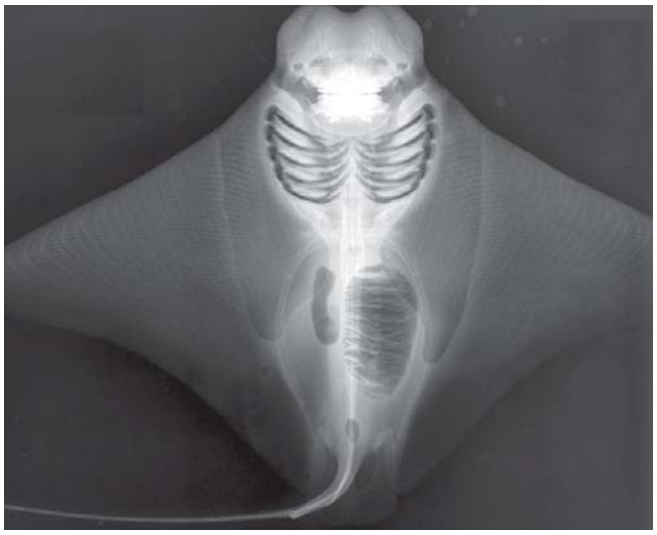


Figure 11. Radiograph of a sub-adult female cownose ray, *Rhinoptera bonasus* (Mitchill, 1815), with gas distention of the gastrointestinal tract. The recently acquired animal was emaciated and found floating at the surface of the aquarium. *Eimeria* sp. was identified in coelomic fluid, but its clinical significance in this case was unknown (images courtesy of Boylan, personal communication).

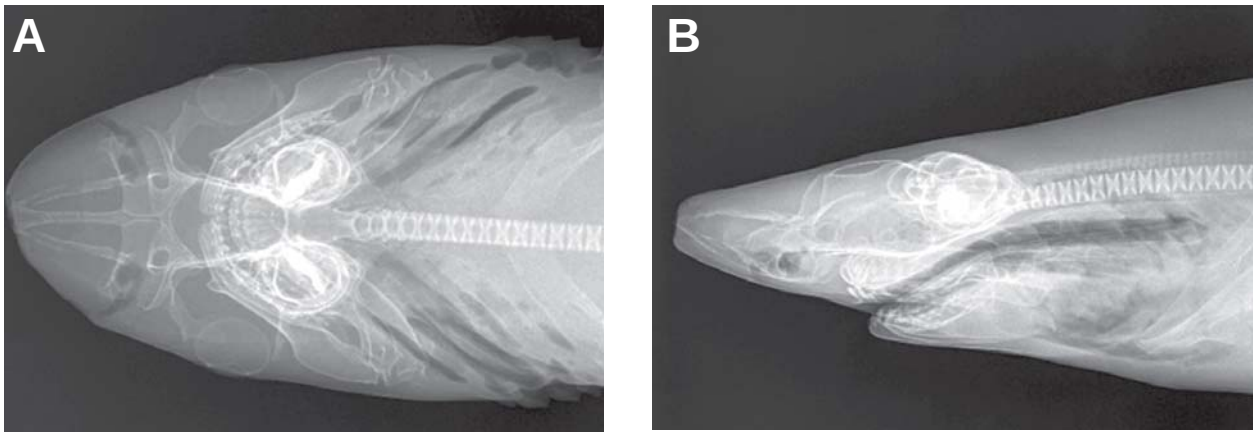


Figure 12. Radiographs (A-B) demonstrating otoliths in an adult blacknose shark, *Carcharhinus acronotus* (Poey, 1860) (Popper and Fay, 1977; images courtesy of Boylan, personal communication).

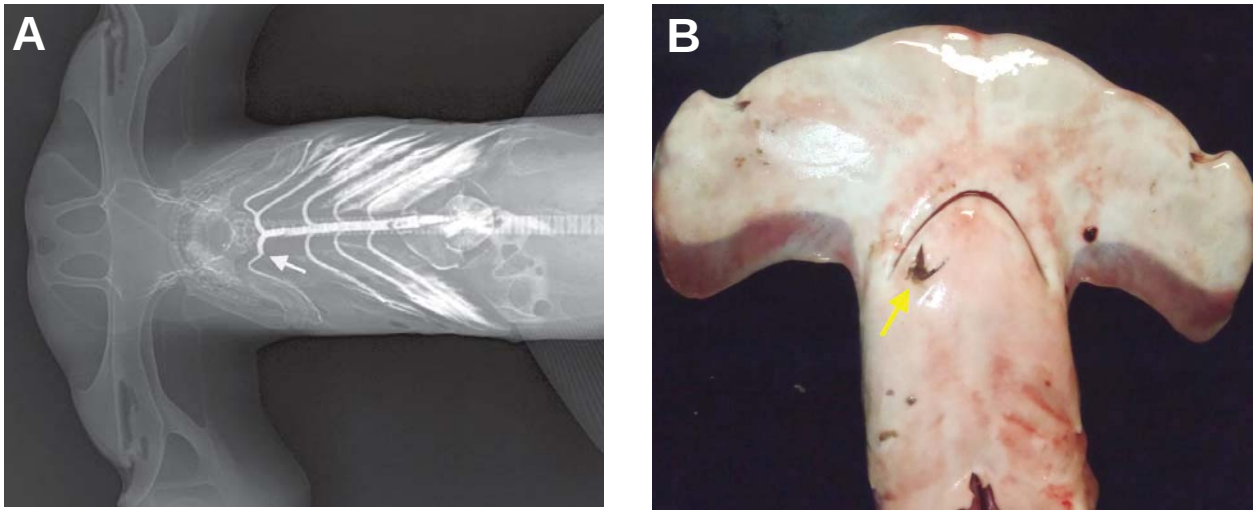


Figure 13. Post-mortem intravenous (IV) non-iodinated contrast study (A) in a scalloped hammerhead, *Sphyrna lewini* (Griffith and Smith, 1834). Note the damaged blood vessel (i.e., lack of vessel presence), indicated by arrow, caudal to the mandible. The injury resulted from a circle hook. A gross image of the same animal (B) shows the hook entry wound (arrow) (images courtesy of Boylan, personal communication).

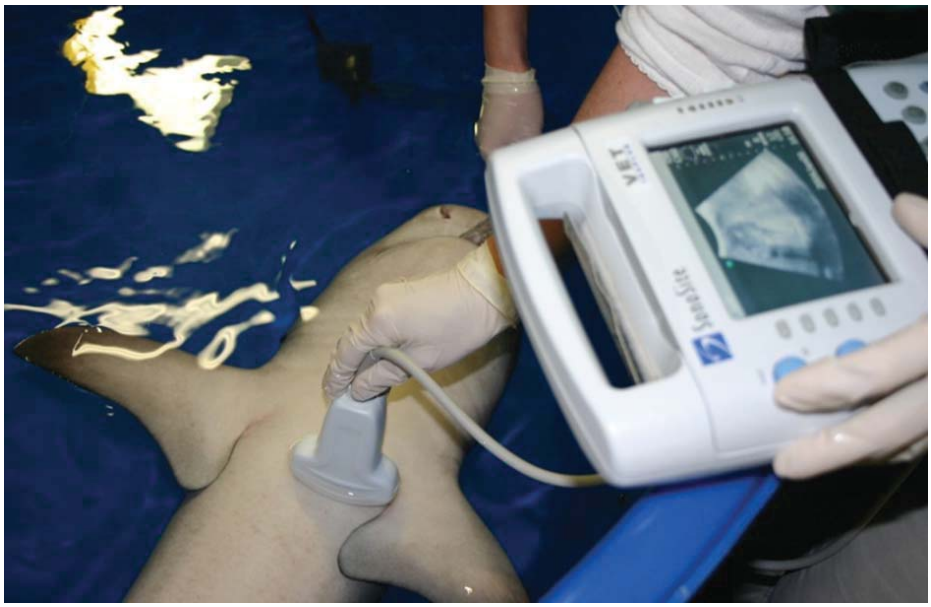


Figure 14. Ultrasound being performed on an adult whitetip reef shark, *Triaenodon obesus* (Rüppell, 1837).

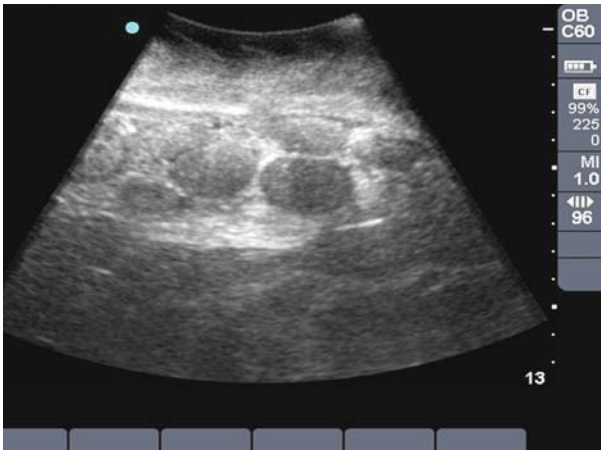


Figure 15. Ultrasound image of an adult giant manta ray, *Manta birostris* (Walbaum, 1792), with sand in its stomach. Refer to Figure 31 for a corresponding endoscopy image.

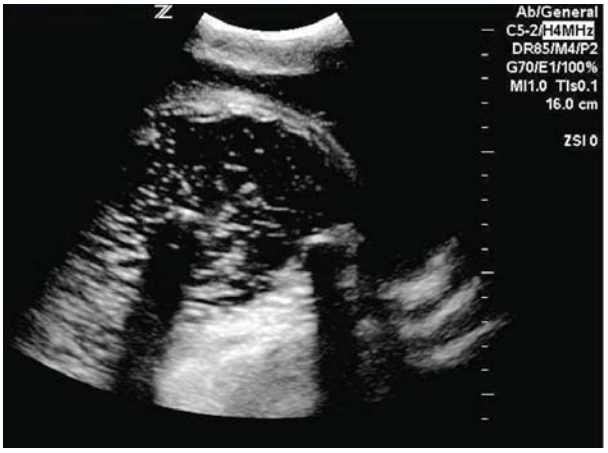


Figure 16. Ultrasound image of an adult female sand tiger shark, *Carcharias taurus* (Rafinesque, 1810), with a normal ovary.

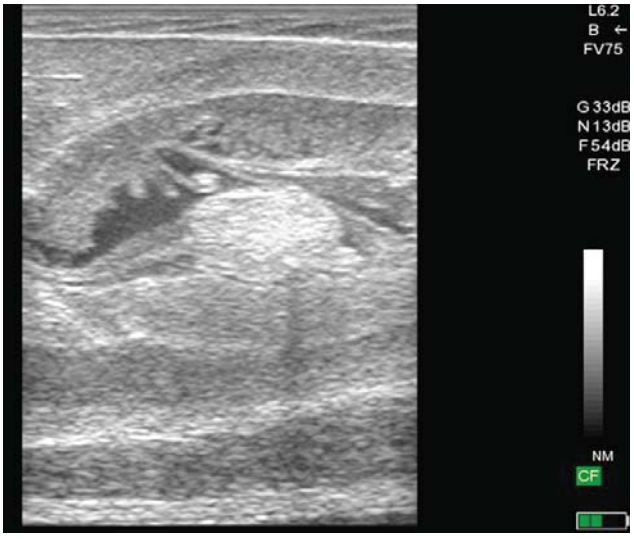


Figure 17. Ultrasound image of a southern stingray, *Dasyatis americana* (Hildebrand & Schroeder, 1928), showing fetus *in utero* with trophonemata over two embryos.

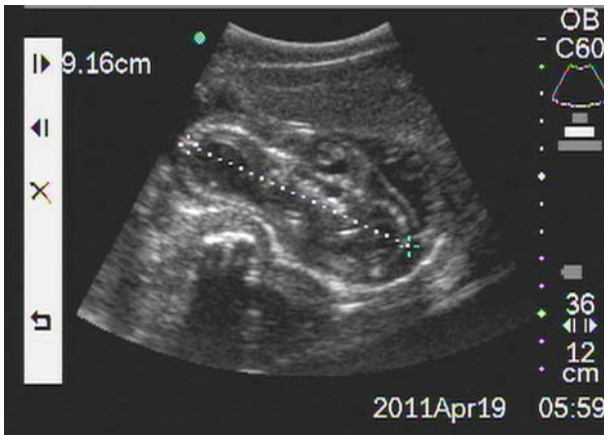


Figure 18. Ultrasound image of a cownose ray, *Rhinoptera bonasus* (Mitchill, 1815), near-term fetus *in utero*.

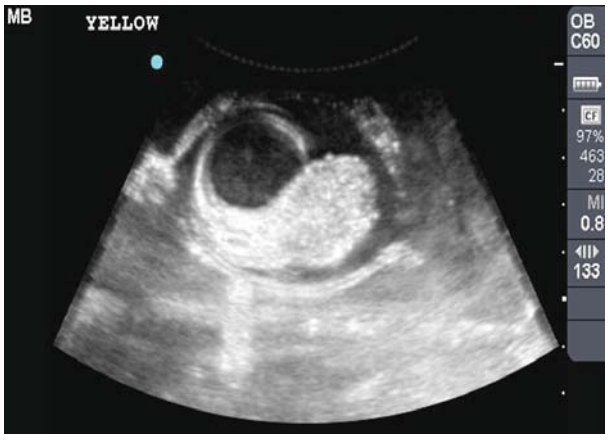
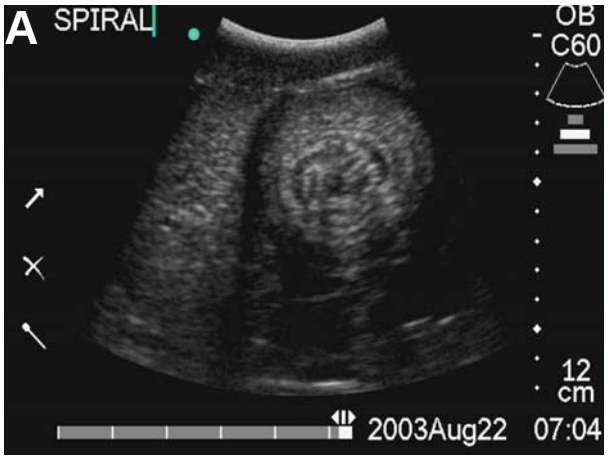


Figure 19. Ultrasound image of a zebra shark, *Stegostoma fasciatum* (Hermann, 1783), fetus in an egg case.



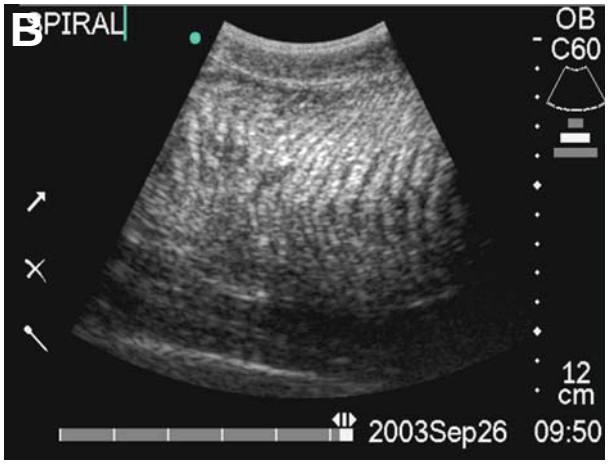


Figure 20. Transverse (A) and sagittal (B) ultrasound images of a normal spiral colon in an adult female zebra shark, *Stegostoma fasciatum* (Hermann, 1783).

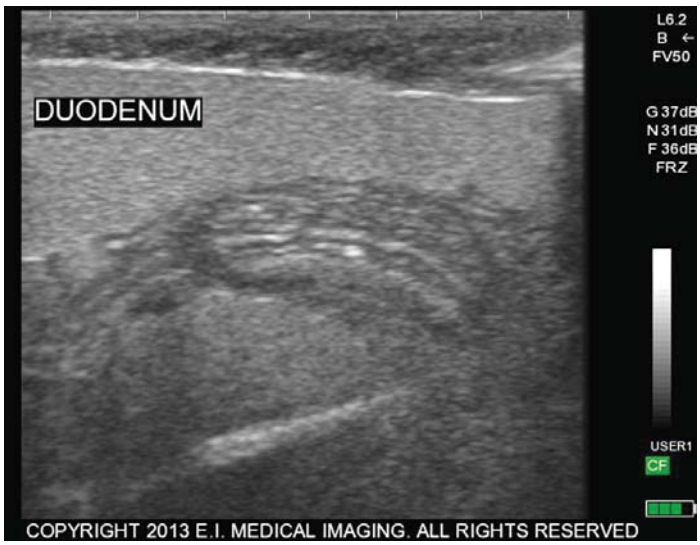


Figure 21. Ultrasound image of normal duodenum in an adult female lesser devil ray, *Mobula hypostoma* (Bancroft, 1831).

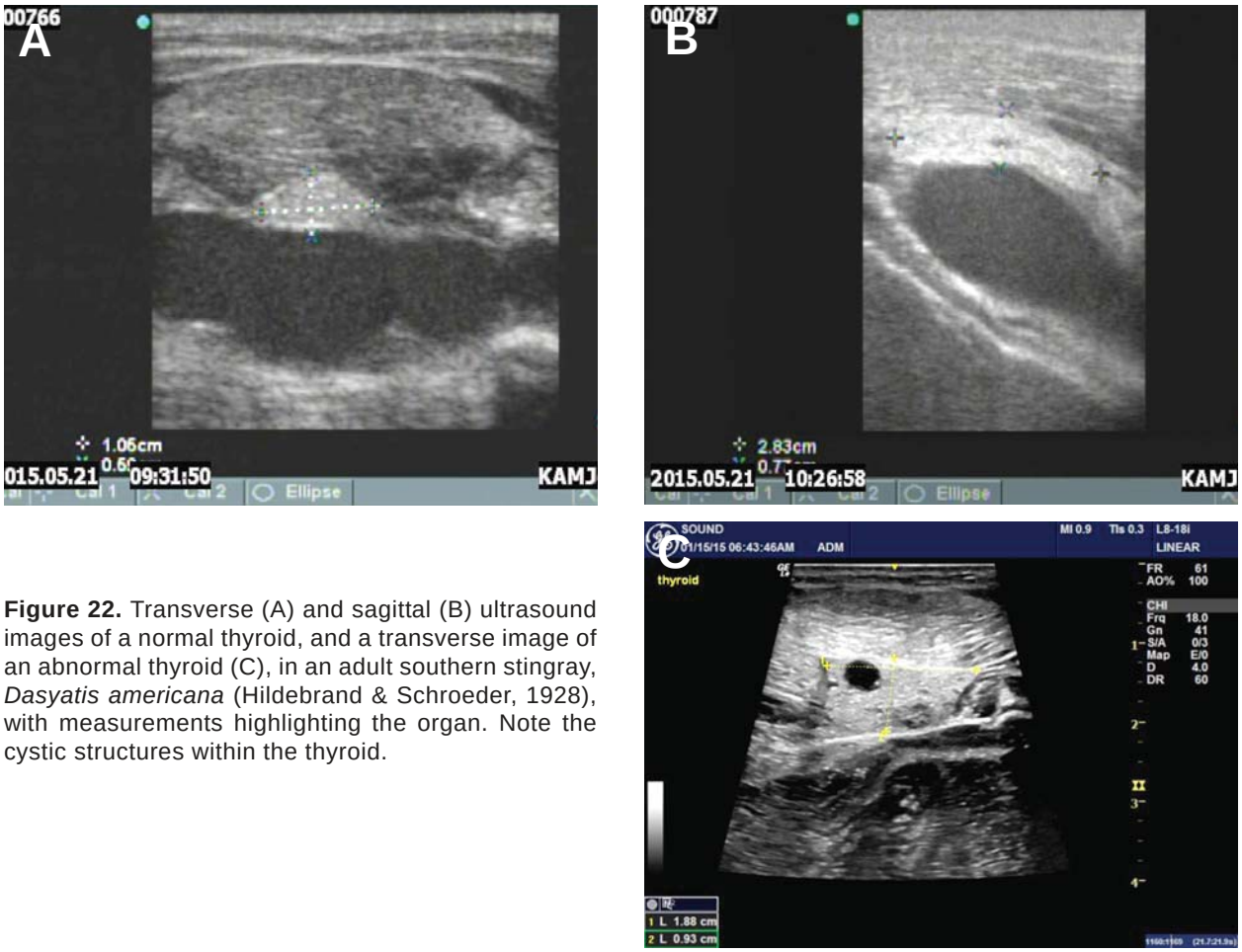


Figure 22. Transverse (A) and sagittal (B) ultrasound images of a normal thyroid, and a transverse image of an abnormal thyroid (C), in an adult southern stingray, *Dasyatis americana* (Hildebrand & Schroeder, 1928), with measurements highlighting the organ. Note the cystic structures within the thyroid.

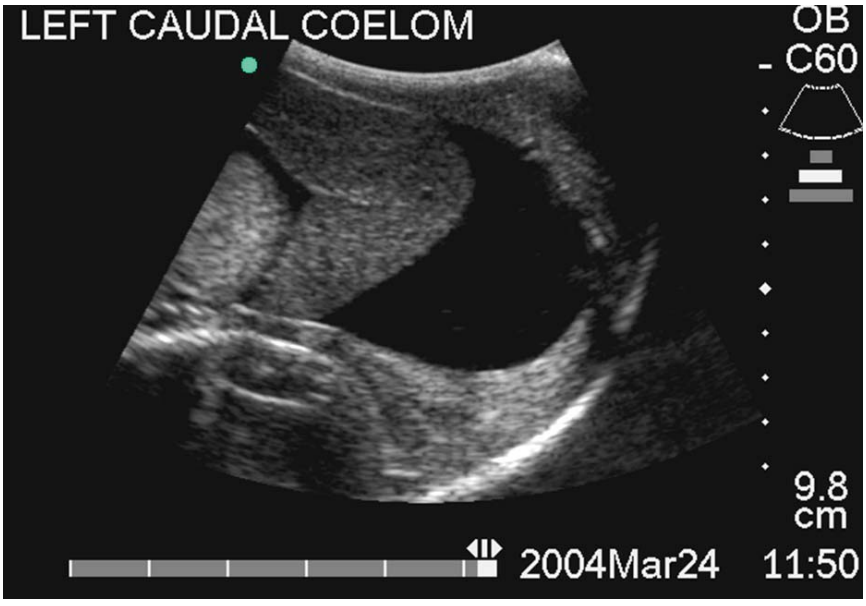


Figure 23. Ultrasound image of an adult male spotted wobbegong, *Orectolobus maculatus* (Bonnaterre, 1788), with renal failure and subsequent fluid accumulation in the coelom. Note: fluid is normal in small amounts; when liver lobes are as prominent as seen in this image it is an abnormal finding.

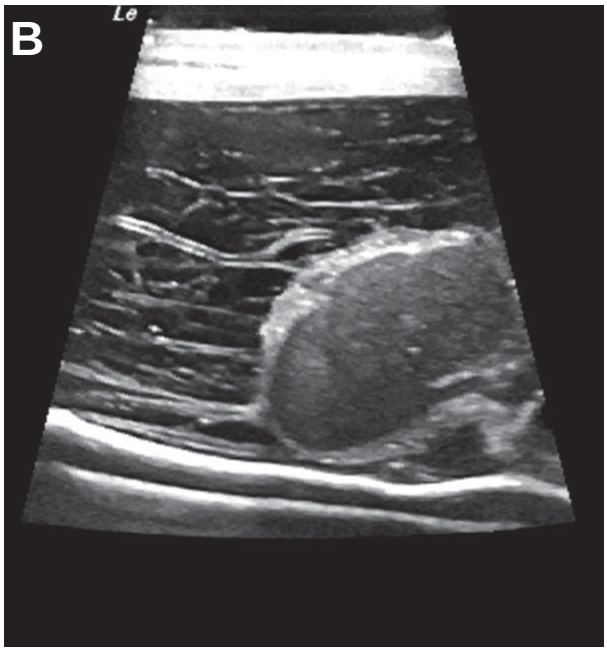


Figure 24. An adult spotted eagle ray, *Aetobatus narinari* (Euphrasén, 1790), post-mortem, showing an example of cerebrospinal fluid (CFS) tap and ultrasound probe placement (A), and an ultrasound image of the brain with a fluid/gel matrix cranial to the cerebrum (B). Note: needle angle varies with the location of the cranial portion of the brain.

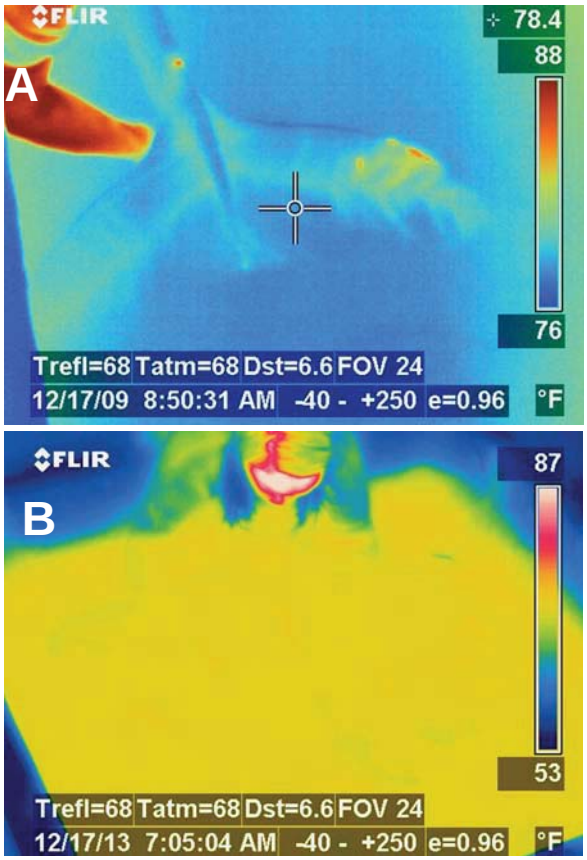


Figure 25. Thermographic images of a bottlenose dolphin, *Tursiops truncatus* (Montagu, 1821) (A), and a cownose ray, *Rhinoptera bonasus* (Mitchill, 1815) (B). Note the two hot spots on the image of the dolphin, which corresponded to a recent venipuncture and a wound on the tail. The *R. bonasus* had multiple superficial wounds at the time of imaging, yet no heat signature was present. An aquarist holding the ray is indicated top of image.

Figure 26. Flexible endoscopic exam/set-up of an inappetant spotted wobbegong shark, *Orectolobus maculatus* (Bonnaterre, 1788).

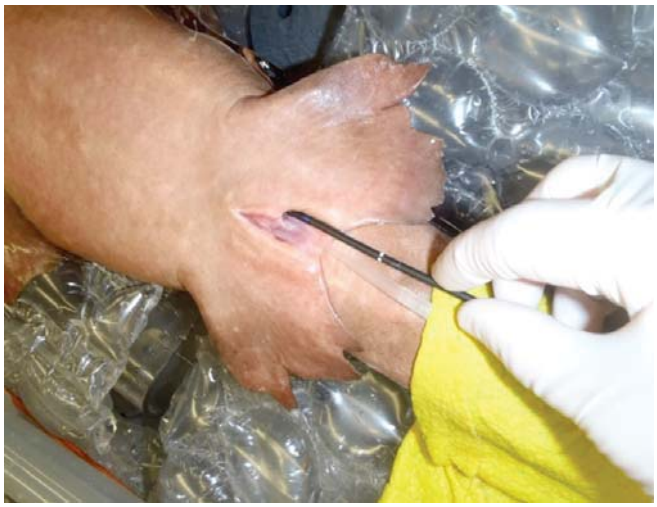


Figure 27. Flexible endoscopic exam through the coelomic (abdominal) pore of an inappetant whitespotted bamboo shark, *Chiloscyllium plagiosum* (Bennett, 1830). In this case, a rigid catheter was placed medial to the scope for infusion of elasmobranch Ringer solution.

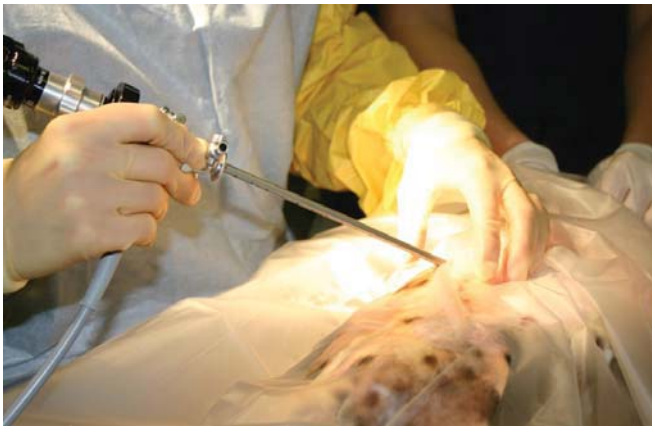


Figure 28. Rigid endoscopic exam of a spotted wobbegong shark, *Orectolobus maculatus* (Bonnaterre, 1788), with inappetance and gastrointestinal stasis.

Figure 29. Flexible endoscopic exam of the uterus (uteroscopy) of a southern stingray, *Dasyatis americana* (Hildebrand & Schroeder, 1928), with abnormal accumulation of fluid.

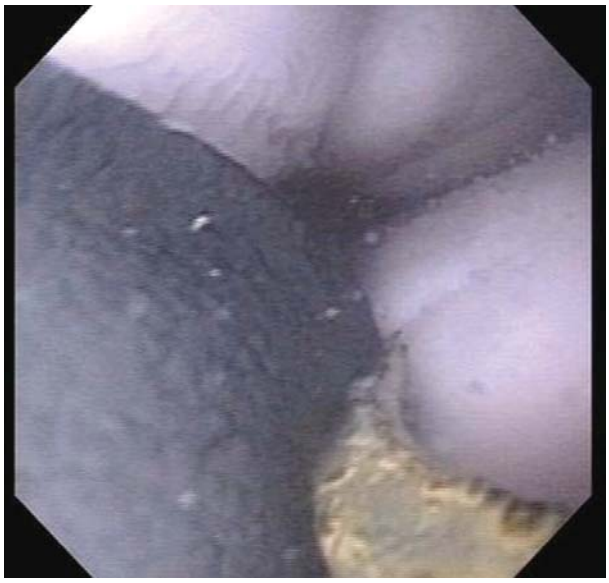


Figure 30. Oral endoscopy of an adult female round ribbontail ray, *Taeniurops meyeni* (Müller & Henle, 1841), with a hook in the esophagus. Refer to Figure 2 for a corresponding radiograph image.

Figures 31. Endoscopic image of an adult giant manta ray, *Manta birostris* (Walbaum, 1792), with sand in its stomach. Refer to Figure 15 for a corresponding ultrasound image.

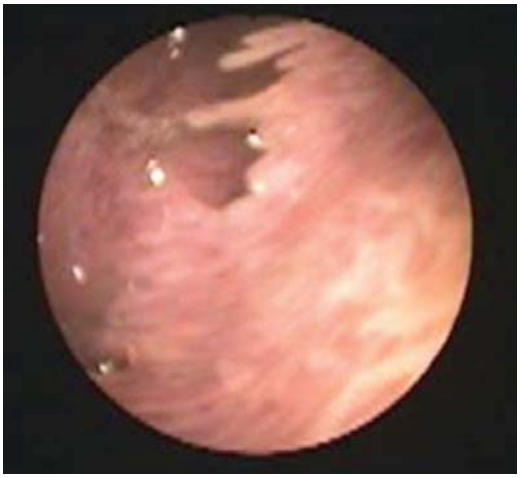


Figure 32. Uteroscopic exam of an adult southern stingray, *Dasyatis americana* (Hildebrand & Schroeder, 1928), which showed normal trophonemata in an air filled uterus. Note that the viscous fluid was abnormal histotroph.

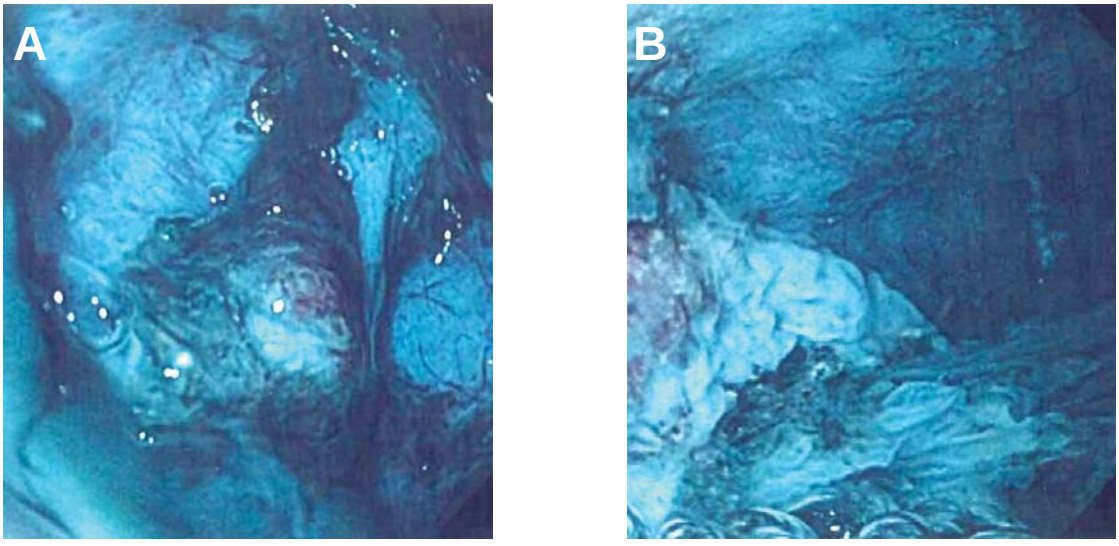


Figure 33. A uteroscopy (A-B) of an adult blacktip shark, *Carcharhinus limbatus* (Müller & Henle, 1839), post-dystocia.

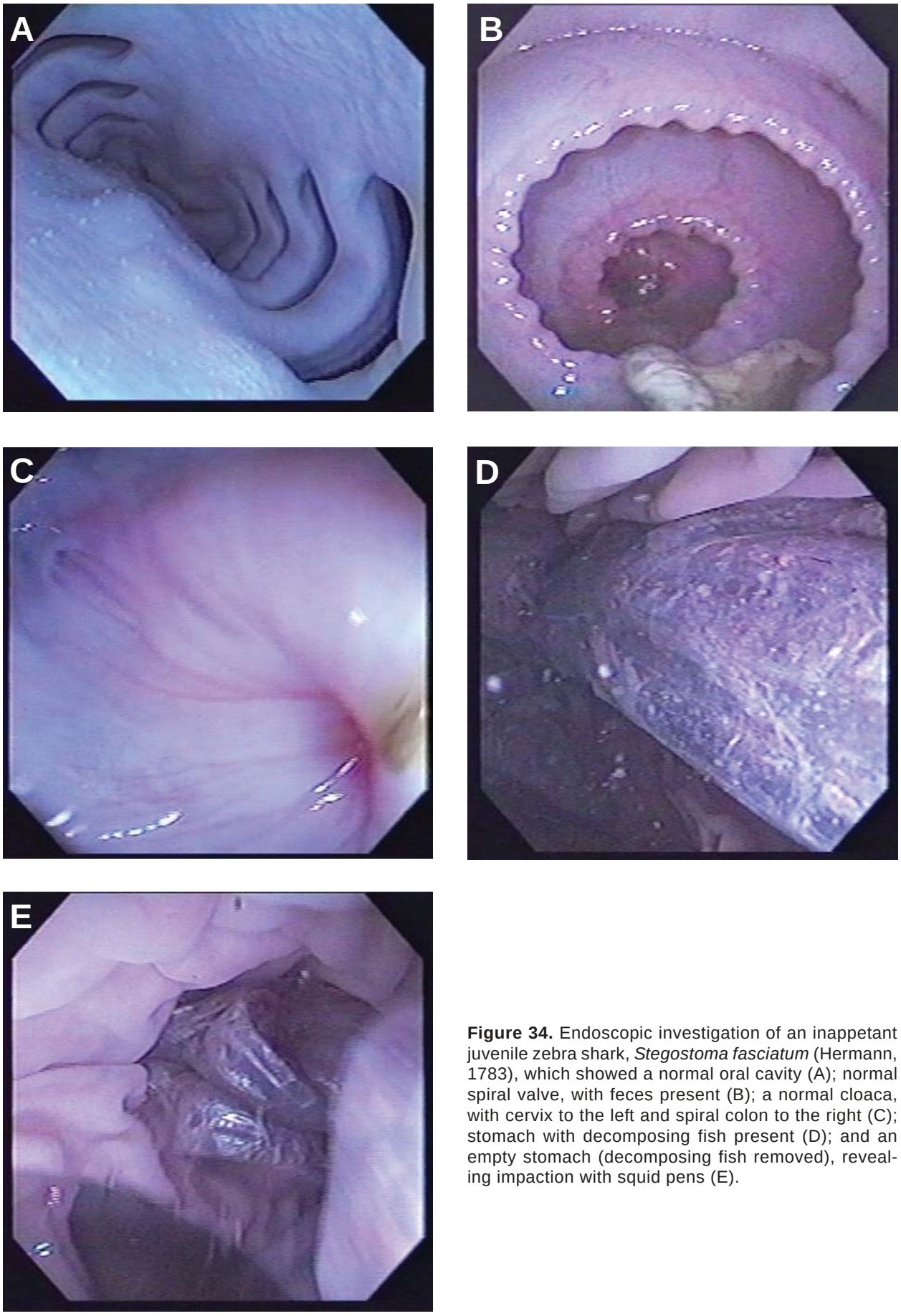


Figure 34. Endoscopic investigation of an inappetant juvenile zebra shark, *Stegostoma fasciatum* (Hermann, 1783), which showed a normal oral cavity (A); normal spiral valve, with feces present (B); a normal cloaca, with cervix to the left and spiral colon to the right (C); stomach with decomposing fish present (D); and an empty stomach (decomposing fish removed), revealing impaction with squid pens (E).

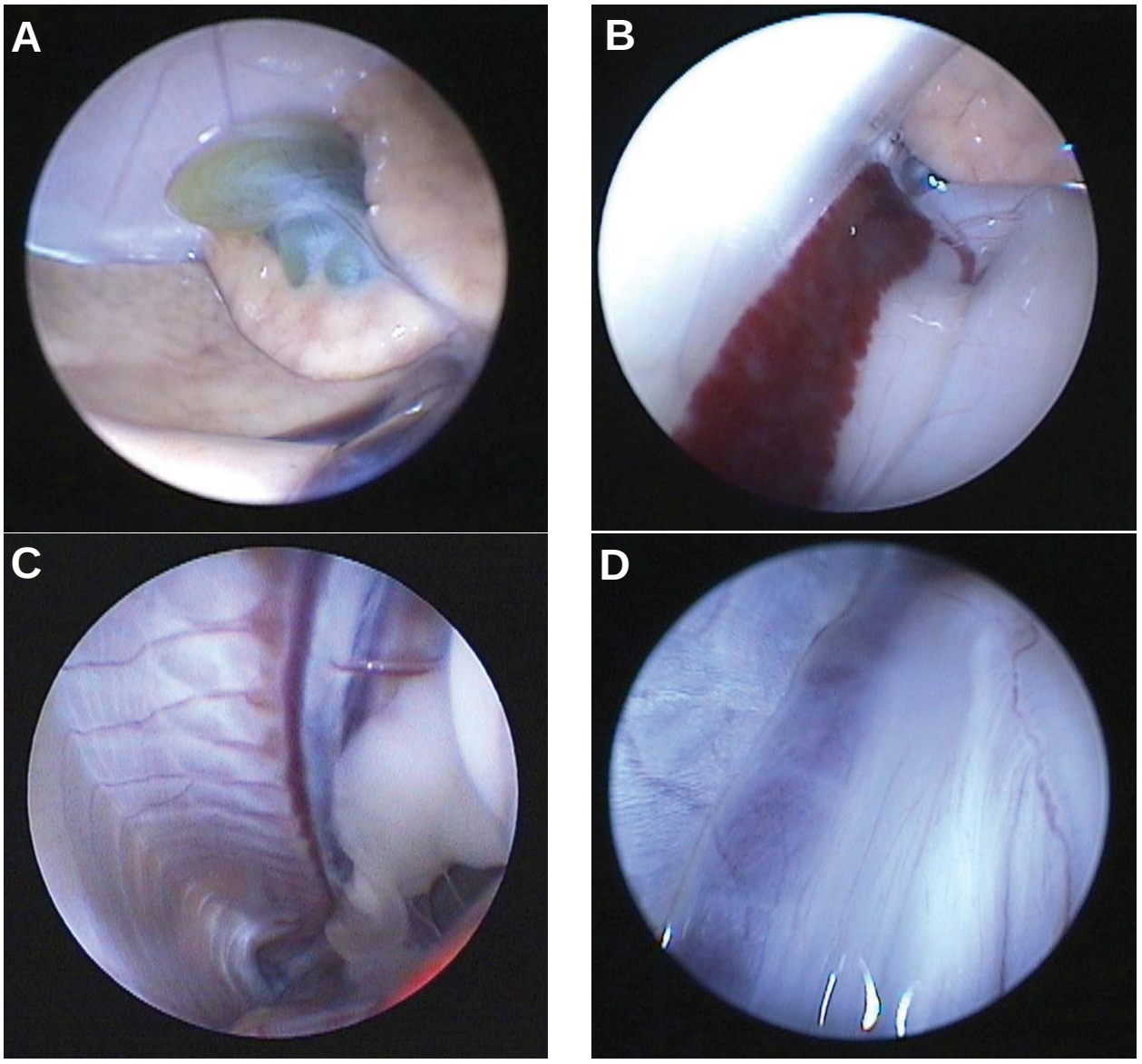


Figure 35. A rigid laparoscopic view of a juvenile male swellshark, *Cephaloscyllium ventriosum* (Garman, 1880), showing liver and gallbladder (A); spleen (B); ventral coelom (C); and kidney (D) (Murray, 2010; Murray, personal communication).

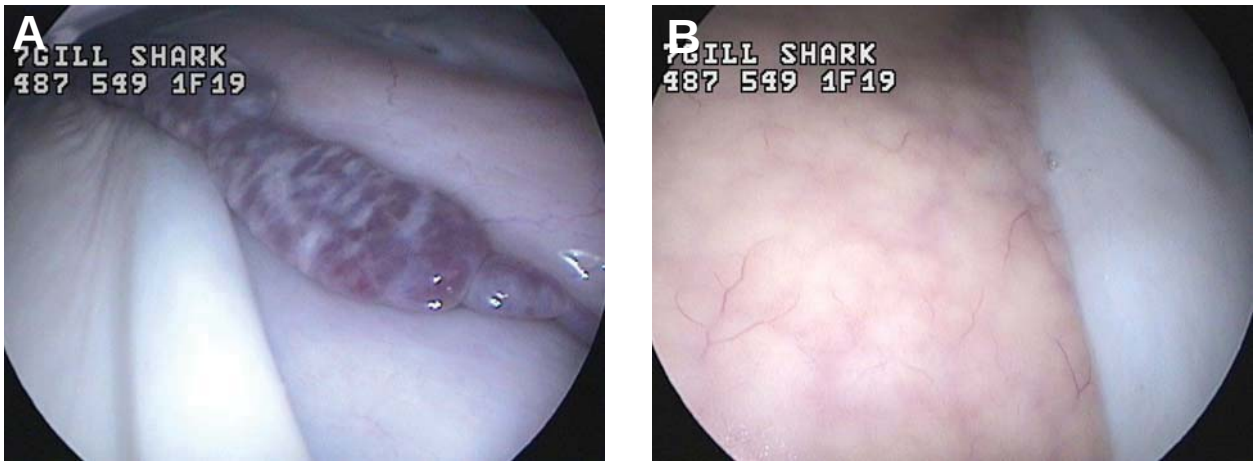


Figure 36. A rigid laparoscopic view of a broadnose sevengill shark, *Notorynchus cepedianus* (Péron, 1807), showing spleen (A) and liver (B) (Murray, 2010; Murray, personal communication).

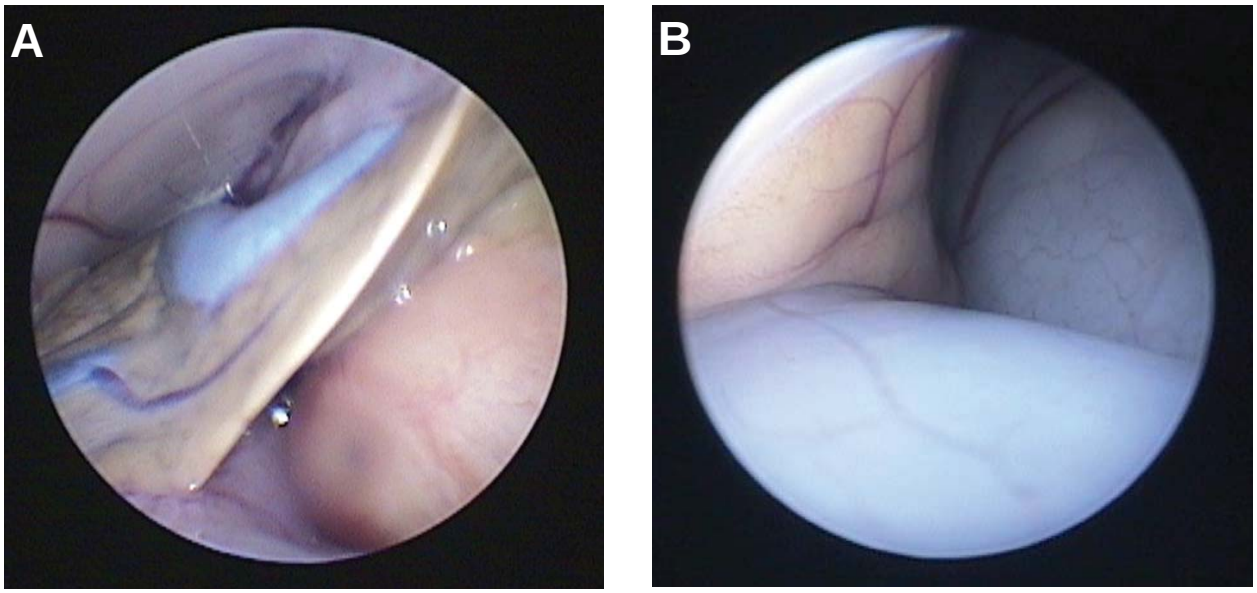


Figure 37. A rigid laparoscopic view of a piked dogfish, *Squalus acanthias* (Linnaeus, 1758), showing liver and gallbladder (A), and liver, stomach, and gravid reproductive tract (B) (Murray, 2010; Murray, personal communication).

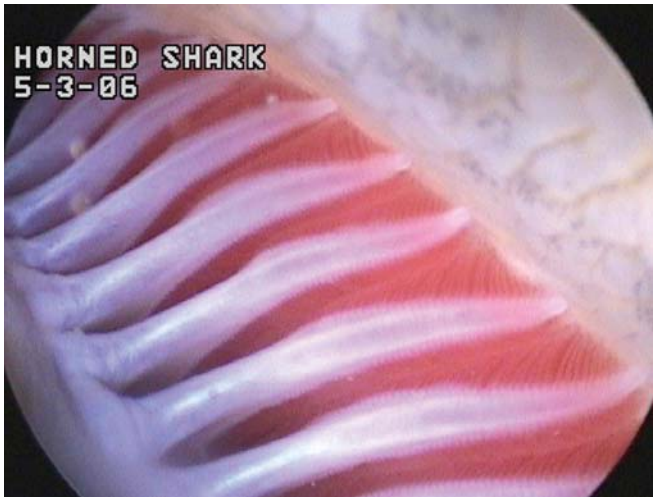


Figure 38. A rigid laparoscopic view of a horn shark, *Heterodontus francisci* (Girard, 1855), showing gill (Murray, 2010; Murray, personal communication).

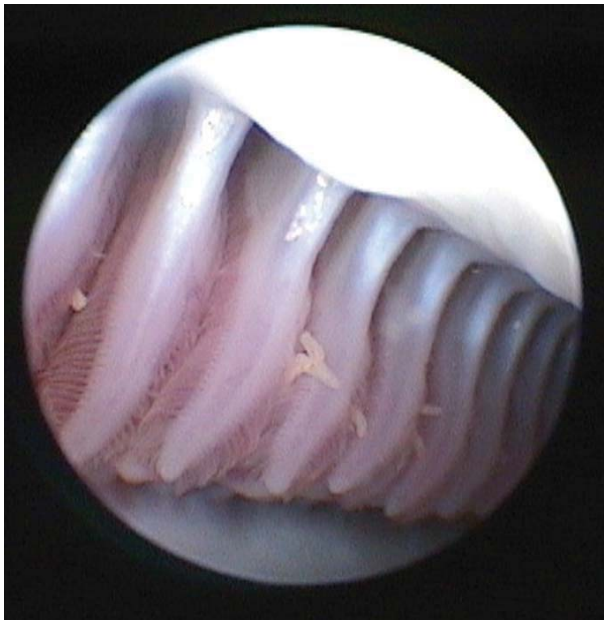


Figure 39. A rigid laparoscopic view of a leopard shark, *Triakis semifasciata* (Girard, 1855), showing gill parasitized with *Erpocotyle* spp. (images courtesy of Murray).

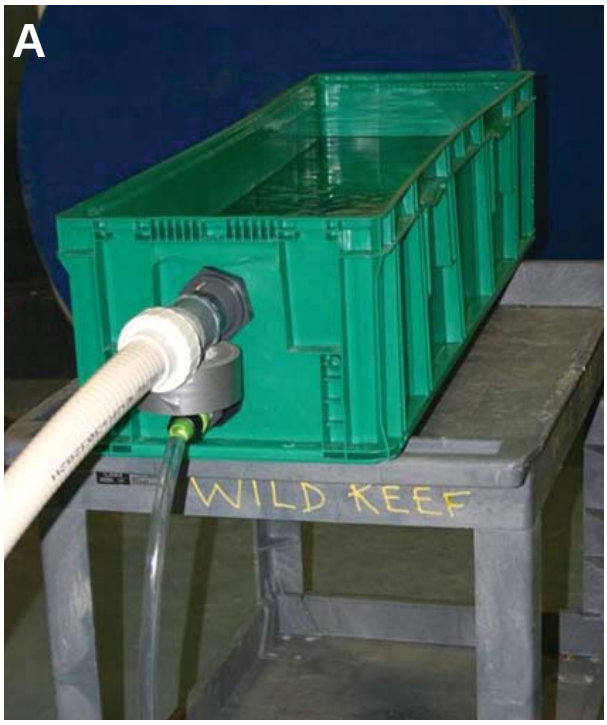


Figure 40. MRI setup (A-C) for a whitetip reef shark, *Triaenodon obesus* (Rüppell, 1837), using a modified flume allowing recirculation of water from a distance. This setup is intended to protect the MRI machine from water, while providing oxygenation to the animal. Note the cling wrap over the tank.

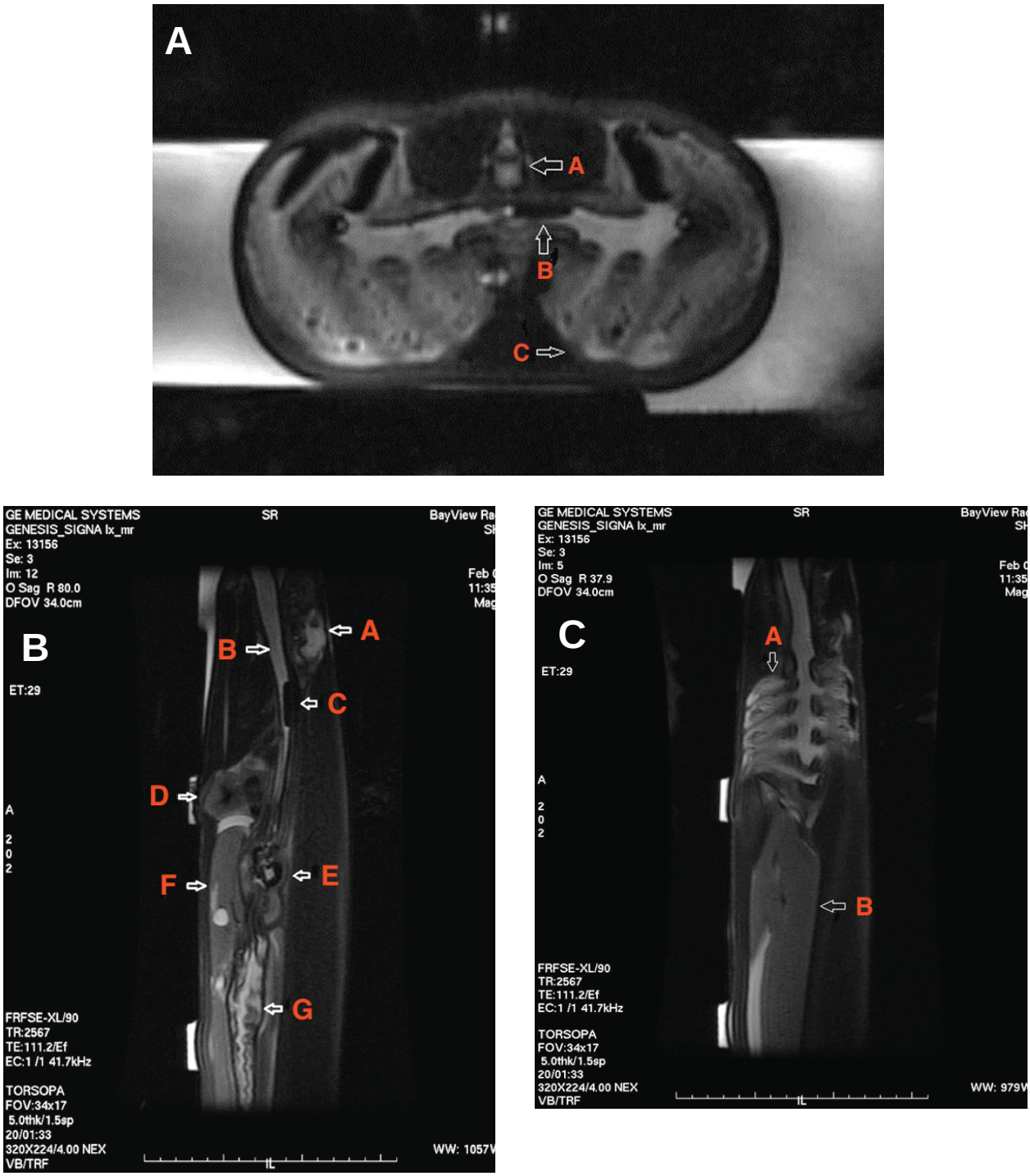


Figure 41. MRI images of a whitetip reef shark, *Triaenodon obesus* (Rüppell, 1837), without the use of contrast (the white shadow density was water): (A) axial view between the brain case and heart, showing spinal cord [A], esophageal filling defect [B], and gills [C]; (B) sagittal view, showing brain [A], esophagus full of water [B], esophageal filling defect [C], heart [D], stomach [E], liver [F] and intestines [G]; (C) sagittal view of the gills [A] and liver [B]. Images courtesy of Dumonceaux; MRI services compliments of Bay View Radiology Center, Tampa, Florida.

The Elasmobranch Husbandry Manual II: Recent Advances in the Care of Sharks, Rays and their Relatives, pages 325-330.

Chapter 31

Ultrasound assessment of pregnant ribbontail stingrays, *Taeniura lymma* (Forsskål, 1775)

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Abstract: There is little documented information on fetal development and gestation in ribbontail stingrays, *Taeniura lymma* (Forsskål, 1775), and reproduction of this species in aquaria is uncommon. *T. lymma* at the Oceanário de Lisboa, Portugal, have bred regularly since 2011, with 12 pregnancies resulting in a total of 16 live pups. The breeding population comprised four females and one male *T. lymma*, introduced into the aquarium between 2007 and 2009. Gestation time was highly conserved in individual *T. lymma*, but varied considerably between individuals. Gestation time ranged from 128 - 203 days. Pregnant *T. lymma* were monitored by serial ultrasound examinations to evaluate fetal vitality, as well as fetal number and growth rate. During ultrasound exams several fetal measurements were taken, including body height (BH), maximum wing height at the main wing internal cartilage (WH), eye diameter (ED), brain cavity height (BC) and yolk sac size (YS), to assess if morphometrics could be used to predict delivery date. BH and ED proved to be the most reliable measures of developmental stage in *T. lymma* fetuses at the Oceanário de Lisboa and, using a multivariate linear regression analysis, were used to predict an approximate date of birth.

INTRODUCTION

The ribbontail stingray, *Taeniura lymma* (Forsskål, 1775), is a tropical dasyatid ray popular in public aquaria and private collections. The species is classified as “near threatened” by the International Union for Conservation of Nature (IUCN), with major threats to the population arising from commercial fisheries and habitat loss (www1). The ability to successfully breed *T. lymma* in aquaria

may be necessary to secure a sustainable source of animals for future exhibition. The majority of *T. lymma* currently displayed in public aquaria were collected from the wild, although some institutions in Europe and the United States are now exhibiting rays that were born in aquaria. Since 2007, when the European Studbook for *T. lymma* was created through the European Association of Zoos and Aquariums (EAZA), the number of *T. lymma* births in aquaria has increased.

Reproduction in *T. lymma* is poorly described. Many of the myliobatiform rays are aplacental viviparous with uterine specialization (i.e., uterine villi or trophonemata) (Dulvy and Reynolds, 1997; Henningsen et al., 2004), with only the left ovary and oviduct functional (Henningsen et al., 2004); *T. lymma* appears to be no different. Male *T. lymma* mature at a disk width (DW) of ~20 cm. Gestation time in dasyatid rays ranges from 3 - 11 months, with shorter gestations generally observed in tropical species (White and Dharmadi, 2007). Wild *T. lymma* can produce up to seven pups per litter (Michael, 1993; Tricas et al., 1997), however a maximum of two pups per litter have been produced in aquaria.

Ultrasonography is a common procedure in human and terrestrial animal veterinary medicine, used to examine and investigate normality and pathological changes in internal organs and soft tissue. While not as common, ultrasound has also been used for animal health (Stetter, 2004; Gore et al., 2007; Whittamore et al., 2010; Mylniczenko and Penfold, 2012) and research purposes in fishes (Stetter, 2004; Daly et al., 2007). However, the use of ultrasound has predominantly been used for reproductive studies (e.g., sex determination and gonadal maturity status) in teleosts (Whittamore et al., 2010). To date, ultrasound technology has had relatively little application in elasmobranchs, although it has been used for diagnosing pregnancy, fetal or embryonic vitality and viability assessment, ovarian follicle assessment, and for general evaluation of reproductive organs (Harper et al., 1999; Carrier et al., 2003; Stetter, 2004; Daly et al., 2007; Whittamore et al., 2010; George et al., 2009; Jirik and Lowe, 2012; Mylniczenko and Penfold, 2012; Daly and Jones, this volume). Routine serial ultrasonographic gestation evaluation can also be used to detect developmental abnormalities, such as intra-uterine death, and to predict parturition dates. The latter application is advantageous in that accommodations could be made for near-term pregnant females, facilitating uncomplicated deliveries and the protection of pups from predation or competition for food.

In this study, serial ultrasound observation was used to monitor gestation and fetal development in *T. lymma* at the Oceanário de Lisboa. Information was collected on *T. lymma* reproductive biology, and a formula was developed, based on fetus morphometrics, to predict delivery dates.

METHODS and RESULTS

Husbandry

T. lymma at the Oceanário de Lisboa were maintained in a multi-species exhibit themed as an Indian Ocean reef. The display was 3.5 m deep and had a volume of 250 m^3. Volumetric water turn-over occurred once every hour. Exhibit life support included a sand filter, a protein skimmer with ozone injection, a degassing tower, a heat exchanger and UV disinfection. Theming consisted of artificial rock and coral sand was used as substrate. Environmental (Table 1) and nutritional parameters were maintained the same for all individuals throughout the duration of the study.

T. lymma were fed a food ration of ~2 - 3% body mass/day. Diet consisted of squid, *Loligo* sp.; Atlantic herring, *Clupea harengus* (Linnaeus, 1758); European sprat, *Sprattus sprattus* (Linnaeus, 1758); Capelin, *Mallotus villosus* (Müller, 1776); European smelt, *Osmerus eperlanus* (Linnaeus, 1758); shrimp of the genera *Litopenaeus* and *Penaeus*; mussels, *Mytilus* spp. and cockles (family: Cardiidae).

Table 1. Water quality parameters in the Oceanário de Lisboa Indian Ocean Reef exhibit.

Water Quality Parameter	Range
Temperature	24.5 - 25ºC
Salinity	32 - 33 g/L
pH	8.20 - 8.25
Dissolved oxygen	6.7 - 6.9 mg/L
Redox	270 - 290 mv
Ammonia	0 mg/L
Nitrite	0.002 - 0.003 mg/L
Nitrate	11 - 12 mg/L
Turbidity	< 0.10 NTU
Phosphate	< 2.8 mg/L

Food was supplemented with vitamins (PSVO 10/3, Premix™, Viana do Castelo, Portugal) and potassium iodide (10 mg/kg) once a week. The *T. lymma* were conditioned to a feeding cue consisting of a visual target. Food was given within a 2 x 2 m square feeding station, at a depth of 25 cm, which facilitated food rationing, monitoring of individual animals and animal capture, when required.

A population of sexually-mature *T. lymma,* comprised of 3 - 4 females and one male, introduced between 2007 and 2009, were maintained in the aquarium during the study period (2011 - 2013). Individual rays were identified through spot pattern body markings and sexual dimorphism.

The first *T. lymma* birth occurred in March 2011, one and a half years after the introduction of the male to the population. A total of 16 pups were born from 11 successful parturition events. One aborted fetus was observed on a separate occasion. Three of the four female stingrays gave birth during the study period, and the number of young per litter varied between one and two. Pregnant *T. lymma* were moved to a quarantine tank towards the end of gestation for parturition. Under normal circumstances the female was returned to the exhibit following delivery, while the pups remained in the quarantine tank for monitoring.

Ultrasonography

Serial ultrasound examinations of three female *T. lymma* were performed during pregnancies. Rays were drawn into a small stretcher using a visual cue at the feeding station and transported to a small tank with oxygen supplementation, where they were manually restrained, or held within a net or stretcher. *T. lymma* were examined with either a Honda Electronics HS/1500 scanner with a HLS-375M 5 - 7.5 - 10 MHz linear transducer, or a Siemens Acuson Cypress scanner with a 5.5 - 6.6 MHz 7L3 linear transducer.

During ultrasound examination, fetal vitality was monitored. A range of fetal measurements was taken, either 'real time' during the examination or from recorded video clips. When feasible, the uterus and ovary were also examined. Handling stress can affect fetus survival (Carrier et al., 2003; Daly et al., 2007), so prolonged stressful procedures were kept to a minimum during serial examinations. Ultrasound examinations were typically conducted within five minutes and, if an animal exhibited increased stress, denoted by resistance to handlers or increased movement, the procedure was terminated. Rays were immediately returned to the exhibit tank at the conclusion of a procedure.

The ultrasound probe was protected from direct contact with water using a disposable shoulder-length polyethylene glove, fixed in place with a rubber band. Ultrasound coupling gel was applied between the surface of the probe and the glove to enable ultrasound transmission. Examinations began with the probe placed transversely on the left dorsal body surface, caudal to the gill cavity, to visualize the left uterus. The coelomic cavity was scanned in an anterior to posterior direction. In single fetus pregnancies, this approach was sufficient to observe the uterine content. When two fetuses were present, it was generally necessary to scan the right dorsal body surface because the left uterus would occupy a larger part of the coelomic cavity. Following fetal assessment, a brief gonadal observation and general overview of the coelomic cavity was conducted. To better understand techniques for elasmobranch ultrasonography, the reader is directed to Mylniczenko, et al. (this volume).

Of the 12 confirmed pregnancies between 2011 and 2013, nine were monitored using ultrasound examinations (36 readings) and embryonic measurements were taken from 12 fetuses as follows: body height (BH; n = 27), maximum wing height at the main wing internal cartilage (WH; n = 21), eye diameter (ED; n = 19), brain cavity height (BC; n = 16) and yolk sac size (YS; n = 6). All measurements were taken transversely, with respect to the fetus, and recorded in mm (Figure 1). BH was measured in line with the eye or branchia, with both approaches yielding similar measurements (e.g., 22.8 mm and 22.9 mm, respectively). ED and BC were not detectable during several examinations. YS became increasingly difficult to visualize as gestation progressed. The latest in gestation that YS was readable via ultrasound was day 117.

Gestation time

Female *T. lymma* were identified as TL1, TL2, TL3 and TL4. Ray TL2 was not pregnant during the period of study. TL4 died six days after her 3rd delivery. Mortality was attributed to postpartum bacterial peritonitis, confirmed by histology. During the 4th pregnancy of ray TL3, two fetuses died near the end of term and an abortion under anesthesia was performed to minimize the risk of complications during parturition. The 5th pregnancy of TL3 produced a nonviable fetus, but a subsequent litter produced

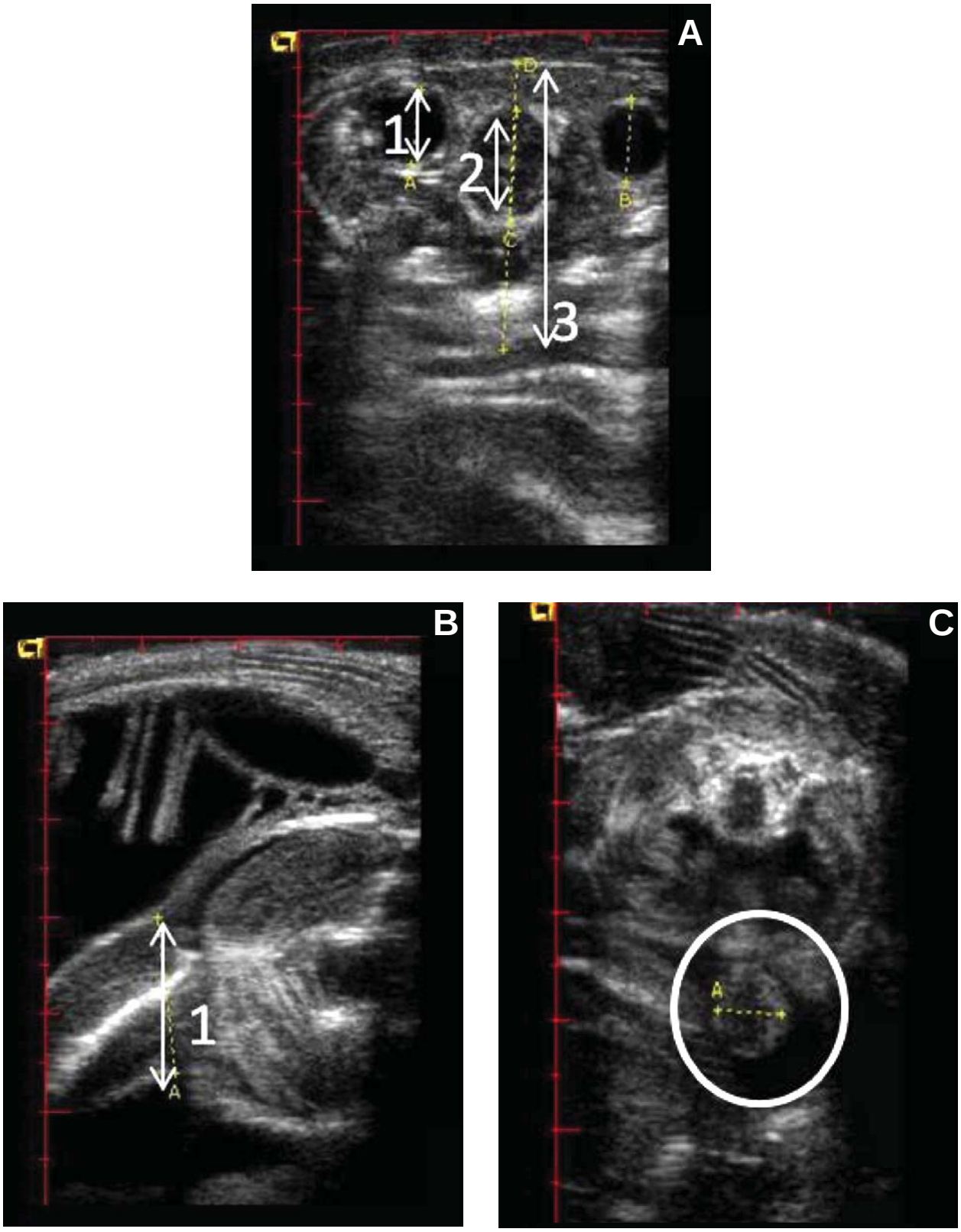

Figure 1. Ultrasound images of ribbontail stingray, *Taeniura lymma* (Forsskål, 1775), embryos showing *in utero* morphometrics. Measurements include: (A) eye diameter (ED) (1), brain cavity height (BC) (2) and body height (BH) (3); (B) maximum wing height at the main wing internal cartilage (WH) (1); and (C) yolk sac size (YS) (white circle).

two healthy pups. Gestation times for TL1, TL3 and TL4 varied from 128 - 203 days, with a mean (± SE) gestation of 157 ± 24 days (n = 11). Although a substantial intraspecific variation in gestation times was observed, gestation times for a single individual were similar in length. It should be noted that copulation was not directly observed, so a precise gestation period could not be determined with 100% certainty. However, prior observations at the Oceanário de Lisboa indicated that copulation occurred immediately following parturition. Parturition was therefore used as the starting point for subsequent pregnancies.

Prediction of birth date

To determine if *T. lymma* birth date could be predicted using *in utero* anatomical morphometrics during pregnancy, WH, BH, ED and BC were correlated with gestation time using Spearman's rank correlation coefficient. BH ($R = 0.92$; $R^2 = 0.85$; $p < 0.01$) and ED ($R = 0.89$; $R^2 = 0.79$; $p < 0.01$) were the most highly correlated parameters and subsequently used for a multivariate linear regression analysis to predict parturition date. Multivariate analysis ($R = 0.93$; $R^2 = 0.87$; $p < 0.05$) confirmed that gestation age of *T. lymma* could be estimated using the formula:

Gestation age =
-76.25 + (4.71 x BH) + (11.27 x ED)

Assuming a mean gestation time for *T. lymma* of 157 ± 24 days, birth date can estimated using the formula:

Days to birth = 157 - Gestation age

Using this tool, the husbandry team at the Oceanário de Lisboa were able to anticipate the approximate birth date of *T. lymma* and take appropriate steps to ensure the safety of the mother and newborn pups.

DISCUSSION

In ectothermic viviparous elasmobranchs, gestation time can be influenced by several factors, including water temperature, salinity, photoperiod and diet (George et al., 2009). In this study, environmental and nutritional conditions were maintained throughout the study and were the same for all animals. These conditions should be taken into account when considering extrapolation of this technique to assess *T. lymma* in other facilities.

Unfortunately, no copulations were observed at the Oceanário de Lisboa, during the period of study, so a precise gestation time could not be definitively determined. It was assumed that copulation immediately followed parturition, or occurred when the male *T. lymma* first had access to a female following parturition. In addition, it was assumed that there was no intra-oviductal sperm storage, that the female *T. lymma* were of equivalent maturity and reproductive status, that a single fetus will develop the same as multiple fetuses, and that body height (BH) was equivalent whether measured at the eye or the branchia. These assumptions, in conjunction with the small sample size, imply that the derived formula should be used with caution.

Despite these restrictions, it can been concluded that the use of ultrasonography to assess the health of a gravid mother and the vitality of the fetus, as well as monitoring embryogenesis, can be a valuable tool for assessing elasmobranch pregnancy. In addition, tracking *in utero* morphometrics holds promise as a mechanism to predict delivery dates.

ACKNOWLEDGEMENTS

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Chapter 32

Chemical immobilization of elasmobranchs at uShaka Sea World, Durban, South Africa

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Abstract: Various circumstances necessitate handling of elasmobranchs, yet physical manipulation can risk injury to the animal or the human carer. The chemical immobilizer 2-phenoxyethanol has been successfully used by the authors to sedate 14 species of elasmobranch, for routine husbandry procedures, at uShaka Sea World (Durban, South Africa) reducing handling risks to both elasmobranchs and husbandry personnel. Optimal anesthesia for the majority of animals sedated was achieved at an immersion concentration of 0.15 mL/L 2-phenoxyethanol. For sedating larger individual elasmobranchs, excellent results have been achieved using a combination of 0.1mg/kg medetomidine and 1.0 mg/kg butorphanol administered intramuscularly (IM). More than 100 sharks and rays, representing eight species, have been successfully sedated and handled using this anesthetic regime.

INTRODUCTION

uShaka Sea World is a large public aquarium based at the uShaka Marine World Park in Durban, South Africa. The aquarium complex holds a total volume of 22,000 m^3 and supports a variety of marine life, including marine mammals, penguins and over 300 species of fishes, including 23 species of elasmobranch.

Elasmobranchs at uShaka Sea World need to be handled for a variety of husbandry procedures. However, handling elasmobranchs presents an opportunity for the animals to damage themselves or their handlers. To reduce the risk of damage, and increase safety for both staff and animals, techniques to immobilize/sedate elasmobranchs have been developed by the team at uShaka Sea World. Suitable drugs have been investigated, and appropriate dosages established and refined over several years.

While many drugs may be used to immobilize, sedate, or anesthetize animals, based on relative dose, for the purpose of this chapter we use the blanket term "sedate" to cover the entire spectrum of effects, unless otherwise specifically noted.

DRUG SELECTION

A number of key factors should be considered when choosing a sedative, which can be summarized as follows:

1. Safety for both animal and user;
2. Previous results for the species in question;
3. Required depth of sedation or anesthesia;
4. Required duration of effect;
5. Need for, and availability of, an antidote; and
6. Any legislative implication for use of the chosen drug.

An 'ideal' sedative should have the following characteristics:

1. An effective dose small enough to be delivered in a quick and safe manner;
2. Rapid absorption into the animal's system;
3. Rapid onset of action, resulting in sufficient sedation;
4. A wide margin of safety;
5. Minimal side effects;
6. Minimal cardiopulmonary depression; and
7. Reduction of the animal's awareness of its surroundings, minimizing fear, distress and pain.

Drugs used for chemical restraint of wild animals can be grouped into four classes (Swann, 1993): anesthetics, opioids, hypnotics and sedatives, and tranquilizers and neuromuscular blockers.

Anesthetics

Anesthetics result in loss of consciousness, with loss of pain sensation and muscle tone. The animal cannot be roused unless drug dosing has ceased, or an antidote or reversal agent has been administered.

Opioids

Opioid analgesics (also called narcotics) interact to a variable extent with the animal's endorphin receptors. Opioids may act as agonists (capable of stimulating a response in the specific nerve receptor), partial agonists or antagonists (blocking a response to the nerve receptor), resulting in great variation in their pharmacological effects. Opioid effects on the central nervous system (CNS) differ from one species to another (Grimm and Lamont, 2007). The effects on the cardiovascular system are highly-variable, depending on the drug, its dose and the species concerned. In general, though, high doses cause bradycardia (Hall et al., 2001).

Hypnotics and sedatives

Hypnotics and sedatives moderately depress the CNS and can produce a physiological state of sleep, from which a subject can easily be aroused. These drugs enable the animal to go to sleep more easily, or are used to intensify the depth of sleep. Hypnotics and sedatives are dose dependent with light sedation achieved at low doses, and hypnotic effects at increased dosages. Some sedatives can produce general anesthesia at high concentrations (Swann, 1993).

Tranquilizers

Tranquilizers are psychotropic agents that result in the suppression of behavioral responses, such as flight or aggression, without affecting spinal reflexes. The predominant action of tranquilizers is relieving anxiety, without producing undue sedation (Hall et al., 2001).

Despite the discrete nature of the four established classes of drug, it is rare that the effects of a drug are limited to a single classification. Many drugs have a sedative effect when administered at low dosages, but work as an anesthetic when administered at a higher dose (Swann, 1993; Hall et al., 2001). Also, many drugs, when used in combination, have a synergistic effect on each other

(i.e., the use of two drugs reduces the amounts of each required to cause effect).

The husbandry team at uShaka Sea World has used both immersion and intramuscular chemical restraint drugs to aid the handling of over 100 individual sharks and rays, representing 19 species (Table 1).

GROUP SEDATION

Various chemical agents (clove oil, tricaine methanesulfonate, isoflurane, 2-methylquinoline, 2-phenoxyethanol) have been tested at uShaka SeaWorld for the simultaneous sedation of a group of animals through system-wide immersion application.

2-phenoxyethanol

2-phenoxyethanol (ethylene glycol mono-phenyl ether) proved to be the most effective sedative. 2-phenoxyethanol is a colorless, oily liquid that has been used as a cooling lubricant, an antibacterial and antifungal preservative in cosmetics, a fixative in perfumes and as a sedative in fishes (Dunn and Koester, 1985; Deacon et al., 1997; Hseu et al., 1998; Inoue et al., 2004; Hajek et al., 2006; Vaughan et al., 2008).

2-phenoxyethanol is a practical sedative, suitable for groups of fishes that require repeated sedation (Deacon et al., 1997). It is considered safe for humans, is relatively inexpensive and remains viable for long-term exposure (e.g., for the duration of a long-distance animal transport) (Kaiser and Vine, 1998). The sedative effects of 2-phenoxyethanol are observed as long as the fish is exposed to the drug. The exact mechanism of action of 2-phenoxyethanol is unknown in fishes, but it has been suggested to involve an expansion of neuronal cell membranes and the suppression of neural activity in higher regions of the nervous system (Zahl, 2011).

Elasmobranch sedation with 2-phenoxyethanol has proven to be highly successful, with a large safety margin, and is now routinely used as an integral part of animal collection management at uShaka Sea World. Optimal sedation with 2-phenoxyethanol was achieved at a concentration of 0.15 mL/L. Higher concentrations of 2-phenoxyethanol did not appear to have negative effects, but animals showed a more rapid and deeper sedation. Work by Weyl et al. (1996) showed that recovery rate of goldfish, *Carassius auratus* (Linnaeus, 1758), sedated with 2-phenoxyethanol, was independent of the duration of exposure to the drug. This finding was corroborated by observed responses of elasmobranchs sedated with 2-phenoxyethanol at uShaka Sea World.

2-phenoxyethanol was administered by mixing the drug with tap water, in large buckets, and then adding the mixture to the seawater within the tank holding the animals (Figure 1). Most sharks and rays became sedated within a few minutes. When steered into a soft PVC stretcher for restraint (Figure 2), the animals rarely attempted to bite and rapidly became subdued. Following husbandry procedures, sedation was reversed by placing animals in clean seawater and animals typically recovered within 10 minutes.

INDIVIDUAL SEDATION

Being a non-selective immersion anesthetic, 2-phenoxyethanol cannot be used to sedate an in-

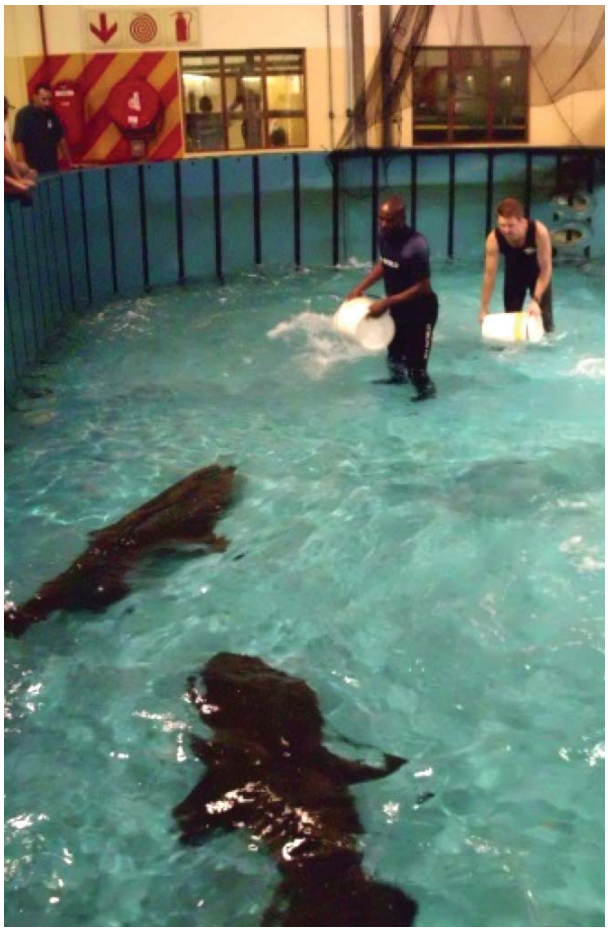

Figure 1. Pre-mixed 2-phenoxyethanol and tap water being introduced into a holding tank to induce sedation in sand tiger sharks, *Carcharias taurus* (Rafinesque, 1810).

Table 1. Species of sharks and rays sedated by staff at uShaka Sea World, Durban, South Africa, showing drug(s) used and route(s) of administration.

Species name	Common name	Sedative type(s)	Administration
Aetobatus narinari (Euphrasen, 1790)	Spotted eagle ray	2-phenoxyethanol / Medetomidine + Butorphanol	Immersion / intramuscular
Aetomylaeus bovinus (Geoffroy Saint-Hilaire, 1817)	Bull ray	2-phenoxyethanol	Immersion
Carcharias taurus (Rafinesque, 1810)	Sand tiger shark	2-phenoxyethanol / Medetomidine + Butorphanol	Immersion / intramuscular
Carcharinus albimarginatus (Rüppell, 1837)	Silvertip shark	Medetomidine + Butorphanol	Intramuscular
Carcharinus brevipinna (Müller & Henle, 1839)	Spinner shark	Medetomidine + Butorphanol	Intramuscular
Carcharinus leucas (Müller & Henle, 1839)	Bull shark	Medetomidine + Butorphanol	Intramuscular
Carcharinus melanopterus (Quoy & Gaimard, 1824)	Blacktip reef shark	2-phenoxyethanol	Immersion
Carcharinus obscurus (Lesueur, 1818)	Dusky shark	Medetomidine + Butorphanol	Intramuscular
Dasyatis marmorata (Steindachner, 1892)	Marbled stingray	2-phenoxyethanol	Immersion
Dasyatis thetidis (Ogilby, 1899)	Thorntail stingray	2-phenoxyethanol	Immersion
Dasyatis uarnak (Gmelin, 1789)	Honeycomb stingray	2-phenoxyethanol	Immersion
Galeocerdo cuvier (Péron & Lesueur, 1822)	Tiger shark	Medetomidine + Butorphanol	Intramuscular
Mobula kuhlii (Müller & Henle, 1841)	Shortfin devil ray	2-phenoxyethanol	Immersion
Mustelus mustelus (Linnaeus, 1758)	Smooth-hound	2-phenoxyethanol	Immersion
Myliobatus aquila (Linnaeus, 1758)	Common eagle ray	2-phenoxyethanol	Immersion
Rhina ancylostoma (Bloch & Schneider, 1801)	Bowmouth guitarfish	2-phenoxyethanol	Immersion
Rhinobatos annulatus (Müller & Henle, 1841)	Lesser sandshark	2-phenoxyethanol	Immersion
Rhynchobatus djiddensis (Forsskål, 1775)	Giant guitarfish	2-phenoxyethanol / Medetomidine + Butorphanol	Immersion / intramuscular
Taeniurops meyeni (Müller & Henle, 1841)	Round ribbontail ray	2-phenoxyethanol	Immersion

Figure 2. A sedated sand tiger shark, *Carcharias taurus* (Rafinesque, 1810), being guided into a restraint stretcher.

dividual animal in a group environment. Husbandry personnel at uShaka Sea World trialled a range of injectable sedatives on their elasmobranch collection, including: medetomidine, butorphanol, etorphine (M99), diazepam, midazolam and ketamine.

Medetomidine and butorphanol

After extensive testing, the drug regime of choice for large elasmobranchs, providing excellent sedation results for individual sharks and rays, was 0.1 mg/kg IM medetomidine, in combination with 1.0 mg/kg IM butorphanol.

Medetomidine is a potent sedative. It stimulates receptors centrally, resulting in dose-dependent sedation and analgesia. Substantial dose sparing properties occur when medetomidine is combined with other anesthetics (Sinclair, 2003). The beneficial effects of medetomidine are reliable sedation, analgesia, muscle relaxation and anxiolysis (a reduction in anxiety), as well as a decrease in the anesthetic requirements of other injectable and inhalant agents (anesthetic sparing) (Sinclair, 2003). Caution must be taken when handling this potent drug, as it is dangerous to humans.

Butorphanol is a synthetic, opioid analgesic that has been used extensively in a wide range of species. Butorphanol has minimal effects on cardiopulmonary function. This drug does not show the usual respiratory depression characteristic of opioid analgesics, but rather reaches a 'ceiling' beyond which supplemental doses do not cause further depression (Swann, 1993).

Medetomidine and butorphanol were typically delivered to elasmobranch at uShaka Sea World using injection darts (Paxarms System, Cheviot, New Zealand). To calculate required dosages of medetomidine and butorphanol, the weight of the animal was estimated prior to darting. In order to keep the volume of drugs to a minimum, sedatives were acquired in high concentrations, ensuring that a minimum number of darts were needed for sedation. Medetomidine and butorpanol were mixed in a single syringe and administered via pressurized dart injection into the dorsal saddle of the animal to be sedated (Figure 3).

Figure 3. A pressurized dart, containing an injectable sedative, in the dorsal saddle of a dusky shark, *Carcharinus obscurus* (Lesueur, 1818).

After successful delivery of the sedative, the elasmobranch was left alone while the drug took effect. Each species reacted slightly differently to the drug combination, but reactions were typically benign. Water temperature and swimming activity had an impact on the efficacy of the drugs. Elasmobranch swimming behavior would change and they would eventually lie recumbent on the floor of the tank after ~40 - 60 min. Once recumbent, divers were able to maneuver the elasmobranch into a large, clear plastic bag of 0.4 - 0.8 mm thickness (Figure 4). The restraint bag was then raised to the surface, where the animal was placed into a PVC stretcher for handling, examination and husbandry procedures.

Atipamizole and naltrexone

Upon completion of handling, antidotes were administered. Each sedative had a companion antidote. Atipamizole, a medetomidine antidote, was administered at 0.5 mg/kg IM (five times the dosage concentration of medetomidine). Naltrexone was administered at 10 mg/kg IM to reverse the effects of butorphanol.

Atipamezole is a potent, selective reversal agent exhibiting activity at both central and peripheral receptor sites. Atipamezole has been developed specifically as an antidote to medetomidine, and is able to reverse behavioral, cardiovascular, gastrointestinal, neurochemical and hypothermic sedative effects (Swann, 1993).

Naltrexone is an opioid reversal agent. Naltrexone has a high affinity for opioid receptors and is able to displace opioid anesthetics. After displacement, the chemical binds to, and occupies, opioid receptors, but does not activate them (Grimm and Lamont, 2007). In this way, it stops and reverses the action of an opioid anesthetic such as butorphanol.

These two antidote drugs were administered separately (IM) into the dorsal saddle of the elasmobranch. Animals were typically left alone to recover. After 10 - 20 min, the elasmobranch was generally able to move, breathe and swim unaided.

CONCLUSIONS

Based on successful trials at uShaka Sea World, we recommend the use of chemical sedation to aid the safe handling of elasmobranchs. The drugs and dosages described in this chapter (summarized in Table 2) satisfy the characteristics identified for the ‘ideal’ sedative. Care should always be exercised when using sedatives as drug concentrations may vary according to supplier. Some drugs employed by the team at uShaka Sea World were specifically formulated by local suppliers, on request, to provide a higher concentration of active ingredient per volume.

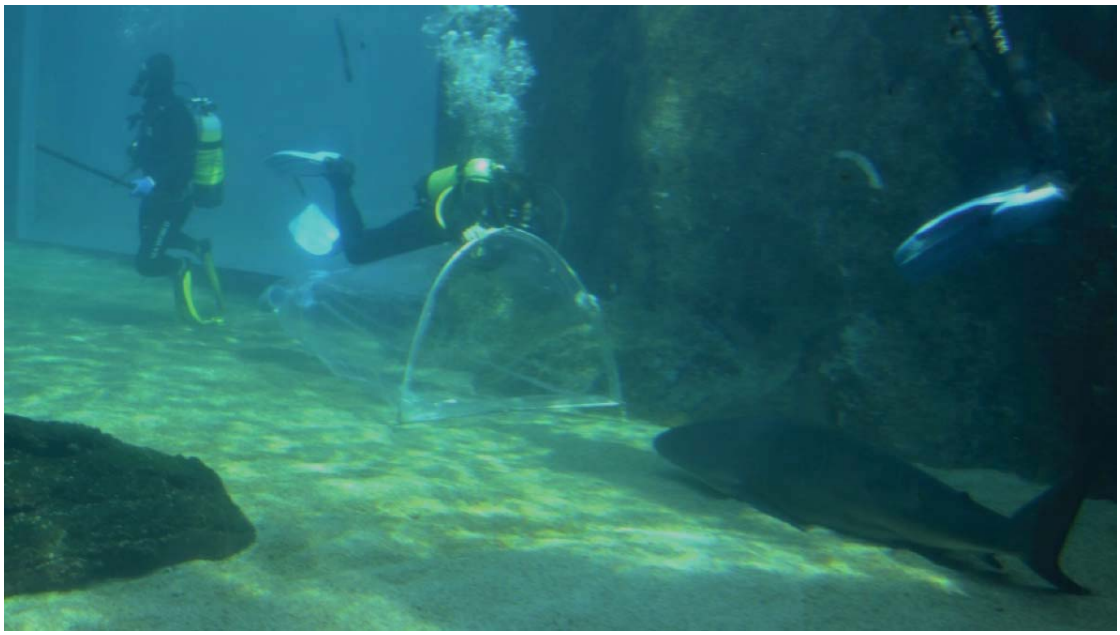

Figure 4. Divers preparing to move a sedated dusky shark, *Carcharinus obscurus* (Lesueur, 1818), into a plastic bag, for transfer to a restraint stretcher at the surface where husbandry procedures were performed.

Table 2. Summary of recommended sedation drugs based on extensive trials at uShaka Sea World, Durban, South Africa. Note: All concentrations listed refer to the active ingredient of the sedative.

Drug	Dosage	Administration
Group sedation		
2-phenoxyethanol	0.15 mL/L	Immersion
Individual sedation		
Medetomidine	0.1 mg/kg	Intramuscular
Butorphanol	1.0 mg/kg	Intramuscular
Antidote		
Atipamezole	0.5 mg/kg	Intramuscular
Naltrexone	10.0 mg/kg	Intramuscular

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Chapter 33

Anesthetic trials using various species of elasmobranch at Nausicaá Aquarium

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Abstract: At Nausicaá Aquarium, Boulogne sur Mer, France, the sedation of many species of elasmobranch has been performed for a variety of medical and husbandry purposes. Combinations of injectable and immersion sedatives, including the rarely used injectable drug etomidate, have been trialed. Etomidate proved to be a useful sedative for short-duration animal transports and handling procedures. Longer immobilization, or a deeper stage of anesthesia, was achieved by using supplemental doses of etomidate, by using etomidate in combination with immersion in eugenol, or by using etomidate in combination with other injectable drugs, such as medetomidine. Etomidate proved to be a reliable

alternative to ketamine and xylazine, a drug combination used historically at Nausicaá. Eugenol showed great promise, either alone or as a maintenance drug after induction with etomdiate.

INTRODUCTION

Elasmobranchs are among the most iconic animals displayed in public aquaria. Unfortunately, elasmobranchs are facing a wide range of anthropogenic challenges in their wild range. As a consequence, elasmobranchs are highly valuable to aquaria as they are representative of many conservation priorities to be communicated to the public. Correspondingly, high-quality management of an elasmobranch collection is central to an aquarium husbandry program.

Anesthetics historically used to capture and transport elasmobranch are increasingly being used for other husbandry procedures, including routine animal examinations, health diagnostics, reproductive studies and surgical treatments. These improved husbandry practices are all contributing toward an increased sustainability of aquarium elasmobranch populations.

A variety of husbandry and research procedures involving elasmobranchs can be facilitated by anesthesia, improving safety for both the animal and the human handler(s). These procedures include: basic morphometrics, transponder insertion or attachment, ultrasonography, biopsies, blood sampling, acite fluid collection, sampling of cerebrospinal fluid, swab and cytology sampling, gastric palpation, fibroscopy, radiography, assisted feeding, treatment of wounds and surgery. The variaous levels of anesthesia are described in Table 1 (McFarlane, 1959), which should be used as a guide in helping to determine the suitability of a drug for a given procedure. An appropriate plane of sedation for elasmobranch transportation or simple clinical procedures is in the range of stage I - plane 2 to stage II - plane 1 (Smith, 1992).

In 1998, the husbandry team at Nausicaá trialed the use of xylazine, alone and in combination with ketamine, as a sedative for large elasmobranchs. The results of these trials were mixed, elasmobranchs responding to the drug(s) in unpredictable ways. In 2001, an alternative sedative, etomidate, was selected and tested. Combining etomidate with medetomidine showed promise as a useful combination for elasmobranch anesthesia. The immersion sedative eugenol was also trialed. Eugenol provided a reliable and repeatable level of anesthesia, usable on a regular basis, and presented minimal risk to the human carer. This chapter reports on the outcome of these anesthetic trials and provides additional recommendations for the sedation of large elasmobranchs.

XYLAZINE

Xylaxine is an alpha 2 agonist. Alpha 2 agonist molecules act on alpha 2 adrenergic receptors and have a strong analgesic activity in vertebrates (Lamont and Grimm, 2014). Atipamezole, a synthetic alpha 2 adrenergic receptor antagonist, can be administered at a dosage ratio of 1:10, atipamezole:xylazine, to reverse the effects of xylazine. The action of alpha 2 adrenergic agonist molecules has been little studied in fishes.

Smith (1992) recommended xylazine at a dosage of 6 mg/kg, in the range 3.5 - 9.5 mg/kg, to sedate large elasmobranchs for up to 5 h. A lethal dose of xylazine was cited as 50 mg/kg. In 1998, the husbandry team at Nausicaá trialed the use of xylazine as a sedative for leopard sharks, *Triakis semifasciata* (Girard, 1855). Six specimens (1 male and 5 females), with a mean (± SE) body mass (BM) of 675.5 ± 147.5 g (range: 310 - 1,350 g), were administered 6 (n = 1), 12 (n = 1) or 18 (n = 4) mg/kg xylazine. No anesthetic effect was observed at any dose, either before or after reversal with atipamezole. A further experiment was conducted to determine if xylazine administered at 40 mg/kg represented a lethal dose to brown smooth-hounds, *Mustelus henlei* (Gill, 1863). Observations of dosed *M. henlei* at 1, 2, 3, 24 and 48 h showed no negative effects, and the sharks continued to swim, eat and behave normally.

Ketamine and xylazine

Ketamine can amplify the effects of xylazine when given concurrently. Ketamine is a psychodysleptic, or dissociative anesthetic, in the arylcyclohexylamine family, which affords the animal some degree of vigilance during anesthesia. The mechanism of action of ketamine involves the antagonism of several neuroreceptors, predominantly the N-methyl-D-

Table 1. Different stages of anesthesia in fishes showing corresponding behavioral changes (after McFarland, 1959).

Stage	Plane	Description	Behavioral responses
Stage 0	Plane 0	Normal	Swimming actively. Reaction to external stimuli normal. Equilibrium and muscle tone normal.
Stage 1	Plane 1	Light Sedation	Voluntary swimming continues. Slight loss of receptivity to visual and tactile stimuli. Respiration, equilibrium and muscle tone normal.
Stage 1	Plane 2	Deep Sedation	Voluntary swimming stopped. Loss of receptivity to visual and tactile stimuli. Respiration and muscle tone slightly depressed. Equilibrium normal. Response to positional changes normal.
Stage II	Plane 1	Light Narcosis	Light narcosis preceded by an excitement phase. Respiration increased. Equilibrium loss increases. Muscle tone decreased. Weak response to positional changes.
Stage II	Plane 2	Deep Narcosis	No response to positional changes. Respiration decreased. Receptivity to strong tactile, vibrational and positional changes only.
Stage III		Surgical Narcosis	Total loss of reactivity. Ventilation rate (opercular rate) very slow. Heart rate very slow. The level for surgical anesthesia.
Stage IV		Medullary Collapse	Respiratory movement ceases. Onset of Cardiac arrest. The level for euthanasia or overdose.

aspartate receptor. During and after recovery, ketamine can cause some cardioventilatory and neurological challenges. The recommended dosage rate for ketamine, based on nominal rates for dogs and cats, is 10 - 20 mg/kg body mass by intramuscular (IM) injection.

The concurrent administration of ketamine and xylazine was assessed for anesthetic effectiveness in eight (2 male and 6 female) *T. semifasciata* of 447.5 ± 1.2 g mean BM (range: 300 - 610 g). Trials with ketamine and xylazine at 7 mg/kg and 3 mg/kg (n = 2), and 15 mg/kg and 6 mg/kg (n = 2), respectively, yielded no effect. When *T. semifasciata* (n = 4) were administered ketamine and xylazine at a dosage of 20 mg/kg and 10 mg/kg, respectively, sedation was induced within 5 - 9 min and achieved a variable result, from stage 1 - plane I to stage II - plane 2. Atipamezole was not used. Recovery occurred 10 - 18 min after sedation was induced.

A further study was conducted (summarized in Table 2) using a combination of ketamine and xylazine for the sedation, capture and transport of sand tiger sharks, *Carcharias taurus* (Rafinesque, 1810), and sandbar sharks, *Carcharhinus plumbeus* (Nardo, 1827). In all cases,

Table 2. Anesthesia of sand tiger sharks, *Carcharias taurus* (Rafinesque, 1810), and sandbar sharks, *Carcharhinus plumbeus* (Nardo, 1827), using a combination of ketamine and xylazine, and reversal with atipamezole. T: time of drug injection. [continued laterally on the facing page]

No	Species	Sex	Estimated body mass (kg)	Xylazine dosage (total) (mg/kg)	Ketamine dosage (total) (mg/kg)	Induction time (min)	Duration of anesthesia (min)	Atipamezole dosage (mg)
1	*Carcharias taurus*	F	200	6	15	20	70	120
2	*Carcharias taurus*	F	200	6	15	30	55	120
3	*Carcharhinus plumbeus*	-	55	6	20	10	15	33
4	*Carcharhinus plumbeus*	M	55	6	10	18	26	33
5	*Carcharhinus plumbeus*	-	55	6	15	70	80	33
6	*Carcharhinus plumbeus*	F	80	6	25	39	Released without chemical reversal	
7	*Carcharhinus plumbeus*	M	50	6	20	15	20	33
8	*Carcharhinus plumbeus*	F	60	6	20	5	10	36
9	*Carcharhinus plumbeus*	-	45	6	20	5	15	33

only stage I - plane 1 anesthesia was attained. Additional doses of anesthetic were required to ensure the sharks remained sedated in most cases. Atipamezole, delivered intra-muscularly (IM), was used as a reversal agent. Recovery, following reversal, was deemed as the time as which the shark was swimming unaided and was not exhibiting emergence reactions.

The collective results from all three trials indicated that xylazine alone, or in combination with ketamine, was not a reliable anesthetic for larger shark species.

Ketamine and medetomidine

Medetomidine is an alpha 2 agonist, but is more selective than xylazine and has a better affinity for alpha 2 adrenergic receptors. In addition, fewer adverse effects on various organs are seen with medetomidine when compared to xylazine (Lamont and Grimm, 2014). Medetomidine is typically combined with ketamine, allowing for a reduced dose of each drug, and has been used for the transportation of elasmobranchs (Snyder et al., 1998). The effects of medetomidine can be reversed using atipamezole at a dosage ratio of 5:1, atipamezole:medetomidine.

The husbandry team at Nausicaá trialed the use of ketamine in combination with medetomidine to sedate five *C. plumbeus* for the purposes of medical examination (Table 3). Sedation was reversed with atipamezole, delivered as a slow intravenous (IV) injection. One of the *C. plumbeus* struggled excessively prior to injection and displayed hyperglycemia once sedated. This specimen died three weeks following the procedure, possibly

Time for recovery (min)	Sedation observations	Recovery observations
30	On second injection (T+3 min), shark twisted and lay on its side; it responded to visual stimuli and presence of divers.	Swam a little and glided to bottom; remained on the bottom without swimming; deceased three weeks after procedure.
12	Ataxia at T+9 min; reacted negatively to light; stopped swimming at T+16 min.	Intermittently swam and rested on bottom over a period of 10 h.
< 1	Ataxia at T+3 min.	Normal behavior.
3	Ataxia at T+8 min; collided with wall.	Normal behavior.
1	Stopped swimming at T+25 min; responded to presence of divers and evaded capture.	Normal behavior.
	Displayed difficulty swimming at T+8 min.	Swam immediately after release; displayed biting at the wall at T+43 min.
2	Slower swimming at T+8 min; stopped swimming at T+10 min.	Displayed difficulty swimming.
4	Collided with wall causing significantly trauma to rostrum; shark caught prior to anesthetic effects observed.	Displayed difficulty swimming; required euthanasia after three days as a result of injury to nose.
< 1	Collided with wall.	Normal behavior.

suffering from profound biochemical changes (i.e., severe acidosis) resulting from pre-capture hyperactivity.

ETOMIDATE

Some anesthetic drugs used in aquaculture (e.g., etomidate and its analog, metomidate) are classified as hypnotics and do not have excitatory effects during sedation. Etomidate acts by fixation of $GABA_A$ receptors (i.e., by improving their receptivity), but does not interact with other receptors, explaining the lack of analgesic activity. Etomidate is less of a cardiorespiratory depressant than other $GABA_A$ agonists, like barbiturates. Etomidate does, however, decrease secretion of cortisol, which can cause severe disturbance of the adreno-cortical axis during a long anesthesia. Etomidate is generally administered via immersion (Amend et al., 2011; Limsuwan et al., 2011), but can be administered IM. In general, it is preferable to administer etomidate in a single injection, as anesthetic induction is generally faster and deeper than achieved when using fractioned injections. This phenomenon is likely due to rapid metabolism of the drug by the liver and kidney (biliary and renal excretion).

The team at Nausicaá trialed the use of etomidate IM to sedate seven *C. taurus* for general husbandry procedures (Table 4). Animals #1, #2, #3 and #4 were administered a low dose of etomidate and only a reduction in reflexes was achieved. Although these animals struggled when handled by the divers, they could be caught and restrained.

Table 3. Anesthesia of sandbar sharks, *Carcharhinus plumbeus* (Nardo, 1827), using a combination of ketamine and medetomidine, and reversal with atipamezole. T: time of drug injection. [continued laterally on the facing page]

Animal	Sex	Body mass (kg)	Ketamine dosage (mg/kg)	Medetomidine dosage (µg/kg)	Induction time (min)	Duration of anesthesia (min)	Atipamezole dosage (mg)
1	M	14.5	2.76	34.5	5	27	2.5
2	M	15.7	2.5	25.4	7	24	2
3	M	12.4	3.23	32.3	7	24.5	2
4	F	12.5	4	32	6.5	27	4
5	M	15.3	3.26	32.6	8.5	21.5	2.5

Table 4. Anesthesia of sand tiger sharks, *Carcharias taurus* (Rafinesque, 1810), using etomidate. BPM: beats per minute. Pp: partial pressure. [continued laterally on the facing page]

Animal	Sex	Body mass (kg)	Number of injections	Time between injections (min)	Etomidate dosage (mg/kg)	Induction time (min)
1	F	77.0	2	18	0.38 (0.19 + 0.19)	32
2	M	52.0	2	25	0.53 (0.38 + 0.15)	50
3	F	61.5	1		0.39	27
4	M	63.0	1		0.38	16
5	F	77.0	2	2	1.20 (0.65 + 0.65)	11
6	F	87.5	2	23	1.60 (1.14 + 0.45)	40
7	M	76.5	1		1.30	7

Animals #5, #6 and #7 were administered a higher dose of etomidate, and a more rapid and deeper level of anesthesia was achieved. These animals slowed, came to a complete stop and did not struggle when handled.

In general, the sharks were captured immediately following administration of the anesthetic, at which point heart rates were elevated. Once the sedated sharks were transferred to a tank, pre-supersaturated with oxygen, and hyperactivity had

Time for recovery (min)	Sedation observations	Time (min)	Glycemia (g/L)	Time (min)	Glycemia (g/L)
6	Injected in two doses, with 1 min interval; shark manually restrained before injection; specimen struggled excessively; specimen died 3 weeks after procedure.	T+9:	0.69	T+26:	0.98
1		T+10:	0.85	T+23:	0.40
7.5		T+10:	0.67	T+26:	0.56
1.25	No sedative effect on first attempt; syringe fell out and drug exited injection site.	T+9:	0.65	T+26:	0.40
5	Additional injection of anaesthetic required after 8 min.	T+17:	0.71	T+29:	0.57

Anesthesia level (stage - plane)	Time	Heart rate (BPM)	Pp O_2 (%)	Time	Heart rate (BPM)	Pp O_2 (%)
I - 1	-	-	-	-	-	-
I - 1	-	-	-	-	-	-
I - 1	-	-	-	-	-	-
I - 1	-	-	-	-	-	-
II - 2	T+24:	106	59	T+26:	94	60
II - 1	T+47:	160	70	T+62:	60	78
II - 2	T+11:	160	40	T+24:	75	74

ceased, they displayed a reduced ventilation rate, reduced heart rate (bradycardia), and the partial pressure of oxygen in their blood increased. Ventilation rate was typically around 35 buccal pumps per minute (BPPM) immediately following injection of etomidate, but decreased rapidly to 16 - 18 BPPM. In one case, ventilation rate dropped to as low as 12 BPPM. Approximately 10 min after sedation, ventilation rates returned to normal.

Table 5. Anesthesia of scalloped hammerhead sharks, *Sphyrna lewini* (Griffith & Smith, 1834), using a combination of etomidate and medetomidine. IV: intravenous. IM: intramuscular. T: time of drug injection. BPM: beats per minute.

Animal	Sex	Body mass	Etomidate dosage	Medetomidine dosage	Method of delivery	Induction time	Duration of anesthesia	Atipamezole dosage	Time for recovery	Time	Heart rate
			(mg/kg)	(µg/kg)		(min)	(min)	(mg/kg)	(min)	(min)	(BPM)
1	F	7.8	0.79	23	IV	3	21	0.13	1	T+18:	70
2	F	4	1.30	50	IM	2	23	0.25	1	T+17:	60

Etomidate and medetomidine

The team at Nausicaá trialed etomidate, in combination with medetomidine (Table 5), to sedate two scalloped hammerhead sharks, *Sphyrna lewini* (Griffith & Smith, 1834). The first animal was caught, placed into tonic immobility and administered the anesthetic via IV injection. The second animal was administered the anesthetic via IM remote injection. Sedation induction via IM injection was rapid, no longer than when the drugs were administered IV. Sedation was reversed with atipamezole, delivered as a slow intravenous (IV) injection, and the sharks were released as soon as voluntary movement of the caudal fin was observed. Both sharks swam at the surface for a few minutes, but were swimming mid-water within six minutes.

Results showed that etomidate, or a combination of etomidate and medetomidine, may be a promising anesthetic regime for sedating, capturing

Table 6. A summary of elasmobranch species anesthetized at Nausicaa Aquarium using eugenol, showing dosage rates, induction times, anesthetic level reached and recovery times. [continued laterally on the facing page]

Species name	Common name	Body weight (kg)
Carcharhinus melanopterus (Quoy & Gaimard, 1824)	Blacktip reef shark	3
Carcharhinus plumbeus (Nardo, 1827)	Sandbar shark	40
Dasyatis pastinaca (Linnaeus , 1758)	Common stingray	4
Orectolobus maculatus (Bonnaterre, 1788)	Spotted wobbegong	9
		7
Pristis microdon (Latham, 1794)	Largetooth sawfish	11
Rhina ancylostoma (Bloch & Schneider,1801)	Bowmouth guitarfish	17
Rhinobatos cemiculus (Geoffroy Saint- Hilaire,1817)	Blackchin guitarfish	20
		20
		20
		21

and handling large sharks. However, it should be noted that the effects of etomidate were short-lived and were not always easy to control.

EUGENOL

Eugenol is a natural oil extracted from cloves (*Syzygium aromaticum*). It comes as a dark yellow liquid with a rich, aromatic odor and flavor. Commercial preparations contain a high content of eugenol (~99%). Eugenol is typically administered by immersion and can be used as an alternative to more conventional drugs used in aquaculture (e.g., tricaine methanesulfonate and phenoxyethanol). The viscosity of the oil can make dispersion of eugenol in water difficult, especially in cold water. Adequate dispersion requires dilution in ethanol, at a ratio of 1:8, and vigorous shaking before it is released into the water.

Although its mechanism of action is not well understood, eugenol has proven its effectiveness during an invasive one-hour surgical procedure on a *C. plumbeus* (Lécu et al., 2011). The husbandry team at Nausicaá trialed the use of eugenol as an anesthetic with a variety of elasmobranchs (Table 6). Whenever possible, sharks were fasted for a few days prior to anesthesia. Before applying eugenol, oxygen saturation in the water was increased to 150 - 200% in order to elevate the partial pressure of oxygen in the blood of the anesthetized animal.

Administration of eugenol via irrigation, through the use of pipes directing medicated water into the mouth and over the gills, facilitated initial induction and control of anesthetic depth, especially during prolonged procedures. Eugenol concentration could be regulated and introduced to the flow of water over the gills to carefully control anesthetic depth.

It is not uncommon for the eugenol concentration required to anesthetize fishes to reach as high as 60 mg/L. However, at Nausicaá, we found that anesthesia was typically achieved in the range 20 - 40 mg/L. Ribbontail stingrays, *Taeniura lymma* (Forsskål, 1775), required a eugenol dosage of only 20 mg/L, while 30 mg/L was sufficient to anesthetize spotted eagle rays, *Aetobatus narinari* (Euphrasen, 1790), brownbanded bamboosharks, *Chiloscyllium punctatum* (Müller & Henle, 1838), nurse sharks, *Ginglymostoma cirratum* (Bonnaterre, 1788), epaulette sharks, *Hemiscyllium ocellatum* (Bonnaterre, 1788), blue-spotted stingrays, *Neotrygon kuhlii* (Müller & Henle, 1841), and bull rays, *Aetomylaeus bovinus* (Geoffroy Saint-

Water temperature (°C)	Eugenol dosage (mg/L)	Induction time (min)	Anesthesia level (stage - plane)	Handling time (min)	Recovery time (min)
26.0	30	16	II - 2	12	30
27.0	30	14	III	81	240
26.0	30	6	II - 1	18	10
26.0	30	20	II - 1	13	11
24.2	20	14	II - 2		11
24.6	30	30	III		6
24.6	30	33	III		7
25.0	35	10	II - 2	17	9
26.0	35	16	III	19	8
26.0	35	15	III	23	6
26.0	35	14	III	26	5

Hilaire, 1817). The following species required a eugenol dosage of 35 mg/L to achieve anesthesia: horn sharks, *Heterodontus francisci* (Girard, 1855), thornback rays, *Raja clavata* (Linnaeus, 1758), lesser spotted dogfish, *Scyliorhinus canicula* (Linnaeus, 1758), nursehound, *Scyliorhinus stellaris* (Linnaeus, 1758), zebra sharks, *Stegostoma fasciatum* (Hermann, 1783) and *T. semifasciata*. Doses of ~2 - 5 mg/L were used, in general, for mild sedation of elasmobranchs during transportation.

Recovery time from eugenol appeared to be influenced by dosage rate and duration of exposure, but water quality, water temperature, species anesthetized, specimen age and specimen condition could also all impact recovery times.

Anesthesia with eugenol was used to aid assisted feeding of *T. lymna* and *A. narinari*. Regurgitation of food was observed on a couple of occasions, so if eugenol is used for assisted feeding it is important to monitor the tank for undigested food and monitor animals for the possibility of obstructed gills. In general, a low dose of eugenol for a short contact time—e.g., 20 mg/L for 19 min—is required for assisted feeding, so the risk of complications is low. Young *T. lymna* were anesthetized with eugenol at 20 mg/L, twice a day for two weeks, to facilitate assisted feeding, and no problems were observed.

Eugenol was used to anesthetize a blackchin guitarfish, *Rhinobatos cemiculus* (Geoffroy Saint-Hilaire, 1817), at a dosage of 35 mg/L. Induction time was 15 min and the animal was handled for 33 min. Ventilation rate at T+9 min was elevated (22 BPPM), characteristic of stage II - plane 3 anesthesia. At T+15 min, ventilation rate had dropped to 4 BBPM suggestive of a transition to a stage III anesthesia. At T+33 ventilation rate had risen back to 25 BBPM.

Eugenol was used to euthanize a piked dogfish, *Squalus acanthias* (Linnaeus, 1758), at a water temperature of 9°C. An initial dose of 40 mg/L eugenol achieved stage III anesthesia. The addition of 40 mg/L eugenol led to a reduction in respiration rate, but an additional 40 mg/L (i.e., a total of 120 mg/L) eugenol was required before stage IV anesthesia was reached and cardiac arrest occurred. Thus, in this single case of euthanizing a *S. acanthias*, the lethal dose was triple the concentration of eugenol required to reach stage III anesthesia.

CONCLUSIONS

Etomidate appears to be a good substitute for ketamine. No excitatory effects were observed with etomidate and, as it suppressed the cortical stress response, it did not induce hyperglycemia. Electrolyte disturbances associated with etomidate were low. As etomidate has no analgesic effect, and its half-life is short, it is useful for brief non-painful procedures only. Combining etomidate with an alpha 2 agonist should yield a longer-lasting level of working anesthesia. Medetomidine may be a more appropriate alpha 2 agonist than xylazine, as it has a higher affinity for the alpha 2 receptor.

Eugenol is an inexpensive, safe and easily applied anesthetic, which can be used repeatedly. The use of pipes to irrigate the gills with water containing regulated doses of eugenol can allow for careful control of anesthetic depth. There is a risk of hypoxia following anesthesia with eugenol, so it is important to ensure the animal is monitored during the recovery phase and that the animal is well ventilated.

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Chapter 34

Removal of an intracoelomic hook via laparotomy in a Sandbar Shark (*Carcharhinus plumbeus*)

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Abstract: A twenty-one-year-old male sandbar shark, *Carcharhinus plumbeus* (Nardo, 1827) demonstrated signs of chronic weight loss even though its appetite remained unchanged. In addition, the gradual development of a red, circular lesion on its ventral body was noted. After months without any significant changes, a ventral fistula appeared revealing the tip of a wire exposed within its center. The shark was immobilized via intramuscular remote injection through dartgun with etomidate; removed from its exhibit tank; and clinically examined with radiographic imaging, a cell blood count/serum chemistry evaluation, and the use of a metal detector along the body wall. A metallic hook was identified in the coelom about 10 cm cranial to the external fistula. The shark was transferred to an isolation pool for one month. A second immobilization via immersion in eugenol was conducted in order to perform a celiotomy. The hook was located in a liver lobe and was surgically removed. After a prolonged recovery from anesthesia, the shark was released into its primary tank and recovered uneventfully, although some of the skin sutures sloughed prematurely.

INTRODUCTION

Shark exhibits are becoming increasingly popular while the husbandry and veterinary care of elasmobranchs has continued to improve in zoos and aquariums (Smith et al., 2004). Medical and surgical procedures used in terrestrial mammals are now more easily applied to sharks due to improved anesthetic protocols (Stamper, 2007; Stamper et al., 2004) and an increased understanding of their unique physiology (Carrier et al., 2004).

Foreign bodies have been reported in a number of free-ranging sharks with different feeding strategies (Borucinska, et al, 2002; Borucinska et al., 2001; Chansue and Monanunsap, 2006) but rarely in those in aquaria. Surgical procedures reported in other fishes (Harms, 2005; Johnson, 2000; Murray, 2002) include celiotomy and surgical manipulation of coelomic organs. This report documents the diagnosis and successful extraction of a coelomic foreign body in a captive shark.

CASE REPORT

A male sandbar shark, *Carcharhinus plumbeus* (Nardo, 1827), estimated to be 20 years old based on size and estimated growth rates, was exhibited in a 1,000 m^3 pool with three other *C. plumbeus* , three sand tiger sharks, *Carcharias taurus* (Rafinesque, 1810), a nurse shark, *Ginglymostoma cirratum* (Bonnaterre, 1788), and approximately 100 bony fishes (primarily damsel fishes, *Dascyllus albisella* (Gill, 1862), and horse-eye jacks, *Caranx latus* (Agassiz, 1831)). The tank's turnover rate of 60 min and the 3% addition of new saltwater daily maintained a pH between 7.9 and 8.0, a nitrate concentration below 35 mg/L and oxygen saturation of approximately 95%. Water temperature was intentionally varied through the year from 23°C (winter) to 27°C (summer) in order to mimic a natural annual gradient. Sharks were fed once or twice a week with thawed Atlantic horse mackerel, *Trachurus trachurus* (Linnaeus, 1758), European conger, *Conger conger* (Linnaeus, 175), or European squid, *Loligo vulgaris* (Lamarck, 1798). A vitamin supplement (Aquavits, Zoovet Product, Keighley Business Centre, Keighley West Yorkshire, BD21 1AG, United Kingdom) consisting of vitamins A, D3 and E, and oligoelements including iodine in the form of kelp, was provided at every meal.

In September 2007, a diffuse red patch was noted ventrally between the pectoral fins of the shark. During the following months, although food intake remained normal (between 0.2 and 1% of body mass daily), subjectively the shark appeared to lose weight and developed a slower swimming pattern in comparison to the other sharks. In May 2008, a fistula was clearly visible medially between pectoral fins on the ventral abdomen. A small tip of foreign material, later identified as a nylon filament, was observed protruding from the fistula. Presence of a fishing gear-related foreign body was suspected, as *C. conger* fed to the sharks were sometimes caught via hook and line. It was theorized that the shark swallowed a hook hidden in a *C. conger* head or esophagus.

A restraint procedure was scheduled in August 2008 (11 months after the initial cutaneous signs were noted) to perform a clinical examination and to formulate a treatment plan. As a perforating foreign body was suspected, prophylactic antibiotic therapy was initiated, two weeks prior to the first procedure, using 250 mg marbofloxacin (Marbocylt 10%, Vetoquinol, BO 189, 70204 Lure Cedex, France) injected intramuscularly via pole syringe by divers, every 5 days for 45 days. The protocol was based upon a body mass estimate of 45 kg and a dosage of 5.5 mg/kg.

The shark was fasted for 7 days prior to the first anesthetic procedure. 40 mg of etomidate (Hypnomidate, Laboratoire Janssen-Cilag, 1 Rue Camille Desmoulins, 92130 Issy-les Moulineaux, France) was administered in two injections of 10 ml per site, into the epaxial muscles lateral to the dorsal fin, via a pole-syringe and 18 gauge needle. Within seconds an observable leak was noted at both injection sites, as previously described in these species (Stamper, 2007; Stamper et al., 2004). Another 40 mg of etomidate was injected 15 min later. Twenty minutes thereafter, when the shark's swimming pace became slow enough, tonic immobility (Watsky and Gruber, 1990) was induced by two experienced divers rotating the shark into dorsal recumbency.

The shark was placed into a sling, which was then placed into a transport tank filled with highly oxygenated water (180% O_2). A clinical exam was completed and a blood sample collected. During the procedure, the pulse rate was monitored via a pulse oximeter (Vet/Ox 4404, Heska, Grand- Places 16, 1700 Fribourg, Switzerland) with a reflectance probe placed 5

to 8 cm deep into the cloacal opening. Heart rate remained between 57 and 70 beats per minute, but a continuous reading was hampered by manipulations of the shark in and out of the water. Radiographs were taken with the shark temporarily placed in ventral and lateral recumbency on soft foam adjacent to the tank. A metal detector (AF03 MD, White's Electronics Ltd., 35J Harbour Road, Inverness, Invernessshire IV1 1UA, Scotland, United Kingdom) confirmed the presence of a metallic object near the ventral fistula. Radiographically, a hook measuring 4 cm x 1.8 cm was observed, but its exact relationship to surrounding organs could not be established, although liver contact was highly suspected. The shark was measured and its actual weight was 40 kg. As a follow-up, 250 mg of marbofloxacin was injected intramuscularly at a dosage of 6.25 mg/kg. In addition, 80 mg of dexamethasone (Dexadreson, Intervet Lab, Angers Technopole, BP 17144, 49071 Beaucouzé Cedex, France) was injected intramuscularly at the end of procedure. The shark was transferred to a cylindrical isolation pool with a strong counterclockwise current, 8 m diameter x 1.5 m deep, with a total volume of 70 m^3.

The blood sample collected from the caudal vein was used for hematologic comparisons (Mylniczenko et al., 2006) and for clinical blood chemistry evaluation (Table 1). Manual cell blood count showed mild elevation of neutrophils when compared to the latest reference ranges for sandbar sharks (Arnold, 2005; Brill, 2008; Stoskopf, 1993). Several liver-related parameters, alanine-aminotransferase and aspartate-aminostransferase, were dramatically increased above published reference ranges. The relationship between aminotransferases and liver pathology is not well defined in elasmobranches, as in mammals, but hepatitis was suspected.

One month after the first anesthetic procedure, the shark was immobilized via immersion anesthesia with a solution of eugenol 99% (Lionel Hitchen [Essential Oils], Gravel Lane, Barton Stacey, Nr Winchester, Hampshire SO 21 3RQ, United Kingdom) and ethanol in a respective 1:8 ratio added to the quarantine tank at a dose of 30 mg/L at time T_0. A submerged pump was added to ensure a homogenous mixing of the solution within the tank. Stage II anesthesia was reached within 5

Table 1. Hematology and serum biochemistry values collected from the caudal vein of a sandbar shark, *Carcharhinus plumbeus* (Nardo, 1827). [1 = Arnold (2005); 2 = Brill et al. (2008); 3 = Stoskopf (1993)].

Parameters	Procedure 1: Etomidate anesthesia (Day 0)	Procedure 2: Eugenol anesthesia (Day 0 + 33 day)	References comparisons[1,2,3]
Hematocrit (%)	24	20	19.8[3]; 17.7 - 21.4[2]
Hemoglobin (g/dL)	5.1	4.7	<4[1]; 4.0 - 4.4[2]
White blood cells (per µL)	25,000	40,000	28,000[3]
Neutrophils (%)	75	85	58[3]
Lymphocytes (%)	20	13	40[3] - 49[1]
Basophils (%)	0	0	0[3]
Eosinophils (%)	0	0	1[3]
Monocytes (%)	5	2	1[3]
Total protein (g/dl)	7.6	5.5	5.8 - 6.7[2]; 2.2[3]
Alanine aminotransferase (UI/L)	414	790	19[3]
Aspartate aminotranferase (UI/L)	550	574	22[3]
Urea (mg/dL)	2,200	2,500	1,814[3]; 2,400 - 2,480[2]
Creatinine (mg/dL)	2.1	1.1	0.45[3]
Gamma glutamyl transpeptidase (UI/L)	<10	<10	—
Creatine kinase (UI/L)	2,100	1,000	

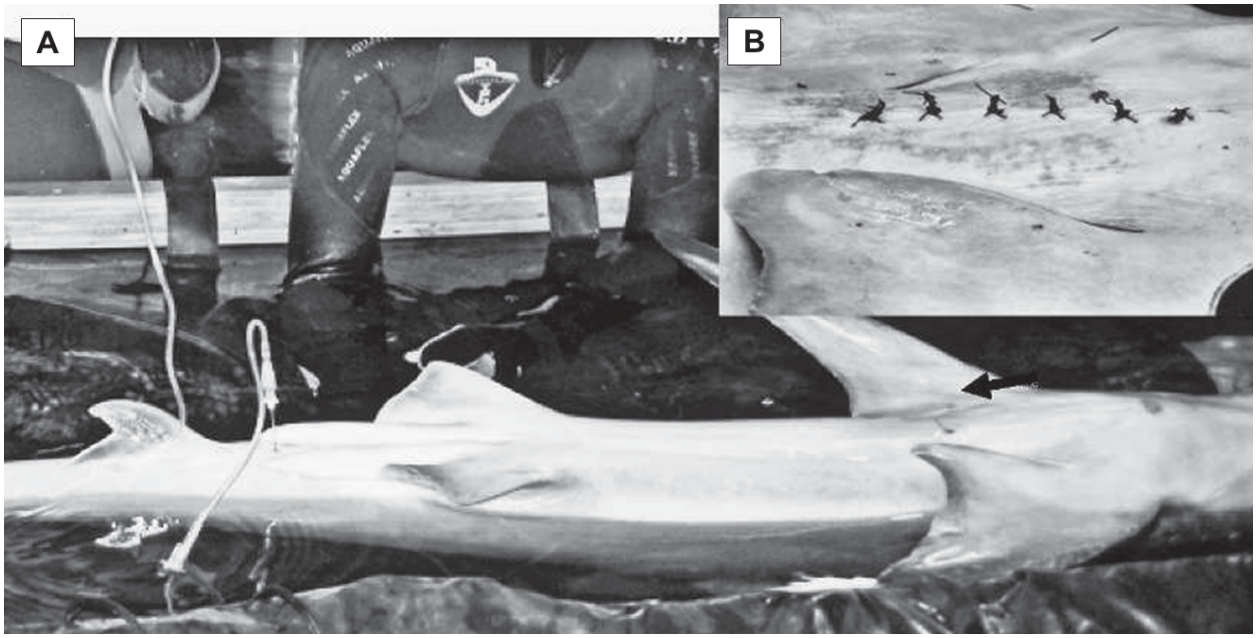

Figure 1. **A.** Presurgical restraint in a stretcher with a spinal needle providing intravenous access. Black arrow is pointing out the fistula between pectoral fins. **B.** Postsurgical wound. (Photos thanks to D. Tirmarche).

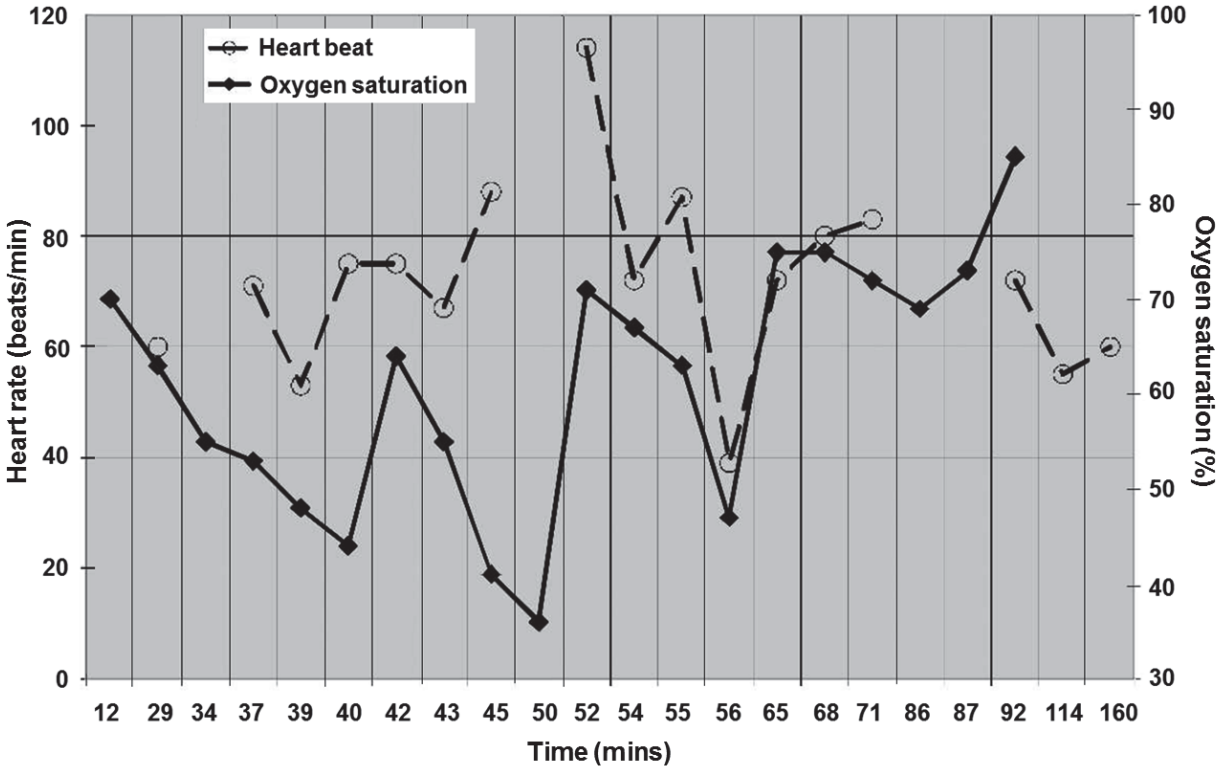

Figure 2. Anesthesia monitoring during surgery. Heart rate (interrupted line) and oxygen saturation (solid line) values from pulse oximeter data. Heart rate was verified by ultrasonographic visualization of the heart.

min and stage III at 10 min after eugenol was added. The shark was placed on a stretcher in dorsal recumbency with the insertion of the pectoral fin a few centimeters above the waterline (Figure 1). The oximeter was set to the same parameters as for the first procedure, and confirmation of this instrument's reported pulse rate was made by frequent visualization of the heart via ultrasonography (DP6600, Mindray, Echomedic France, D. Royer Rue de Vaugirard 107, 75006 Paris, France) using a 7.5 MHz linear probe placed on the ventral side between pectoral fins. Results from this monitoring are reported in Figure 2.

A spinal needle (18g, 110 mm) was inserted ventrally, 10 cm caudal to the cloaca, into the caudal tail vein for use as an intravenous catheter as previously described (Smith, 1992). Through this needle, a perfusion of shark modified Ringer's solution (SMR) (Andrews and Jones, 1990) was infused at a rate of 3 mL/min. An atraumatic tubing (25 mm) was placed into shark's mouth to direct water from the anesthesia tank (at 30 mg/L eugenol) over the gills. At T_{0+40} min postinduction, a second tube was added to deliver anesthesia-free tank water from the aquarium's main circuit so that the dosage of eugenol presented was decreased to 15 mg/L until the end of the procedure.

A manual inspection of the esophagus and stomach was conducted through a plastic tubular speculum of 20 cm in diameter placed in the jaw to protect the inspector's arms. No lacerations or scars were detected on the gastric or esophageal walls, but the hook could be palpated through the stomach and its location was confirmed to be within the coelom. Ultrasound also confirmed the presence of the hook several centimeters cranial to the fistula.

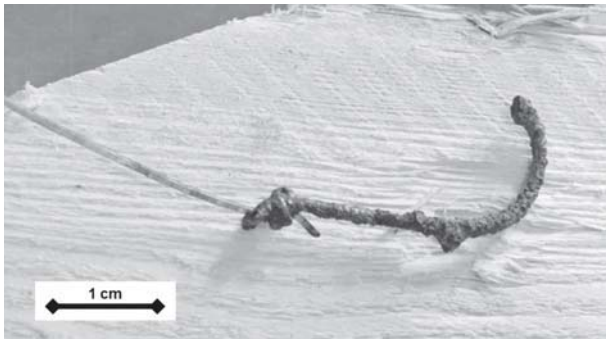

Figure 3. Corroded hook removed from liver lobe. (Photo thanks to D. Tirmarche).

Following gentle cutaneous disinfection using a solution of chlorhexidine (1:40), a 10-cm length incision was made in the ventral midline, caudal to the pectoral girdle, extending over four to five mytotomes of the ventral bundle. Mild necrosis of the muscle surrounding the fistula was noted. Changes in the liver were consistent with a focal inflammatory process in the region where the hook was imbedded. The hook appeared corroded (Figure 3) and was carefully removed by manually advancing it in the direction of the gastrohepatic ligament to avoid hepatic hemorrhage. Damaged and ischemic liver and muscle tissues were surgically excised. The ventral coelomic cavity was flushed with SMR and marbofloxacin powder was applied to the surgical site. The muscular layer and peritoneum were closed with an interrupted cruciate mattress pattern of polydioxanone suture (USP 2) (Ethicon Inc., Somerville, New Jersey 08876, USA). Another continuous pattern was used to close the most superficial fascia before closing the skin in a cruciate mattress pattern (Figure 1).

The surgical procedure was completed 86 min following induction at which point eugenol administration was discontinued. The marbofloxacin dosage was increased to 10 mg/kg and 400 mg was hand-injected intramuscularly at the end of the procedure. The shark also received dexamethasone (2 mg/kg IM). The shark was then transferred to the recovery area, designed as a shallow pre-enclosure (80 cm deep) within the main exhibit, at T_{0+124} min. Although recovery was prolonged, the respiratory rate (gill slit movement) increased progressively from 4/min to 25/min (respectively from T_{0+124} to T_{0+180}). Spontaneous swimming occurred at T_{0+195} min. During recovery, the shark was periodically aroused by direct digital stimulation of its cloacal pouch. Autonomous swimming was consistent at T_{0+280} min and the shark was returned to the main exhibit. The shark never stopped swimming following release. Food was offered the next day, but feeding did not occur until 72 hr after the surgery (1.2 kg Atlantic salmon, *Salmo salar* (Linnaeus, 1758)). At that point, the feeding schedule returned to its presurgical frequency and quantity and the shark ate regularly. Antibiotic therapy was administered every other day with oral or parenteral enrofloxacin (10 mg/kg, Baytril 10%, Bayer Pharma, 13 rue Jean Jaurès, 92807 Puteaux Cedex, France) for 10 days postsurgery.

Within the first 3 days postsurgery, several ligatures sloughed and the laparotomy site appeared partially opened. However, a second intention healing process quickly progressed from the inner edges of the wound and the skin was totally closed 15 days after surgery. During the first few days after surgery, a few teleost fishes were observed picking at the wound, but the shark managed to avoid them and to discourage any further interest. The other sharks did not appear interested in the wound. Skin healing was considered complete at 22 days postsurgery. Visual evaluation of the shark was performed thereafter by observation from divers and through windows. One year after the surgery, the animal's subjective weight had returned to normal and reproductive behavior was observed during the next breeding season.

DISCUSSION

Two different chemical immobilization techniques were used in this case. The initial choice was parenteral injection of etomidate. At this time, immersion anesthesia was impractical because of the large tank size and the presence of other fishes. Etomidate is a hypnotic anesthetic, previously described for use in fishes either via immersion (Limsuwan, et al., 1983; Plumb, et al., 1983) or by injection. Despite its poor analgesic properties, etomidate at 1 mg/kg appeared adequate to manipulate *C. taurus* into tonic immobility for nonpainful procedures (Herbert et al., 2003). Etomidate also provides a relatively short recovery time due to its quick elimination (Herbert et al., 2003). The adrenal inhibition potential of etomidate should be taken into account during its use and clinicians should consider use of supplemental corticosteroids at the end of a procedure to mimic the beneficial actions of endogenous corticosteroids.

For the second immobilization and surgery, immersion in a eugenol (clove oil) bath was selected. Eugenol has a proven antagonist effect on nociception in various species and appeared adequate to achieve good analgesia when mixedwith tonic immobility (Lécu, unpublished data). Dosages used in various fish species usually range between 20 and 100 mg/L (Ross and Ross, 2008) mixed with alcohol in a ratio of 1 volume of eugenol to 9 volumes of alcohol (Gemma et al., 2000). The maintenance dose used in this case was 15 mg/L, which is comparatively low. In addition to the typical anesthesia circuit (Lewbart and Harms, 1999), connection of an ambient water circuit to the eugenol source allowed modulation of the anesthetic delivery dose, as needed, based upon monitored parameters or shark reactions during surgery. The prolonged recovery was potentially the result of the use of eugenol (Sladky et al., 2001) combined with the duration of tonic immobility. Restraint, prolonged inversion, and maintenance of tonic immobility can affect both blood pressure (Davie et al., 1993) and acid-base balance, inducing acute acidosis (Manire et al., 2001; Skomal, 2007; Smith, 1992). Recovery times of more than 3 hours are reported in pelagic sharks immobilized for more than 1 hour (Skomal, 2007).

C. plumbeus are obligate ram ventilators, therefore, forced water administration through the gills (Stamper et al., 2004; Young et al., 2002) and SMR fluid perfusion (Andrews and Jones, 1990; Greenwell et al., 2003.) were beneficial in limiting the detrimental effects of prolonged immobilization in dorsal recumbency. SMR infusion contained urea and other salts and its shelf life is unknown. Therefore, the authors recommend SMR preparation 24 hours in advance, storage at 4°C, and administration at ambient water temperature. The perfusion rate was set according to estimated total blood volume in order to prevent a detrimental effect of lactate buildup from hypoperfusion of tissues. A tankside portable blood gas analyzer would have been useful to more precisely monitor and subsequently control blood pH during surgery by an ability to adjust perfusion rates.

Proper monitoring of the shark throughout the anesthetic event and induction to recovery is very important. Monitoring vital signs during anesthetic procedures in sharks is more complicated than in mammals. Oximetric probes should be placed deep into the cloaca in order to have the most accurate values possible, considering the heterogenic distribution of blood flow within the shark body (Carrier et al.,2004). Moreover, ultrasonic evaluation of the heartbeat is a useful (Walsh et al., 2005) and reliable method to validate oximetric pulse values and assess the character of the heartbeat.

The fish hook and associated adnexa was likely inadvertently ingested during a routine feeding

event while on exhibit. Perforation through the gastric wall is not uncommon with hooks of this shape and has been previously described in the blue shark, *Prionace glauca* (Linnaeus, 1758), by Borucinski et al. (2002) in a 2002 case report that described similar pathologic findings for embedded fish hooks, including the presence of fibronecrotic tissue. Without proper surgical treatment, the outcome would likely lead to peritonitis and septicemia (Borucinska et al, 2001; Borucinska et al., 2002).

Sharks are known to recover quickly from minor coelomic surgery (Sundström and Gruber, 1997) with tremendous skinhealing capacity and speed. Temporary connection between the coelom and the external water column could probably be sustained, as long as the liver lobe is acting as a biologic barrier separating coelom from the external water. Antibiotic therapy should be continued as long as the peritoneum is exposed. In this case, the need for a broad spectrum antibiotic with a good expected liver diffusion led to the choice of fluoroquinolones. Marbofloxacin has been used in bony fishes (Zhu, 2009) and sharks (Firmin, 2006) with good results. Enrofloxacin was also used in this present case (Dehart and Stoops, 2004; Smith et al., 2004) because the variety of available oral forms that facilitated delivery became difficult when remote injection was required at the end of the treatment.

CONCLUSION

This is the first report of an intracoelomic foreign body removal in a shark. Anesthetic and surgical procedures of large sharks are not common. In this case, the two immobilizations, coupled with a major coelomic surgery, demonstrate that large sharks can be effectively managed in a fashion similar to that employed in terrestrial carnivores. The diagnostic and therapeutic paradigm may include methods for diagnostic imaging, anesthetic monitoring and postoperative care. Anesthesia with eugenol proved to be safe and effective in this case, but its use may be subject to regulatory control in some circumstances.

For that reason, other immersion drugs, such as tricaine methanesulfonate, should also be considered for use in elasmobranchs (Mylniczenko et al, 2006). Operant conditioning may mitigate some of the more stressful aspects of the immobilization process such as dart-delivered parenteral agents and physical restraint. Prevention of hook ingestion may be achieved by the use of a more diligent pre-feeding inspection process and the use of metal detectors with food fish.

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Chapter 35

Diagnosis and treatment of common reproductive problems in elasmobranchs

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Abstract: Elasmobranchs in aquaria may encounter a number of reproductive problems, the most common of which include egg retention metritis in sand tiger sharks, *Carcharias taurus* (Rafinesque, 1810), cystic ovaries and mucometra in southern stingrays, *Dasyatis americana* (Hildebrand & Schroeder, 1928), and dystocia in cownose rays, *Rhinoptera bonasus* (Mitchill, 1815). If left untreated these problems often result in death, so early detection is fundamental to their resolution. Diagnostic tools for these conditions may include endoscopy, ultrasonography and radiology. Techniques such as uterine lavage, ovariectomy and cesarean section are useful in preventing, as well as treating, these reproductive disorders.

INTRODUCTION

The establishment of natural habitats in aquaria, coupled with advances in our understanding of animal biology, has resulted in an increasing number of elasmobranch species reproducing while under human care. Despite these advances, a number of aquarium elasmobranch species have not successfully reproduced. While unsuccessful reproductive efforts are the result of a variety of factors, some of these cases are a direct result of complications associated with the elasmobranch reproductive tract. By understanding commonly diagnosed reproductive problems associated with aquarium elasmobranchs, it is possible to increase the health and longevity of these fishes. In this chapter, we review three commonly diagnosed reproductive problems seen in aquarium elasmobranch collections, and present examples of diagnostic methods and treatments for these challenges.

EGG-RETENTION METRITIS IN SAND TIGER SHARKS

Sand tiger sharks, *Carcharias taurus* (Rafinesque, 1810), are one of the most commonly displayed

large sharks in public aquaria. In the wild, female *C. taurus* mature at 9 - 10 years of age, and estimates of longevity for wild sharks range from 16 - 17 years (Goldman et al., 2006). *C. taurus* in aquaria often live for 10 - 15 years, although they have been reported to live for well over 20 years in a number of cases (Mohan et al., 2004). During such a long time on display, female *C. taurus* may go through many reproductive cycles. Although there have been several successful reproductive events in open and semi-closed systems around the world, no pregnancies have been recorded for *C. taurus* on display in the United States, excluding animals that were gravid prior to capture. Regardless of the extent of other reproductive activity, female *C. taurus* continually produce non-fertilized eggs, which are often released into the tank. However, eggs produced and retained, in reproductively unsuccessful females, can lead to potentially lethal consequences. Specifically, eggs retained in one or both uterine horns eventually become necrotic, serving as a nidus for bacterial metritis, which can result in septicemia and death.

A recent study showed egg retention metritis to be an important cause of death in female *C. taurus* in aquaria. Septic sharks often display depression, anorexia and diffuse dermal abscesses, or draining fistulas. Metritis can be diagnosed by detecting necrotic eggs using radiology, ultrasonography (Figure 1), endoscopy, or cannulation and uterine lavage. Antibiotic treatment alone is generally ineffective. Treatment should therefore consist of flushing out the eggs using a uterine lavage of an antibiotic saline solution, followed by the administration of an appropriate parenteral antibiotic. Bacteria cultured from necrotic eggs have most commonly been *Pseudomonas* and *Enterococcus* species. Appropriate parenteral antibiotics should be determined by bacterial culture.

C. taurus have two cervical openings, which may be open or closed. In the event that a cervix is not open, the operator can gently work one finger through the cervix and then a second finger. Once two fingers gain entrance, they can be spread to allow for insertion of the lavage hose and provide an exit for the eggs. In some cases, it may be necessary to keep the cervix manually dilated throughout the procedure. If retained eggs were detected in both uterine horns, the procedure must be repeated on the remaining horn. Readers considering the use of this procedure should thoroughly familiarize themselves with the anatomy of a female *C. taurus.* Female *C. taurus* subjected to uterine lavage may lose the air from their gastrointestinal tract, resulting in a loss of buoyancy. If this loss of air occurs, the stomach may need to be manually re-

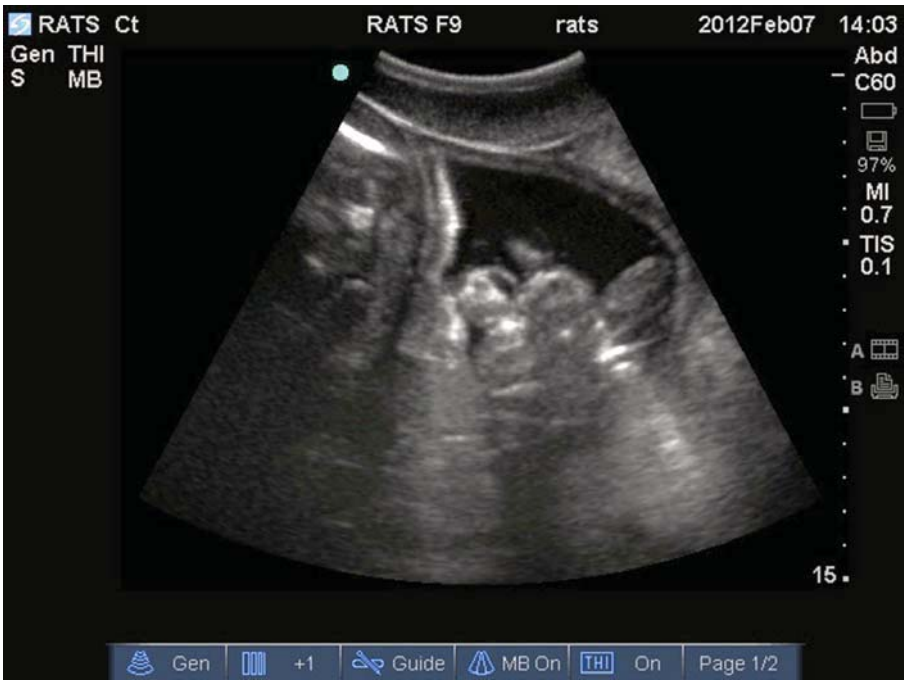

Figure 1. Ultrasound of necrotic eggs in the uterus of a sand tiger shark, *Carcharias taurus* (Rafinesque, 1810).

inflated before the animal is released. With early treatment, sharks with bacterial metritis have a good prognosis.

Uterine lavage case history

Uterine lavage was successfully employed to treat egg retention metritis in a female *C. taurus*. In preparation, a variable-speed submersible pump was placed in an 18.9 L bucket containing approximately 11.5 L of clean, but not sterile, saline solution. Ceftazidime (3 g) was added to the water making a solution of 260 mg/L ceftazidime. A sterilized 2.5 cm diameter polyvinyl chloride (PVC) semi-rigid hose, with a flame-smoothed end, was attached to the pump. Uterine lavage was performed on the submersed, un-anesthetized shark, calmed using oxygen narcosis and tonic immobility, which was achieved by restraining the shark, placing it in dorsal recumbence, and pumping low-pressure water, saturated with atomized oxygen, into its mouth. A gloved hand was gently and slowly inserted into the cloaca and directed dorsal to the plenum, separating the urogenital sinus from the entrance to the spiral colon. In this case, the cervix was open, so the hose was inserted and gently advanced until it hit the anterior end of the uterine horn. The saline/antibiotic solution was then pumped under low pressure into the uterine horn to expel purulent debris and decomposing eggs from the horn and cloaca. Throughout the procedure, expelled solid material was collected with a small fish net to maintain system water quality. Retained eggs were manually expressed to accelerate the process using gentle external compression and working in a cranial to caudal direction. Once the lavage fluid became clear and no more eggs were seen, the hose was removed. Following the procedure, the shark was returned to an upright position for several minutes and treated with parenteral antibiotics. Once the shark was alert and responsive, it was released back into the display. Several female *C. taurus* have been successfully treated in the manner described.

OVARIECTOMY OF SOUTHERN STINGRAYS

The southern stingray, *Dasyatis americana* (Hildebrand and Schroeder, 1928), is a large ray commonly displayed in public aquaria. In both aquaria and in the wild, female *D. americana* are reproductively fecund and can produce two to 10 pups a year in one or two litters. Female *D. americana* are seldom, if ever, not gravid, as they are impregnated within hours to days of parturition (Henningsen, 2000). Because *D. americana* are constantly pregnant, the uterus never involutes, and villus trophonemata do not atrophy. As a result, the trophonemata continue to produce thick mucoid histotroph and the uterus remains dilated. It appears that *D. americana* are induced ovulators, so copulation must occur in order to stimulate hormonal signals for ovulation. If copulation does not occur, the mature follicles are not ovulated and remain on the ovary, irrespective of previous ovulations.

To decrease the production of rays in captivity, many aquaria have resorted to single-sex exhibits, inhabited by large adult female rays. Even though there are no males in these exhibits, to induce ovulation and pregnancy in the females, the female rays continue to produce a large amount of histotroph (Myleniczenko and Penfold, 2012). The accumulation of histotroph in the uterus leads to marked mucometra (Figure 2). Female rays that develop this condition appear distended, however, there are no pups present when the uterus is examined via ultrasound. Instead, ultrasound often reveals a large hypoechoic fluid-filled uterus and some hyperechoic particles floating in the histotroph (Figure 3). If left untreated, this condition will worsen as histotroph accumulates. The ray may become distended ventrally, as well as dorsally, and may develop pressure sores when resting on the aquarium floor. If these pressure sores become severe, the coelomic wall can rupture resulting in salt water intrusion, with death generally occurring shortly thereafter. While the mucometra may be

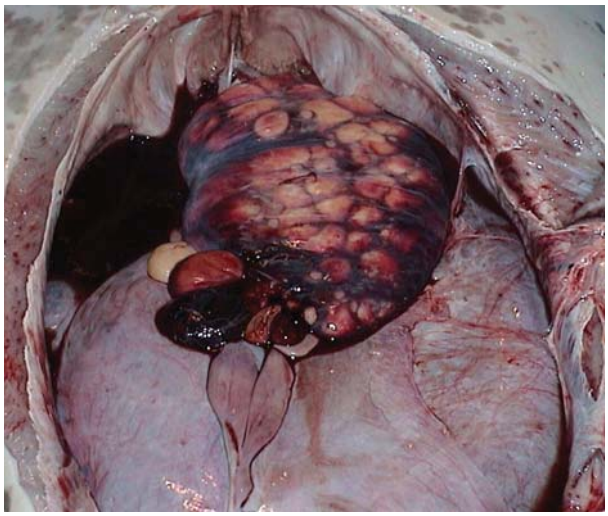

Figure 2. Large cystic ovary and mucometra in a southern stingray, *Dasyatis americana* (Hildebrand and Schroeder, 1928).

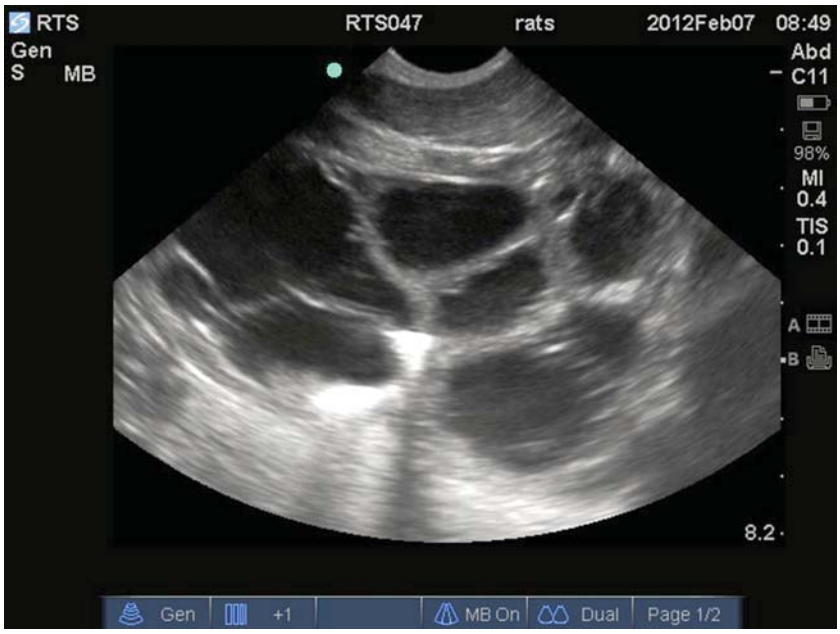

Figure 3. Ultrasound image of a cystic ovary in a southern stingray, *Dasyatis americana* (Hildebrand and Schroeder, 1928).

symptomatically treated by cannulating the cervix and evacuating the uterus, fluid accumulation will recur within a few months.

Similar to continued uterine production of histotroph, the left ovary in *D. americana* continues to produce follicles, despite absence of pregnancy. Continuous follicle production, without induced ovulation, causes the ovary to accumulate a large number of mature follicles over a period of time. The ovary will become cystic and can achieve huge dimensions, weighing up to a kilogram. Ovarian tissues are thin and friable, so when ovaries attain such a large size they are prone to tearing (Figure 2), which can lead to hemorrhage and death.

In an effort to prevent reproductive complications in an all-female collection, surgical ovariectomy in juvenile *D. americana* can be employed. Patient size is critical to the success of the procedure. If the animals are too large or too small it can be challenging to safely complete the surgery. The ideal size for a candidate *D. americana* is ~60 cm disc width (DW). The ovary is too large, vascular and difficult to remove in *D. americana* with a DW >70 cm, while it is difficult to identify the ovarian tissue embedded in the cranial aspect of the epigonal gland in rays with a DW <50 cm. To unequivocally confirm developmental status during the selection of candidates for ovariectomy, ovarian size should be evaluated pre-operatively with ultrasonography.

Ovariectomy in *D. americana* represents a safe option for controlling reproduction and population size in a display exhibit, however, as this is a new procedure, it is too early to tell if removal of the ovary in sub-adult animals causes subsequent growth, metabolic or reproductive problems. Until the long-term safety and side effects of this procedure are firmly established, ongoing hormonal studies and routine health examinations should be performed in treated animals.

Ovariectomy case history

We have performed ovariectomy under anesthetic, induced and maintained with tricaine methanesulfonate at 75 mg/L, in a recirculating water system. The left para-lumbar area was disinfected with an iodine surgical prep solution and a 6 - 8 cm incision was made parallel to the spinal column and 2 cm lateral to the dorsal lumbar muscles. The skin incision started just caudal to where the fin rays joined the epaxial muscles. After incising the muscle and the peritoneum, the cranial portion of the ovary, and the enveloping oviduct, were dissected free from the dorsal suspensory ligament. The thick anterior pedicle, which contained the majority of the blood supply to the ovary, was clamped and ligated with a single encircling ligature using 3-O polydioxanone (PDS) absorbable suture (Ethicon US LLC, New Jersey, USA). The thin suspensory ligament running along the dorsal midline between the ovary and the body wall was relatively avascular, and could be transected

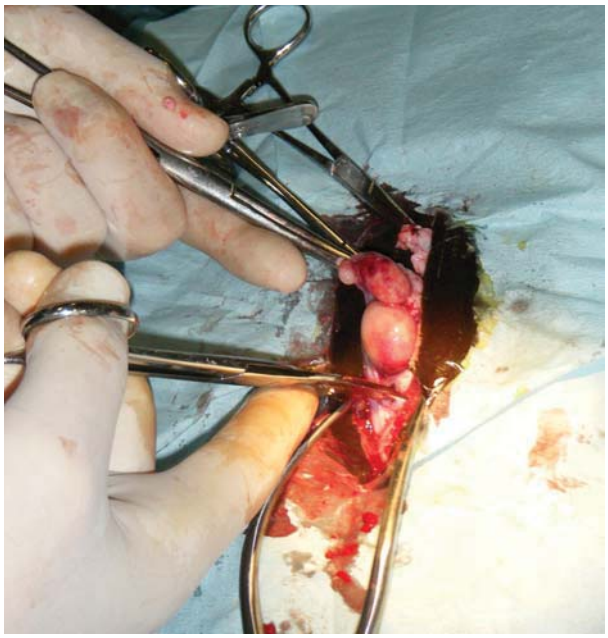

Figure 4. Ovariectomy in a southern stingray, *Dasyatis americana* (Hildebrand and Schroeder, 1928).

with scissors or blunt dissection (Figure 4). Mosquito forceps were used for hemostasis, as necessary. The caudal pole of the ovary is broadly attached to the cranial end of the epigonal gland. Thus, an encircling suture was placed around the epigonal gland with the assistance of a clamp placed across the gland, caudal to the ovary/epigonal gland junction. The ovarian tissue was then transected and removed.

The peritoneum was closed with 3-O PDS using a half-round taper needle in a simple interrupted pattern. The muscular layers were not directly sutured, as the tissues were too weak to support the suture. The skin was sutured with 3-O PDS using a cutting needle in a simple interrupted pattern. Because elasmobranch tissues do not swell, care was taken to place the sutures relatively close together and they were drawn tighter than in mammals. Tissue apposition was critical to avoid water intrusion, which could result in suture line dehiscence, or rupturing.

Post-operatively, ceftiofur at 8 mg/kg (Excede, Zoetis, New Jersey, USA) and meloxicam at 0.3 mg/kg (Boehringer Ingelheim Vetmedica, Inc., Missouri, USA) were administered. Recovery from anesthesia was rapid and the *D. americana* resumed feeding within 3 h of the procedure. Sutures were removed one month after surgery. Other than mild bleeding, no additional complications were observed intra- or post-operatively.

As an aside, the left para-lumbar surgical approach described has also been successfully employed to perform laparotomies and cesarean sections in cownose rays, *Rhinoptera bonasus* (Mitchill, 1815), as well as other dasyatids.

DYSTOCIAS IN COWNOSE RAYS

Dystocia is a condition that occurs when a female cannot successfully deliver her offspring. This condition may occur for a variety of reasons, including the offspring being too large or presenting abnormally, the presence of twins, or because the female cannot initiate a normal birthing process to expel the offspring. When a dystocia occurs, it is a medical emergency for both the mother and the fetus. While dystocias may occur in any elasmobranch species, they are most commonly noted in *R. bonasus*. Because physical signs of labor may not be obvious, it may be difficult to determine the presence of a dystocia. Typically, a dystocia is suspected when the ray has achieved a large girth, but an expected pup has not appeared. Upon examination, a pup may be palpated in the urogenital sinus or by pushing at a dilated cervix. The cloacal tissues may be bruised and edematous. In some cases, the pup can be manually removed without further intervention, but in most cases an episiotomy is necessary to aid extraction through the cloaca. In other cases, a cesarean section may be indicated. In either case, the *R. bonasus* should be anesthetized for the procedure (see below). The clinician is urged to carefully study the case before a decision about the chosen methodology is made.

In general, it is recommended that pregnant *R. bonasus* receive monthly physical and ultrasound examinations, enabling staff to predict a parturition window. This practice will allow the husbandry team to monitor pregnant animals, manage the collection by segregating animals where appropriate and intervene in the event of pupping complications (George and Schuder, 2007).

Dystocias case history

A *R. bonasus* with a suspected dystocias was sedated with tricaine methanesulfonate at 60 mg/L and examined. A pup was palpable, through an open cervix, confirming that the birthing process had begun, and strongly suggestive of a dystocia.

In this case cesarean section was indicated and the left paralumbar approach was employed, using a ~7 cm incision. Uterotomy and pup extraction was similar to cesarean procedures in other species. Routine closure of the uterus and body cavity were followed by the administration of parenteral antibiotics and pain medications (as per the section on ovariectomy in *D. americana* above). Recovery was rapid and the *R. bonasus* resumed normal swimming and feeding within 24 hours.

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Chapter 36

The use of reproductive technologies in breeding programs for elasmobranchs in aquaria

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Abstract: Despite the use of reproductive technologies in many terrestrial and aquatic species, little work has been done with elasmobranchs. Reproductive technologies, such as sperm collection and sperm quality assessment, sperm cryopreservation, artificial insemination, and monitoring female reproductive condition and gestation, could potentially complement or enhance existing breeding programs for elasmobranchs in aquaria. As greater emphasis is placed on self-sustaining elasmobranch aquarium populations, reproductive technologies will become an increasingly important component of associated breeding programs.

INTRODUCTION

Elasmobranch breeding programs in aquaria

It has been estimated that over 210 elasmobranch species are maintained in major aquaria worldwide, for display, conservation and education purposes (www1). With the exception of species that are endangered in their natural habitat, elasmobranchs held in aquaria have traditionally been caught from the wild as required. Breeding programs for elasmobranchs in aquaria are not as extensive or developed as those for terrestrial species held in zoological parks, and have traditionally relied on naturally occurring mating. While this approach has been successful for some species (Henningsen et al., 2004), there are many that have never reproduced in aquaria, e.g., broadnose sevengill sharks, *Notorynchus cepedianus* (Peron, 1807), and others that only reproduce sporadically, e.g., sand tiger sharks, *Carcharias taurus* (Rafinesque, 1810) (Henningsen et al., this volume; Willson and Smith, this volume). A range of factors, including suboptimal population structures and non-specific environmental influences, created by the need to cater for multiple species in a single display or system, can limit the success of aquarium breeding programs for elasmobranchs in general and, in particular, for large sharks. Yet, captive breeding programs for elasmobranchs are becoming increasingly important, especially for display species that are threatened in their natural habitat. This situation is exemplified by *C. taurus*, which has been held in aquaria for over one hundred years (Koob, 2004) and remains a popular aquarium species today. Despite its long history as an aquarium species, *C. taurus* has traditionally been wild-caught for display, with relatively few individuals born in aquaria. However, *C. taurus* is currently classified by the International Union for Conservation of Nature (IUCN) as threatened worldwide, and the Australian east coast population is classified by the Australian federal government as critically endangered. Therefore, breeding programs for *C. taurus* are likely to be a crucial

management tool for their future as an aquarium species.

Reproductive technologies—i.e., monitoring reproductive cycles, collecting semen and artificial insemination—would provide greater control over managed elasmobranch breeding programs within aquaria. The use of sperm cryopreservation to store the genetics of individual males would also be of benefit, enabling male sharks to contribute to the gene pool of a population beyond their normal reproductive lifespan. This technology will allow the transfer of genetic material between aquaria for selective breeding, ensuring genetic diversity within captive populations. Although well established in many mammalian and teleost species, reproductive technologies have had relatively little application for elasmobranchs to date.

Definition of reproductive technologies

Reproductive technologies are a broad group of techniques used to assist reproduction in animals. A distinction should be made between the reproductive technologies that are currently used in elasmobranchs (and discussed here), and the more complex and well-established assisted reproductive technologies (ARTs) used in humans. The World Health Organization (WHO) defines ARTs as "all treatments or procedures that include the *in vitro* handling of both human oocytes and sperm, or embryos, for the purpose of establishing a pregnancy" (Zegers-Hochschild et al., 2009). The definition of ARTs provided by the WHO specifically excludes artificial insemination, as it does not involve the manipulation of both male and female gametes *in vitro*. We, therefore, use the term "reproductive technologies", which is more appropriate in the context of elasmobranchs. For the purposes of this chapter "reproductive technologies" is defined as techniques used to monitor or manage reproduction in elasmobranchs and includes the monitoring of reproductive cycles with ultrasound or hormone analysis, hormone treatments to alter reproduction, semen collection, sperm cryopreservation, artificial insemination and artificial rearing of viviparous embryos. These techniques have all been used to varying degrees in elasmobranchs and offer great potential for the reproductive and genetic management of shark and ray populations in aquaria.

MONITORING REPRODUCTION

Reproduction in elasmobranchs is usually seasonal, with the breeding season occurring during spring/summer (Hamlett and Koob, 1999). Vitellogenesis and follicular growth generally occur over a period of months leading up to the breeding season. However, there is variation among species, most notably in some oviparous species that deposit eggs year round (Hamlett and Koob, 1999). Similarly, in male elasmobranchs, spermatogenesis in most species occurs seasonally, leading up to the breeding season (Parsons and Grier, 1992). While there are some elasmobranch species that reproduce regularly in aquaria, and for which reproductive cycles of individuals can easily be inferred, the reproductive conditions of many species are not so immediately apparent and require routine monitoring in order to differentiate. This situation may be due to reproductive asynchrony of individuals within the population, single sex populations where reproductive activity cannot be observed, or, simply, a lack of reproductive activity within a population. Techniques that assess and monitor reproductive condition in male and female elasmobranchs, such as ultrasound, hormone analyses and semen assessment, can be useful to help manage the reproductive health of a population. While all three techniques are useful, they are somewhat limited in the amount of information they can provide from a single examination. If the reproductive state of an elasmobranch is unknown, a number of regular examinations may be required to build a pattern of reproductive activity. In the absence of regular examinations, it can be difficult to determine where findings from one examination fit within the overall reproductive cycle of an individual or species. For this reason, assessment of reproductive status should be performed on a regular basis as part of routine health examinations.

Ultrasound

Ultrasound is a non-invasive means of directly visualizing reproductive structures and has been used to monitor ovarian changes and pregnancy in several species of elasmobranchs. Ultrasonography has also been used to visualize embryos developing inside the egg case of oviparous Port Jackson sharks, *Heterodontus portusjacksoni* (Meyer, 1793), and horn sharks, *Heterodontus fransisci* (Girard, 1855) (Pereira, personal communication), both of which produce thick, opaque egg cases that are not conducive to 'candling'. One of the earliest published reports on the use of ultrasound for elasmobranchs was from Walsh et al. (1993). These authors assessed the potential diagnostic applications of ultrasound for nurse sharks, *Ginglymostoma cirratum* (Bonnaterre, 1788), lemon sharks, *Negaprion brevirostris*

(Poey, 1868), and bonnethead sharks, *Sphyrna tiburo* (Linnaeus, 1758), and were able to obtain images of fetal spines in a pregnant *S. tiburo*. The utility of reproductive ultrasound in elasmobranchs was further demonstrated by Carrier et al. (2003), who captured female *G. cirratum*, previously observed mating in the wild, and monitored their progress throughout gestation using ultrasonography. The use of ultrasound on elasmobranchs in aquaria has since been reported for the assessment of folliculogenesis in *N. cepedianus* (Daly et al., 2007) and detection of pregnancy in whitetip reef sharks, *Triaenodon obesus* (Ruppell, 1837) (Schaller, 2006).

Although published reports on the use of ultrasound in elasmobranchs are limited, ultrasonography is the most commonly used reproductive technology, within aquaria, and has been used for reproductive assessment in at least 39 species (Table 1). Ultrasound has now become a regular part of routine examinations in many aquaria and is typically used to monitor reproductive cycles in female elasmobranchs to enable management of individuals within a population. By measuring the diameter or volume of follicles in the ovary over time, it is possible to follow folliculogenesis in individual females (Figure 1). Further, by monitoring the growth of fetuses with ultrasound it is possible to estimate pupping dates (e.g., ribbontail stingrays, *Taeniura lymma*, (Forsskal, 1775); Pereira, personal communication; Pereira et al., this volume), and enable the transfer of pregnant females to nursery tanks prior to parturition, providing neonates with a better chance of survival (e.g. cownose rays, *Rhinoptera bonasus* (Mitchill, 1815); George, personal communication).

Sharks are generally placed in dorsal recumbence for ultrasound examination as this induces tonic immobility and imaging of internal organs is usually easiest via the ventral surface. Tonic immobility (Henningsen, 1994) enables examination without the use of anesthetic agents, although sedation may still be preferred for longer exams (Mylniczenko, personal communication). In the case of rays, ultrasound examination can be conducted either ventrally or via the dorsal surface, particularly in the case of large rays that are difficult to place in dorsal recumbence. Sedation or anesthesia is often necessary for batoids, large or small, to minimize the risk of an animal causing injury to itself or the person conducting the examination (Daly and Jones, unpublished results). In some cases, where regular examination is required, the barb may be removed either partially, by clipping, or completely, by excision of the barb and germinal plate to prevent re-growth (George, personal communication; Mylniczenko, personal communication).

Hormone analysis

The measurement of circulating reproductive hormones in serum or plasma by radioimmunoassay (RIA) provides an accurate means of determining the reproductive status of individual animals. Several reproductive hormones are present in the blood of elasmobranchs and these can be associated with reproductive parameters. The main hormones associated with reproductive cycles in elasmobranchs are estradiol, progesterone and testosterone in females, and testosterone in males (Hamlett, 1999; Hamlett and Koob, 1999). Early work on developing RIA for use in elasmobranchs involved collecting blood from sharks, maintained for a short period in aquaria, and comparing the concentrations of reproductive hormones with the condition of reproductive organs following dissection (Koob et al., 1986). RIA has since been used to compare circulating reproductive hormone levels with ovarian and testicular cycles in several elasmobranch species from wild populations, either with or without dissection, allowing for the assessment of reproductive organs (Rasmussen and Gruber, 1993; Tricas, 2000; Awruch et al., 2008).

To date, the analysis of reproductive hormone levels has had limited application in elasmobranch populations in aquaria. RIA has been used to monitor circulating hormone levels to assess the reproductive status of individual sandbar sharks, *Carcharhinus plumbeus* (Nardo, 1827), bull sharks, *Carcharhinus leucas* (Muller & Henle, 1839), juvenile *N. brevirostris* (Rasmussen and Murru, 1992), clearnose skates, *Raja eglanteria* (Bosc, 1800) (Rasmussen et al., 1999), *C. taurus* (Henningsen et al., 2008), and *S. tiburo* (Gelsleichter et al., 2002). More recently, reproductive hormone analysis has been used in aquaria to monitor the reproductive cycle of bowmouth guitarfish, *Rhina ancylostoma* (Bloch & Schneider, 1801) (Hanna, personal communication), and blackchin guitarfish, *Rhinobatos cemiculus* (Geoffrey Saint-Hilaire, 1817) (Lécu and Herbert, personal communication), and has been used to assist in the diagnosis of reproductive disease in southern stingrays, *Dasyatis americana* (Hildebrand & Schroeder, 1928) (Mylniczenko and Penfold, 2012).

Although there are many gaps in the knowledge of elasmobranch reproductive endocrinology

Table 1. Elasmobranch species for which reproductive ultrasound has been reported or observed. All references are personal communications unless indicated by a date of publication.

Species name	Common name	Reference
Aetobatus narinari (Euphrasen, 1790)	spotted eagle ray	George; Mylniczenko
Carcharhinus plumbeus (Nardo, 1827)	sandbar shark	Mylniczenko; Hadfield
Carcharhinus limbatus (Muller & Henle, 1839)	blacktip shark	Mylniczenko
Carcharias taurus (Rafinesque, 1810)	sand tiger shark	Mylniczenko; Daly and Jones, unpublished results
Chiloscyllium punctatum (Muller & Henle, 1839)	brownbanded bambooshark	Mylniczenko; Daly and Jones, unpublished results
Dasyatis americana (Hildebrand & Schroeder, 1928)	southern stingray	Mylniczenko; Hadfield; Pereira
Dasyatis sabina (Lesueuer, 1824)	Atlantic stingray	Hadfield
Ginglymostoma cirratum (Bonnaterre, 1788)	nurse shark	Carrier et al. (2003); Hadfield
Gymnura altavela (Linnaeus, 1758)	spiny butterfly ray	Hadfield
Hemiscyllium ocellatum (Bonnaterre, 1788)	epaulette shark	Daly and Jones, unpublished results
Heterodontus francisci (Girard, 1855)	horn shark	Lécu and Herbert
Heterodontus portusjacksoni (Meyer, 1793)	Port Jackson shark	Daly and Jones, unpublished results
Himantura uarnak (Gmelin, 1789)	honeycomb stingray	Hadfield
Himantura dalyensis (Last & Manjaji-Matsumoto, 2008)	Australian freshwater whip ray	Hadfield
Manta alfredi (Krefft, 1868)	Alfred manta	Tomita et al. (2012)
Mobula hypostoma (Bancroft, 1831)	lesser devil ray	Mylniczenko
Myliobatis aquila (Linnaeus, 1758)	common eagle ray	Pereira
Myliobatis freminvillii (Lesueur, 1824)	bullnose eagle ray	Hadfield
Notorynchus cepedianus (Péron, 1807)	broadnose sevengill shark	Daly et al. (2007); Grassman
Orectolobus ornatus (De Vis, 1883)	ornate wobbegong	Otway and Ellis (2011)
Platyrhinoidis triseriata (Jordan & Gilbert, 1880)	thornback guitarfish	Hadfield
Potamotrygon motoro (Muller & Henle, 1841)	South American freshwater stingray	Daly and Jones, unpublished results
Pteroplatytrygon violacea (Bonaparte, 1832)	pelagic stingray	Hadfield
Raja clavata (Linnaeus, 1758)	thornback ray	Whittamore et al. (2010)
Raja eglanteria (Bosc, 1800)	clearnose skate	Hadfield
Rhinobatos cemiculus (Geoffrey Saint Hilaire, 1817)	blackchin guitarfish	Lécu and Herbert
Rhinoptera bonasus (Mitchill, 1815)	cownose ray	George; Mylniczenko; Hadfield
Rhynchobatus laevis (Bloch & Schneider, 1801)	smooth nose wedgefish	Hadfield
Scyliorhinus canicula (Linnaeus, 1758)	lesser spotted dogfish	Whittamore et al. (2010)
Sphyrna tiburo (Linnaeus, 1758)	bonnethead shark	Walsh et al. (1993); Hadfield
Stegostoma fasciatum (Hermann, 1783)	zebra shark	Hadfield; Daly and Jones, unpublished results
Taeniura grabata (Geoffrey Saint-Hilaire, 1817)	round stingray	Pereira
Taeniura lymma (Forsskal, 1775)	ribbontail stingray	Pereira
Taeniurops meyeni (Muller & Henle, 1841)	round ribbontail ray	Hadfield
Triaenodon obesus (Rüppell, 1837)	whitetip reef shark	Schaller et al. (2003); Daly and Jones, personal observation
Triakis megalopterus (Smith, 1839)	sharptooth houndshark	Smale, 2003
Triakis semifasciata (Girard, 1855)	leopard shark	Stetter, 2004; Lécu and Herbert
Urobatis halleri (Cooper, 1863)	Haller's round ray	Jirik and Lowe (2012)
Urobatis jamaicensis (Cuvier, 1816)	yellow stingray	Mylniczenko; Hadfield

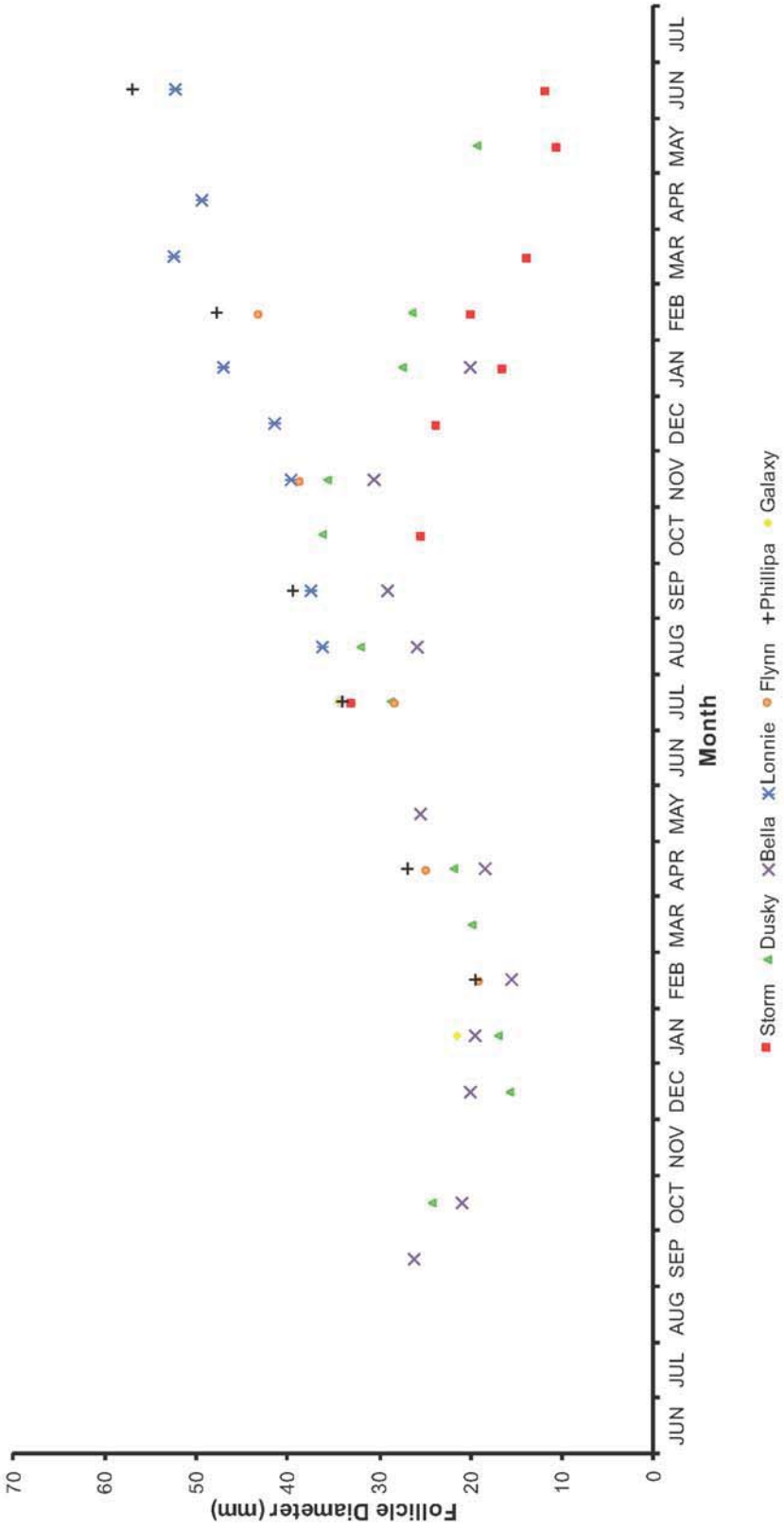

Figure 1. Changes in ovarian follicle diameter in seven broadnose sevengill sharks, *Notorynchus cepedianus* (Péron, 1807), as measured using ultrasound.

(Awruch, 2013), hormone analyses have a lot to offer in regards to the reproductive management of elasmobranchs in aquaria. Monitoring reproductive hormones has already been used to assist in the management of reproductive populations (Henningsen et al., 2008) and could be used either in place of, or in conjunction with, ultrasound to monitor female reproductive cycles. Hormone assays could also be used to monitor reproductive cycles in male elasmobranchs, for which ultrasound monitoring is of limited use. It may also be possible to use RIA to predict the timing of ovulation in female elasmobranchs, which would be of great benefit to artificial insemination studies. At present, the ranges of estradiol, progesterone and testosterone during the reproductive cycle in male and female elasmobranchs are yet to be established for most species. In most cases, regular assay of reproductive hormones over a one to two year period will be required for the establishment of 'normal' ranges, which will be essential for the utility of hormone analyses in breeding programs for elasmobranchs in aquaria.

Semen collection and sperm quality assessment

Traditionally, researchers and aquarists have focused on monitoring the female reproductive cycle, and in particular, the detection and monitoring of pregnancy. As a result, there is comparatively little information available on mechanisms to monitor reproductive cycles in male elasmobranchs. In many elasmobranch species, the testes undergo annual seasonal changes to spermatogenesis, which are often accompanied by changes in the gonadosomatic index (GSI) (Parsons and Grier, 1992; Maruska et al., 1996; Lucifora et al., 2005). In some species, seasonal changes in GSI correspond with the annual breeding season, while other species breed all year round irrespective of GSI, or have a defined mating season with no appreciable change in GSI (Parsons and Grier, 1992). As a result of this situation, and relatively minor changes to the size of the testes throughout the reproductive cycle, compared to changes in the size of the ovaries in females, ultrasound is of limited use for monitoring reproductive cycles in male elasmobranchs. Indeed, there are no published reports on the use of ultrasound to monitor reproductive cycles in male elasmobranchs.

Semen collection and sperm quality assessment have been used as a means of monitoring male elasmobranch reproductive function in aquaria (Daly, 2008). Semen can be collected from male elasmobranchs during the breeding season, either by exerting gentle pressure on the distal reproductive tract, proximal to the cloaca (e.g. Masuda et al., 2003; Masuda et al., 2005), or by passing a thin tube or catheter into the ampulla of the ductus deferens via the urogenital papilla (e.g., Minamikawa and Morisawa, 1996). Both methods have successfully been used to collect semen from a number of elasmobranch species in aquaria (Table 2). Semen collection and sperm quality assessments have been used to monitor seasonal reproductive cycles in male *N. cepedianus* and male short-tailed stingrays, *Dasyatis brevicaudata* (Hutton, 1875) (Daly, 2008). Preliminary observations in other species, including brownbanded bamboosharks, *Chiloscyllium punctatum* (Müller & Henle, 1838), South American freshwater stingrays, *Potamotrygon motoro* (Müller & Henle, 1841), and Australian bull rays, *Myliobatis australis* (Macleay, 1881), indicate that this method will be useful for monitoring reproductive activity in a wide variety of elasmobranch species held in aquaria (Daly and Jones, unpublished results).

The simplest way to assess the quality of a semen sample is by observing motility under a light microscope, however, there is little information available in the literature on the handling conditions required for observing the activity of elasmobranch sperm. The high osmotic pressure of elasmobranch body fluids means that sperm should be assessed in solutions with an osmolality of approximately 900 - 1100 mOsm/kg in order for them to be maximally active. Most studies to date have used ionic solutions based on elasmobranch biological fluids. Jones et al. (1984) reported that sperm from *H. portusjacksoni* were active in a phosphate-buffered elasmobranch ringer solution based on the ionic composition of elasmobranch blood, and similar solutions have been used to observe the activity of elasmobranch sperm from a range of species (Daly and Jones, unpublished results). Work by Minamikawa and Morisawa (1996) showed that sperm from banded houndsharks, *Triakis scyllium* (Muller & Henle, 1839) could maintain activity in solutions that replicate blood or uterine conditions. These investigators also suggested that the presence of hexoses, such as glucose, are important for sperm activity (Minamikawa and Morisawa, 1996). A slightly different approach has been used successfully by Luer et al. (2007), who reported that sperm from *R. eglanteria* maintained high motility in a poultry semen extender that had been modified for elasmobranch conditions by the addition of urea, trimethylamine oxide and NaCl, to raise the osmotic pressure. These authors also found that sperm were highly active in a mixture

Table 2. Elasmobranch species for which semen collection has been reported or observed. All references are personal communications unless indicated by a date of publication.

Species name	Common name	Reference
Chiloscyllium plagiosum (Anonymous [Bennett], 1830)	whitespotted bamboo shark	Masuda et al., 2005; Adams, personal communication
Scyliorhinus torazame (Tanaka, 1908)	cloudy cat shark	Masuda et al., 2003
Hemiscyllium ocellatum (Bonnaterre, 1788)	epaulette shark	Janse, personal communication; Daly and Jones, unpublished results
Carcharias taurus (Rafinesque, 1810)	sand tiger shark	Daly and Jones, unpublished results
Urolophus paucimaculatus (Dixon, 1969)	sparsely-spotted stingaree	Daly and Jones, unpublished results
Urolophus gigas (Scott, 1954)	spotted stingaree	Daly and Jones, unpublished results
Chiloscyllium punctatum (Muller & Henle, 1839)	brownbanded bambooshark	Daly and Jones, unpublished results
Notorynchus cepedianus (Péron, 1807)	broadnose sevengill shark	Daly and Jones, unpublished results
Dasyatis brevicaudata (Hutton, 1875)	short-tail stingray	Daly and Jones, unpublished results
Potamotrygon motoro (Muller & Henle, 1841)	South American freshwater stingray	Daly and Jones, unpublished results
Triaenodon obesus (Rüppell, 1837)	whitetip reef shark	Daly and Jones, unpublished results
Myliobatis australis (Macleay, 1881)	Australian bull ray	Daly and Jones, unpublished results
Triakis scyllium (Müller & Henle, 1839)	banded houndshark	Minamikawa and Morisawa, 1996
Stegostoma fasciatum (Hermann, 1783)	zebra shark	Janse, personal communication
Raja eglanteria (Bosc, 1800)	clearnose skate	Luer et al., 2007

of seminal fluid and fluid from the alkaline gland, suggesting that secretions from male secondary sexual organs may be important for sperm activity. From the limited information available, it appears that the osmotic pressure of solutions in which sperm are held is more important than the exact ionic composition of those solutions. However, it is important to note that most solutions used for diluting and handling shark semen have contained high amounts of NaCl and urea to mimic conditions naturally found in elasmobranch biological fluids.

Another method that has been used to assess the quality of elasmobranch sperm is "viability" staining. This method involves the use of a dye that is membrane impermeant—meaning that it is excluded by normal, "viable" cells, but is able to stain the nucleii of "non-viable" cells with compromised plasma membranes. The fluorescent dyes SYBR® 14 and propidium iodide (LIVE/DEAD® Sperm Viability Kit, Molecular Probes, Eugene, USA) have been used in conjunction with flow cytometry to assess the viability of sperm from sparsely-spotted stingarees, *Urolophus paucimaculatus* (Dixon, 1969), and *M. australis* (Daly and Jones, unpublished results), as well as assessing seasonal changes in semen quality in *D. brevicaudata* (Daly, 2008). The disadvantage of assays that use fluorescent dyes is that they require access to specialized equipment (i.e., a flow cytometer or fluorescent microscope) for analysis, limiting their application in aquaria for routine semen assessment and situations where immediate sperm quality analyses are required.

An alternative to fluorescent stains is viability stains, which can be viewed through standard light microscopy. One such viability stain is Nigrosin-Eosin, which is commonly used to assess mammalian and avian sperm, but, to date, has had limited application for elasmobranch sperm. The simplicity of this staining method makes it a suitable means of assessing sperm quality in the aquarium setting, as a complement to motility assessments. It is important to note that motility assessment and viability staining evaluate two different aspects of sperm function, so wherever possible, these methods should be used in combination to maximize the accuracy of sperm quality assessments.

METHODS FOR CONTROLLING REPRODUCTION

Artificial insemination

An area of research that is gaining increasing attention within aquaria is the use of artificial insemination to achieve pregnancy in elasmobranchs (Adams, personal communication; Janse, personal communication; Daly and Jones, unpublished results). Although still in the early stages of development, results achieved so far indicate that this technique will be an important component of future aquarium elasmobranch breeding programs. Research on artificial insemination procedures is ongoing, but, in general, artificial insemination of elasmobranchs involves depositing sperm in the female reproductive tract with a tube or catheter attached to a syringe or pipette.

There are only three published accounts of artificial insemination in elasmobranchs (Masuda et al., 2003; Masuda et al., 2005; Luer et al., 2007), although there have been other unpublished attempts conducted by aquaria around the world. Masuda et al. (2003) achieved a fertilization rate of 76.9% in cloudy catsharks, *Scyliorhinus torazame* (Tanaka, 1908), when sperm were deposited in the cloaca of the female. A follow-up study using whitespotted bamboosharks, *Chiloscyllium plagiosum* (Anonymous [Bennett], 1830), reported a fertilization rate of 23.3% when sperm were deposited in the uterus of the female (Masuda et al., 2005). An unpublished study using *C. plagiosum* found that oviduct insemination resulted in a fertilization rate of 100%, but that insemination of a second female, by depositing semen in the cloaca, did not result in fertile eggs (Adams, personal communication). After re-insemination, using the oviduct method, the second female began to produce fertile eggs. A study by Luer et al. (2007) tested different insemination methods in *R. eglanteria* and found that fertilization occurred when sperm were deposited in the cloaca or right uterine horn of the female, but did not occur when sperm were deposited in the left uterine horn. When compared to insemination of the right uterine horn, cloacal insemination resulted in a slightly higher fertilization rate during the first six weeks following insemination (100% compared to 77%), but a slightly lower fertilization rate from six to ten weeks after insemination (10% compared to 33%). There have also been unsuccessful attempts to artificially inseminate *N. cepedianus* (Daly and Jones, unpublished results), *C. taurus* (Daly and Jones, unpublished results), and zebra sharks, *Stegostoma fasciatum* (Hermann, 1783) (Janse, personal communication). It appears from these studies that the site at which the sperm are deposited during the artificial insemination procedure has an effect on fertilization success, and that outcomes may be affected by species differences, the timing of insemination or by the person performing the procedure.

Artificial insemination has also been attempted using semen that has been stored for a period of hours to days prior to insemination (Adams, personal communication). The motility and fertilizing ability of semen collected from *C. plagiosum* and stored at room temperature (21°C) or refrigerated (4°C) was assessed at 24, 48, and 72 h of storage. Fertilization was achieved using sperm stored at 4°C for 24 h, but not using semen that was stored at 4°C for 48 h, or semen that was stored at room temperature. Successful artificial insemination of a female *C. punctatum* at SEALIFE Melbourne Aquarium, Australia, was achieved using sperm collected at Underwater World SEALIFE Aquarium, Mooloolaba, Australia, and stored at 4°C for 8 h during transport (Daly and Jones, unpublished results). Attempts were made to artificially inseminate a female *C. punctatum*, using cryopreserved semen, but successful fertilization was not achieved (Daly and Jones, unpublished results).

Artificial rearing of embryos from viviparous species

Although the incubation of eggs from oviparous species is relatively common among aquaria, there is only one account of an attempt to artificially rear developing fetuses from a viviparous species (Otway and Ellis, 2011). In this study, investigators removed late term fetuses with no external yolk sac from a pregnant ornate wobbegong, *Orectolobus ornatus* (De Vis, 1883), and transferred them to an artificial uterus for the final 18 days of gestation. All fetuses survived until "birth" and followed similar growth patterns to *O. ornatus* pups born naturally. Artificial rearing of embryos or fetuses from earlier developmental stages is likely to be restricted by difficulties in accurately replicating the elasmobranch uterine environment and embryo nutrition in species with matrotrophy. With further research it is possible that artificial uteri could have an application in aquarium elasmobranch breeding programs, however, it is unlikely that this will occur in the near future.

Sperm cryopreservation

Sperm cryopreservation involves the dilution of semen in an extender solution containing cryoprotectants, which help protects the sperm from freezing injury, packaging samples in straws or vials, freezing at a controlled cooling rate, and storing the samples in liquid nitrogen at -196°C. Despite the common use of sperm cryopreservation in breeding programs for a wide range of aquatic animals (Tiersch and Green, 2011), there has been very little work done with elasmobranchs. The only published account of cryopreservation of sperm from an elasmobranch was in *U. paucimaculatus* (Daly et al., 2011). This study reported post-thaw membrane integrity of 23.4 ± 0.4% and an initial motility of 37.5 ± 3.4% when sperm were frozen in an extender containing elasmobranch ringer solution (257 mM NaCl, 7 mM Na_2SO_4, 2.5 mM $NaHCO_3$, 4 mM KCl, 2 mM $CaCl_2$, 3 mM $MgSO_4$, 70 mM trimethylamine N-oxide, 0.27 mM Na_2HPO_4, 0.01 mM NaH_2PO_4, 400 mM urea, 30 mM glucose) with 20% v/v egg yolk and 10% v/v glycerol. Sperm frozen using a tris-sucrose-potassium solution (30 mM Tris, 25 mM sucrose, 600 mM KCl) with 20% v/v egg yolk and 10% v/v glycerol or dimethyl sulfoxide (DMSO) had slightly lower post-thaw membrane integrity (21.7 ± 0.5% and 18.4 ± 0.4%, respectively) and motility (35.8 ± 4.0% and 30.8 ± 3.3%, respectively), but a longer duration of motility compared to other treatments.

Recent experiments on cryopreservation of sperm from *M. australis* have achieved a post-thaw motility of 30% (50% of pre-freeze motility) and a membrane integrity of 57.5% using elasmobranch ringer solution containing 10% v/v glycerol. The higher post-thaw motility and membrane integrity in this recent study may have been due to the faster cooling rate used (>10°C/min) compared to the earlier study in *U. paucimaculatus* (3°C/min), but may also be due to species differences in sperm freezing tolerance. It is plausible that there will be variation in sperm cryopreservation requirements between elasmobranch species. Recent experiments on cryopreservation of sperm from *C. punctatum* have found that glycerol is toxic to sperm from this species, despite having protective effects on sperm from *U. paucimaculatus* and *M. australis* (Daly and Jones, unpublished results). Although some components of the sperm cryopreservation protocol will remain the same for all elasmobranch species, some aspects, including cryoprotectant type, will need to be trialed for each species.

Decreasing reproduction

It is sometimes necessary to reduce the reproductive capacity of an individual or population in an aquarium. This need may be for population management, to mitigate interactions among conspecifics or to improve the health of an animal. In elasmobranch species such as *C. taurus*, seasonal changes in water temperature are important for reproductive behavior and mating (Henningsen et al., 2004; Henningsen et al., 2008). For these species, interactions among conspecifics and reproductive activity can be re-

duced by maintaining a constant water temperature (Henningsen et al., 2008). For other species, the simplest and most common way to reduce reproduction is by housing single sex populations, although this can have negative health consequences in some species. Female *D. americana* housed in single sex populations are known to develop reproductive disease characterized by increased estrogen levels, ovarian pathologies and an overabundance of histotroph in the uterus (Mylniczenko and Penfold, 2012; George, personal communication; Mylniczenko, personal communication). Depo-Provera® (medroxyprogesterone acetate, 400 mg/mL, Pfizer) has been used successfully to reduce the incidence of reproductive disease in single sex populations of *D. americana* (Mylniczenko, personal communication). Ovariectomy has also been used in subadult females of this species as a more permanent means of eliminating reproductive function and preventing the development of reproductive disease (George, personal communication; George, this volume), although the long term effects of this procedure are currently unknown.

Hormonal implants containing deslorelin, a GnRH agonist, have been tested as a means of reducing reproduction in elasmobranch species, with mixed success. Suprelorin™ implants (deslorelin, 4.7 mg, Peptech Animal Health) have been tested in male *D. brevicaudata*, but were unsuccessful in reducing reproductive function (Daly and Jones, unpublished results). Suprelorin™ implants have also been trialed in a male *S. fasciatum* (Mylniczenko, personal communication). In female largespot river stingrays, *Potamotrygon falkneri* (Castex & Maciel, 1963), Suprelorin™ implants (deslorelin, 4.7 mg) produced mixed results, with one of two females producing pups while on the treatment (Hanna, personal communication). Reasons for the varied success of hormonal implants in elasmobranchs are unknown, but may be related to variation in the physiological response to hormonal preparations or differences in the application of the implants. Therefore, the utility of hormonal implants to control reproduction in elasmobranchs should be carefully assessed for each species.

FUTURE DIRECTIONS

Reproductive technologies are a relatively new addition to aquarium breeding programs for elasmobranchs, but they have already contributed greatly to the management of various species. While technologies, such as ultrasound, have gained relatively widespread use, others, such as sperm cryopreservation and artificial insemination, are still in the early stages of development. Sperm cryopreservation protocols have so far been tested in only three species, yielding mixed results. Many factors can affect sperm cryopreservation, including base medium composition, cooling rate, cryoprotectant type, cryoprotectant concentration and equilibration time. These factors will need to be optimized for each species in order for sperm cryopreservation to be a useful technique for aquarium breeding programs and for gene banking of elasmobranch sperm.

Preliminary studies on artificial insemination have given an indication of this technology's potential, but there is still a lot of research required before it will become common practice among aquaria. It is worth noting that, so far, all successful attempts at artificial insemination have been in oviparous species, in which ovulation can be easily visualized. Further research is needed to adapt this technique for viviparous species and to identify the optimal time for insemination. Although sperm storage has been reported for many elasmobranch species, the receptivity of the female reproductive tract to sperm storage may be hormonally modulated and variable throughout the reproductive cycle. For species with a well-defined annual or biennial cycle, it may be possible to predict the timing of artificial insemination based on behavioral cues or ultrasound examination, while other species may benefit from hormonal monitoring to predict the timing of ovulation, or even the induction of ovulation through administration of exogenous hormones.

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Chapter 37

Reproduction of the sand tiger sharks, *Carcharias taurus*, in aquaria: a framework for a managed breeding program

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Abstract: Although a large, sexually mature, meta-population of sand tiger sharks, *Carcharias taurus* (Rafinesque, 1810), is maintained in aquaria worldwide, breeding success in this species has been limited. To date, successful reproduction—including the birth of live, healthy young—as a result of copulation in captivity, has not been achieved outside of Australian, South African, and recently, Middle Eastern aquaria. Using examples of successful reproductive events in these aquaria, we provide a framework from which a successful sand tiger shark breeding program can be developed. Until recently, no organized, collaborative breeding effort had been attempted for sand tiger sharks in aquaria. Such a directed program must take into account the biology of the species; including reproductive biology and physiology, behavior, social factors, and environmental parameters and cues. In addition, the incorporation of technology, integrated communication, detailed record keeping and active collaboration are critical to the success of a program. The primary goal of a sand tiger shark reproduction program should be to sustain stock levels for public display, while also supplementing knowledge about the biology and husbandry of this species.

INTRODUCTION

The reproduction of animals in human care may be either opportunistic or the result of a concentrated effort. Over 100 species of chondrichthyan fishes have successfully reproduced in aquaria and much of the early information on reproduction in elasmobranchs came from aquarium animals (Henningsen et al., 2004b; Koob, 2004). In recent years, however, increased husbandry efforts, more natural display settings and greater knowledge of species' biology, have resulted in reproductive success in species that had previously been considered challenging. Examples include the smalltooth sawfish, *Pristis pectinata* (Latham, 1794), at Atlantis Paradise Island Resort in the Bahamas (www1), and the manta ray, *Manta birostris* (Donndorff, 1798), at the Okinawa Churaumi Aquarium. Aquarium breeding programs should focus on species of conservation concern that have not yet been bred in aquaria, and those that are highly desired for public display or for scientific research (Table 1). Here, we focus on one such species, the sand tiger shark, *Carcharias taurus* (Rafinesque 1810), as a case study to build a framework for a successful aquarium breeding program.

THE SAND TIGER SHARK

The *C. taurus* is a large (to 3.2 m total length; TL), wide-ranging, coastal lamniform shark. It is found in warm temperate to tropical waters, although absent in the central and eastern Pacific (Compagno, 2001; Castro, 2011; Ebert et al., 2013), and undertakes seasonal migrations that are coupled with its reproductive cycle (Gilmore et al., 1983; Gilmore, 1993; Parker and Bucher, 2000). Like most elasmobranchs and other K-selected species, life history constraints, such as delayed sexual maturity, slow growth, long lifespan and particularly low fecundity, make this species extremely vulnerable to exploitation (Dulvy et al., 2014). The biennial or triennial reproductive cycle of female *C. taurus* (Pollard and Smith, 2005; Pollard and Smith, 2009), and their unique reproductive mode and methods of embryonic nutrition (embryophagy and oophagy), result in the species' low replacement rate of a maximum of two young per female, every other year. As a consequence, *C. taurus* is globally listed as vulnerable. However, regionally, this status ranges from near threatened on the west coast of Australia, to critically endangered in the southwest Atlantic and on the east coast of Australia (Pollard and Smith, 2009). Although the most recent status review for *C. taurus* in the northwestern Atlantic by Carlson et al. (2009) suggested that it be retained as a species of concern, as a precautionary measure, it is not listed as endangered in the region. Information to date suggests very limited genetic exchange across ocean basins or even between the east and west coasts of Australia (Stow et al., 2006; Ahonen et al., 2009).

In order to formulate appropriate and effective species management strategies, a thorough and comprehensive understanding of the species' reproductive biology is essential. The reproductive mode of *C. taurus* contributes to a unique mating system, where behavioral polyandry may still result in genetic monogamy due to intrauterine cannibalism (Chapman et al., 2013).

C. taurus are commonly maintained in public aquaria due to their hardiness, large body and "toothy" appearance. According to the American

Table 1. Plan of action for an aquarium elasmobranch breeding program.

Plan of action

Aquarium elasmobranch breeding program

General considerations:

1. Public aquaria intending to develop a aquarium breeding program should consider which species represent a conservation priority, specifically:

   a. Is the species listed as endangered or critically endangered on the IUCN Red List of Threatened Species?
   b. Is the species regionally endemic, little studied, or even undescribed, and at risk of losing its habitat?
   c. Is the species in demand for public aquariums, e.g., sand tiger shark, *Carcharias taurus* (Rafinesque, 1810), zebra shark, *Stegostoma fasciatum* (Hermann, 1783), spotted eagle ray, *Aetobatus narinari* (Euphrasen, 1790) etc; and
   d. Does the aquarium have the requisite expertise?

2. Public aquaria should consider the long-term objectives of the breeding program, specifically:

   a. Will breeding and inter-aquarium distribution of the species reduce pressure on wild populations?;
   b. Will the breeding program contribute towards the collective knowledge of elasmobranch reproduction?; and
   c. Is the intention to breed a pool of animals for future release into the wild and, if so, is this a fitting objective?

3. Public aquaria should discourage the breeding of common species excess to current requirements. Instead, surplus animals may be considered for invasive reproduction research studies (e.g., investigation of organ development studies).

Priority aquarium breeding objectives:

1. Establish an aquarium elasmobranch breeding specialist group.
2. Develop a databank of aquarium elasmobranch breeding information, detailing relevant aspects of species successfully reproduced, or exhibiting reproductive behavior, in public aquariums.
3. Establish zoological studbooks for those species that have bred successfully in captivity and that require a management program.
4. Develop a common system of identification to track individual animals within a breeding meta-population.
5. Establish a centralized breeding facility to support the development of collaborative breeding programs for key species (e.g., *C. taurus, S. fasciatum*).
6. Establish a tissue bank as a resource for reproduction studies. Support genetic and hormonal research by making tissue samples available for appropriate projects.

Elasmobranch Society International Captive Elasmobranch Census, 137 male and 105 female *C. taurus* were held in aquaria worldwide in 2008, and the 2009 United States (US) census reported 99 males and 62 females held in US institutions alone (Firchau, personal communication). The Association of Zoos and Aquariums (AZA) studbook (December 2014) recently reported a population of 181 *C. taurus*, consisting of 114 males and 67 females, maintained in 33 AZA accredited facilities (www2). The population size increased to 201 (114 males and 87 females) in December 2015, when formerly non-AZA accredited institutions were included (Littlehale, personal communication). Despite the species' hardiness and its popularity within the aquarium industry, few aquarium births have been achieved (Henningsen et al., 2004b). However, reproductive behaviors and sexual

conflict in *C. taurus* are fairly common in aquaria. In fact, copulation has been documented in many cases, and reproductive behavior in this species was first described from an aquarium population (Gordon, 1993; Henningsen et al., 2004b).

HISTORY OF AQUARIUM REPRODUCTION IN THE SAND TIGER SHARK

There are records of premature *C. taurus* born in aquaria, resulting from mating in the wild, at Marineland of Florida, as far back as 1958, and Sea World Australia, in 1988 (Smith, personal communication). However, to date, reproductive behavior of *C. taurus* in human care has only led to the successful production of live, healthy young at four institutions: Underwater World SEA LIFE Mooloolaba (Australia), Manly Sea Life Sanctuary (formerly known as Oceanworld Manly) (Australia), Sea World South Africa Durban / UShaka Marine World (South Africa) and the Scientific Center of Kuwait (Middle East) (Willson and Smith, this volume). Details of *C. taurus* born in aquaria are provided in Table 2, along with their relative genealogies in Figures 1-3. The basic physical characteristics of aquaria that have achieved successful *C. taurus* reproduction are provided (Table 3), as well as some comparative parameters from AZA-accredited facilities that maintain *C. taurus*, but have not had successful births (Table 4).

Despite the rarity of *C. taurus* births in aquaria, observations of reproductive behaviors have been documented at several institutions. Based on a 2010 survey of AZA institutions housing *C. taurus*, 15 institutions reported observing reproductive behavior, whereas an equal number had not (although 12 of those institutions maintained single sex populations). Six institutions reported bite wounds only, three reported changes in behavior, such as “avoidance” or “following”, and six reported copulation in *C. taurus*. A further survey with specific questions on reproduction and reproductive behavior/sexual conflict in *C. taurus* was returned by nine institutions. The limited results obtained in this survey (Figures 4-5) corroborated previous findings that behaviors associated with reproduction in *C. taurus* were observed more commonly than successful reproduction (Henningsen et al., 2004b; Nicholson, 2009). UnderWater World SEA LIFE Mooloolaba has recorded the greatest success of aquarium reproduction of *C. taurus* over a 20 year period, as further described by Willson and Smith (this volume).

C. taurus represents an ideal model species to use as a framework for an aquarium breeding program, due to its conservation status, popularity and abundance in public aquaria, hardiness in captivity, unique mode of embryonic development, documented and described reproductive cycle and behavior, and the relative paucity of reproductive success.

Factors linked with successful aquarium reproduction of *C. taurus*

Environmental (i.e., temperature, photoperiod), architectural and social factors were identified as being highly relevant to successful mating, gestation and parturition in *C. taurus*. This finding is unsurprising, as temperature and photoperiod are generally considered to be two of the most important environmental factors influencing elasmobranch reproduction in general (Demski, 1990a; Demski, 1990b). Seasonal variation in temperature and photoperiod were both employed by institutions that have successfully bred *C. taurus* (Table 3). Furthermore, equal, or female-skewed, sex ratios were maintained by these institutions and adequate space to allow for the separation of individuals (i.e., to provide refuge for females away from courting males) was available. Conversely, the absence of a suitable area to limit social interactions and sexual conflicts has occasionally resulted in traumatic intra- and inter-sexual interactions (Henningsen et al. 2004b; Claus, 2014).

ASSESSMENT OF MATURITY AND REPRODUCTIVE STATUS

The success of a aquarium breeding program relies upon several intrinsic factors, not least of which includes a mixed-gender population of healthy, mature individuals. A thorough understanding of the life history characteristics of the target species is also highly advantageous, as is knowledge about the source of the specimens, the age and morphometrics of individuals within the population, and their maturity and reproductive status (Henningsen et al., 2004a). It is beyond the scope of this chapter to cover life history and assessment of maturity in elasmobranchs, however, a wealth of general and species-specific reviews and publications on the topic are available (Hamlett, 1999; Hamlett and Koob, 1999; Conrath, 2004).

It is also important to assess how human care activities and the display environment may affect breeding. There is strong evidence suggesting

Table 2. Aquarium births of sand tiger sharks, *Carcharias taurus* (Rafinesque, 1810), showing institution, date of birth, gender and survivorship. All pups were the result of aquarium insemination with the exception of Julie (*) and Juliette (*), born the day after a gravid female was collected. Sex ratio is expressed as (x.x.x.) = (male.female.unknown). + indicates that the shark was alive at the time of writing.

Sea World South Africa (uShaka Marine World), Durban

Parturition events: 2
Pups (total): (1.3.0) = 4
Live: (1.3.0) = 4
Stillborn: (0.0.0) = 0

Shark name	Sex	Date born	Survival (years)	Comments
unnamed	F	10 Nov 1998	1.2	Eaten by *Carcharhinus leucas*
unnamed	F	10 Nov 1998	16.4 +	Released to wild (12 Mar 2015)
Storm (uShaka)	F	09 Mar 2013	2.7 +	Transferred to National Marine Aquarium, UK (1 Nov 2015)
Toni (Mandela)	M	04 Dec 2014	0.9 +	Transferred to National Marine Aquarium, UK (1 Nov 2015)

Scientific Center of Kuwait, Middle East

Parturition events: 4
Pups (total): (1.4.0) = 5
Live: (0.1.0) = 1
Stillborn: (1.3.0) = 4

Shark name	Sex	Date born	Survival (years)	Comments
unnnamed	F	11 Feb 2003	0	Live pup born during fourth parturition event

Manly Sea Life Sanctuary, Sydney , Australia

Parturition events: 9
Pups (total): (3.2.8) = 13
Live: (3.2.0) = 5
Stillborn: (0.0.8) = 8

Shark name	Sex	Date born	Survival (years)	Comments
Maia	F	21 Dec 2001	10.1	
Apollo	M	21 Dec 2001	2.9	
Phebos	F	03 Nov 2003	0	
Atlas	M	29 Jun 2006	0	Pup premature (Townsend et al., 2015)
Murdoch	M	06 Feb 2007	8 +	

Underwater World SEALIFE Mooloolaba

Parturition events: 8
Pups (total): (5.8.4) = 17
Live: (1.6.0) = 7
Stillborn: (4.2.4) = 10

Shark name	Sex	Date born	Survival (years)	Comments
Julie (Ali) *	F	24 Jul 1992	16.5	Transferred to Melbourne Aquarium (2 Jun 2000)
Juliette *	F	24 Jul 1992	12.4	
Fatty (Georgie)	F	08 Nov 1997	11.6	Transferred to Melbourne Aquarium (5 Oct 2001)
Bent Spine	F	08 Nov 1997	10.9	
Unnamed	F	Nov 1999	0.3	Mortality associated with transport
Unnamed	M	Nov 1999	0.3	Mortality associated with transport
Freckles	F	18 Sep 2009	6 +	Transferred to Manly Sea Life Sanctuary

that unnatural or inconsistent temperature and photoperiod profiles may shift the seasonality of elasmobranch reproductive cycles. This hypothesis has been corroborated both behaviorally and from endocrine measures in aquarium animals (Henningsen et al., 2004a; Henningsen et al., 2004b), and in *C. taurus*, specifically (Henningsen et al., 2015). This is a phenomenon that is not unique to aquarium animals, however, as the Atlantic sharpnose

shark, *Rhizoprionodon terraenovae* (Richardson, 1836), has shown a shift in seasonal reproductive cycle in response to rising sea temperatures (Hoffmayer et al., 2010).

Reproductive status

Hormone levels serve as a valuable tool to determine shark maturity, reproductive status and current stage of reproductive cycle. Both peptide and steroid hormones play a role in the regulation of the reproductive tract in both sexes of sharks (Gelsleichter, 2004; Callard et al., 2005). Published information on normal baseline levels is available for many species of elasmobranchs, however, a dataset of hormone levels over the entire reproductive cycle of both sexes is incomplete (Manire et al., 1999; Henningsen et al., 2004a).

Levels of reproductive steroid hormones obtained from plasma, serum or muscle tissue, have been used as a non-lethal means of determining maturity and reproductive status in elasmobranchs (Sulikowski et al., 2007; Prohaska et al., 2013). In general, male elasmobranchs, with an annual cycle, have androgen levels (chiefly testosterone and dihydrotestosterone) that rise prior to the onset of mating behavior and mating, accompanied by lower estrogen (estradiol) and progestin (progesterone) levels. Trends in female hormone level are more variable and are largely dependent on reproductive mode. However, elevated estradiol levels are typically associated with the growth of the oocytes (follicular phase, during vitellogenesis) and decrease following ovulation. In oviparous species, progesterone peaks peri-ovulatory and, in viviparous species,

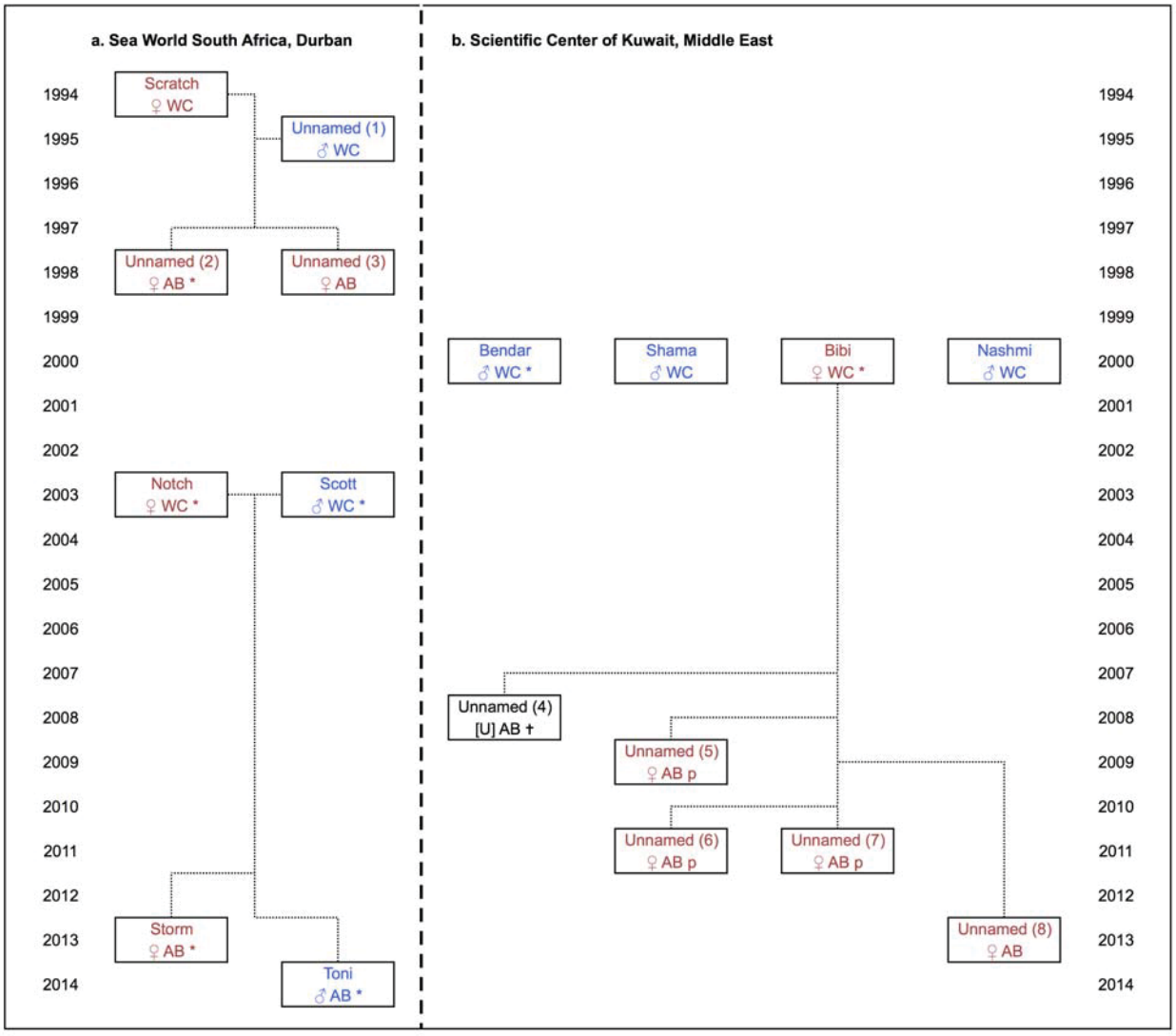

Figure 1. The genealogy of sand tiger sharks, *Carcharias taurus* (Rafinesque, 1810), successfully bred in aquaria from: (a) Sea World South Africa, Durban and (b) Scientific Center of Kuwait, Middle East [WC = wild caught. AB = aquarium bred. † = still born. p = premature. * = shark is alive at time of writing].

persists through early- to mid-gestation (Callard and Koob, 1993; Henningsen, 1999; Gelsleichter, 2004; Callard et al., 2005). Progesterone titers decrease as estradiol levels rise towards the end of gestation, prior to parturition (Hamlett and Koob, 1999; Callard et al., 2005; Gelsleichter, 2004).

Non-steroid hormones involved with reproduction include gonadotropins, thyroid hormones, the tropic hormones, gonadotropin releasing hormone (GnRH), thyroid stimulating hormone (TSH), oxytocin-like nonapepetides (asvatocin and glumatocin), and possibly prolactin and similar peptide hormones (Gelsleichter, 2004). These, hormones, however, have not received as much attention as the steroid hormones and greater investigation on their levels may be useful in improving breeding efforts. Analogs of GnRH have been used to regulate, promote, and inhibit reproduction and sexual conflicts in a variety of vertebrate taxa (e.g., Atkinson et al., 1998; Felberbaum et al., 2000), and may be agonistic or antagonistic. In other vertebrates, agonists act by engaging the feedback system to reduce gonadal steroid synthesis, whereas antagonists act by competitively binding the GnRH receptor (Felberbaum et al., 2000). An investigation carried out in male *C. taurus* in aquaria demonstrated that when incorporated in time-release implants, an antagonist was maintained in systemic circulation for at least eight months (Henningsen et al., 2015). Further research is needed to document the effect of GnRH analogs on circulating steroid levels and behavior in elasmobranchs.

Diagnostic imaging techniques, such as sonography, are useful when assessing the reproductive status of elasmobranchs (Whittamore et al., 2010; Pereira et al., this volume; Mylniczeniko et al., this volume). Stetter (2004) detailed the use of ultrasound imaging for gross

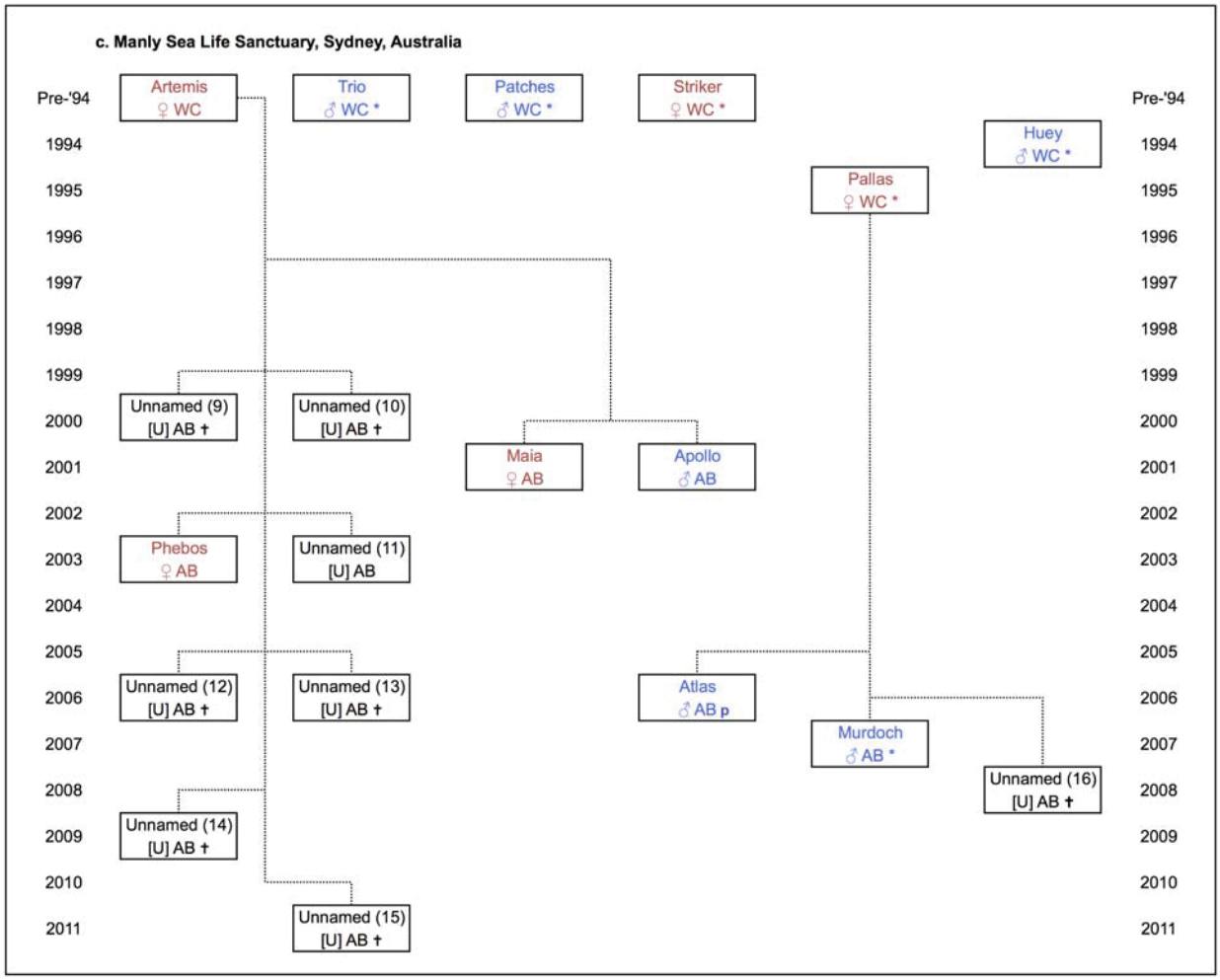

Figure 2. The genealogy of sand tiger sharks, *Carcharias taurus* (Rafinesque, 1810), successfully bred in aquaria from Manly Sea Life Sanctuary, Sydney, Australia [WC = wild caught. AB = aquarium bred. † = still born. p = premature. * = shark is alive at time of writing].

Table 3. Basic physical characteristics of aquarium systems where sand tiger sharks, *Carcharias taurus* (Rafinesque, 1810), have successfully reproduced. All exhibits were supplied with natural seawater. Sex ratio of population is expressed as (x.x.x.) = (male.female.unknown).

	Tank volume (m^3)	Photoperiod (light / dark)	Lighting type	Temperature (°C)	Population (m.f.u)
Manly Sea Life Sanctuary Sydney, Australia	4,000	10 / 14	Artificial	14.0 - 24.0	4.5.0
Underwater World SEALIFE Mooloolaba, Australia	2,302	10.5 / 13.5	Artificial	17.8 - 25.0	50:50
Sea World South Africa Durban (Exhibit 1)	1,450	Seasonal	Natural	20.0 - 26.0	2.5.0
Sea World South Africa Durban (Exhibit 2)	750	Seasonal	Natural	20.0 - 26.0	2.2.0

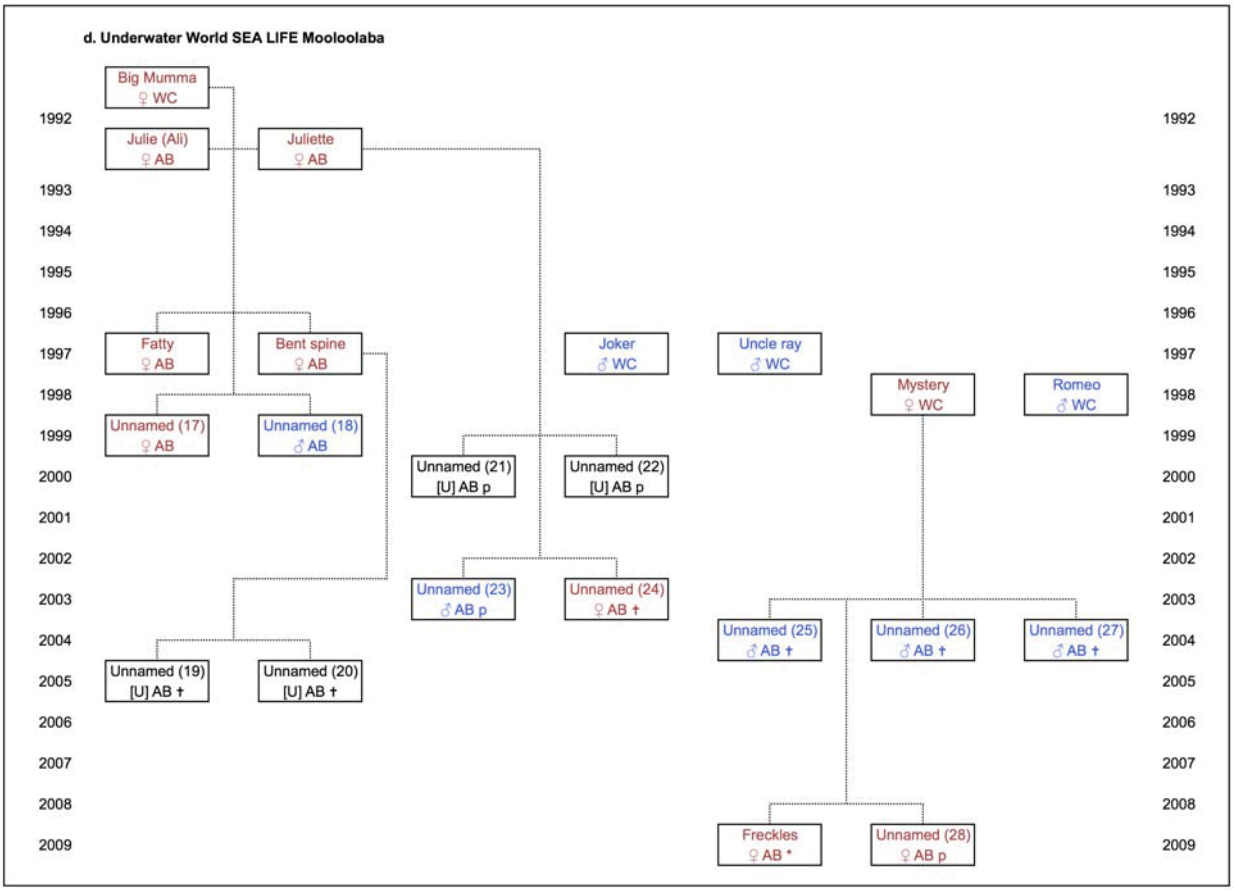

Figure 3. The genealogy of sand tiger sharks, *Carcharias taurus* (Rafinesque, 1810), successfully bred in aquaria from Underwater World SEA LIFE Mooloolaba (d). WC = wild caught. AB = aquarium bred. † = still born. p = premature. * = shark is alive at time of writing.

Table 4. A comparison of the basic physical characteristics of international aquarium systems, where sand tiger sharks, *Carcharias taurus* (Rafinesque, 1810) have successfully reproduced, versus AZA-accredited facilities in the USA, that maintain *C. taurus* but have not had successful reproduction.

	Saltwater type	Temperature	Photoperiod	Lighting type
International institutions: aquarium breeding observed (n = 3)	natural (x3) artificial (x0)	constant (x0) seasonal (x3) manipulated (x0)	night > day (x2) night = day (x0) night < day (x0) variable (x0) seasonal (x1)	natural (x1) artificial (x2)
AZA institutions: reproductive behavior observed (n = 15)	natural (x4) artificial (x11)	constant (x10) seasonal (x2) manipulated (x3)	night > day (x3) night = day (x3) night < day (x3) variable (x5) seasonal (x1)	natural (x4) artificial (x11)
AZA institutions: copulation observed (n = 6)	natural (x1) artificial (x5)	constant (x5) seasonal (x1) manipulated (x0)	night > day (x0) night = day (x2) night < day (x1) variable (x2) seasonal (x1)	natural (x2) artificial (x4)

examination, as well as for the assessment of testicular, ovarian and uterine maturity, in aquarium and wild elasmobranchs. The reproductive cycle of female elasmobranchs is more variable than that of males, ranging from continuous (year around) to punctuated cycles, i.e. annual, biennial or triennial (Hamlett and Koob, 1999).

Levels of reproductive steroid hormone in *C. taurus* are available from only three published studies (Rasmusssen and Murru, 1992; Henningsen et al., 2008; Henningsen et al., 2015). Estradiol ranged from 450 - 690 pg/ml in two serially-sampled immature sharks, and from 600 - 2,000 pg/mL in two mature females (Rasmussen and Murru, 1992). Published values for steroid levels in *C. taurus*, across the reproductive cycle, demonstrated an annual cycle in aquarium males (Henningsen et al., 2008; Henningsen et al., 2015), and a biennial cycle in an aquarium female (Henningsen et al., 2008).

Reproductive technologies, such as artificial insemination, are currently being investigated in *C. taurus* (Daly and Jones, 2013; Daly and Jones, this volume) and these technologies may eventually be incorporated into the framework of a breeding program. However, further knowledge, including a greater understanding of sperm dynamics in males and ovarian and uterine dynamics in females, will vastly improve the success rate of reproductive technologies. For successful fertilization, sperm must be mature, viable and mobile, and female follicles must be post-vitellogenic and pre-ovulatory. Further, uterine conditions must be appropriate at the cellular, biochemical and physical levels. Recent work carried out in near-term ornate wobbegongs, *Orectolobus ornatus* (de Vis, 1883), which completed development in an artificial uterus (*ex utero)* (Otway and Ellis, 2010), may lead to further advances in reproductive technologies in *C. taurus* and other species. However, significant adjustments will need to be made to accommodate the wide variation in intraspecific developmental modalities (e.g., embryonic nutrition, uterine chemistry).

SEXUAL CONFLICT AND MATING SYSTEMS

Sexual conflict can be defined as any behavioral or biological interaction between or within sexes, including those that influence the genetic makeup of the offspring (Henningsen et al., 2004b; Portnoy, 2010). Mating systems can be broken down into behavioral and genetic, the former being observed mating behavior in terms of numbers of each sex involved in mating with the opposite sex, while the latter is best understood as paternity (i.e., single or multiple). The incor-

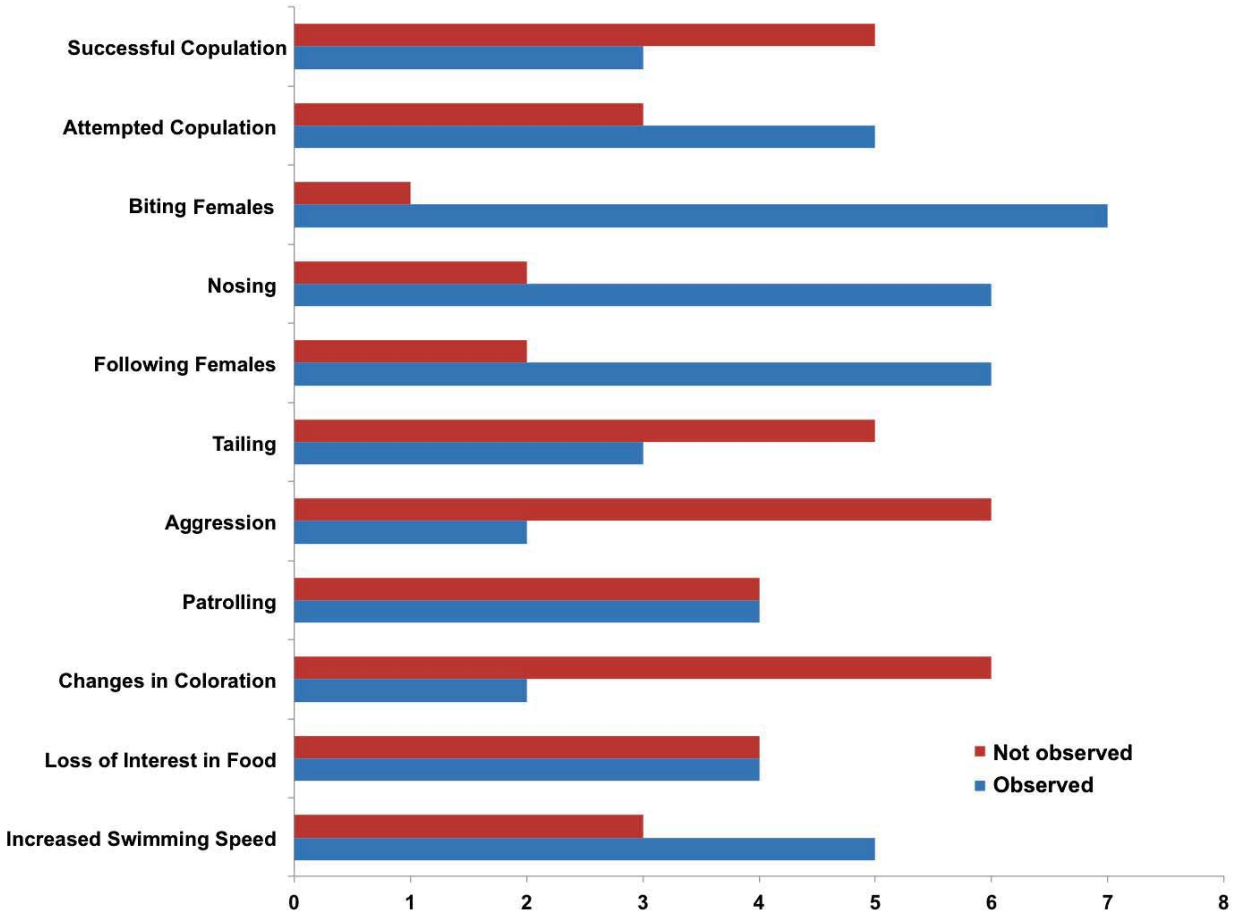

Figure 4. Reproductive behaviors observed (or not observed) in male aquarium sand tiger sharks, *Carcharias taurus* (Rafinesque, 1810), from nine AZA institutions.

poration of molecular genetics and, specifically, highly polymorphic molecular markers (HPMM), has greatly enhanced our understating of the genetic mating systems of many taxa, including elasmobranchs (Heist and Feldheim, 2004). While its application in aquaria is not as critical as in the wild, an understanding of mating systems is, nonetheless, important for factors such as kinship among litter-mates, predicting paternity and maintaining genetic diversity within zoological institutions (Heist and Feldheim, 2004; Portnoy, 2010). Of the species investigated to date, polyandry, or a litter sired by multiple males, is the most common modality employed by elasmobranchs. Information on genetic mating systems has been published for ten species of sharks and one skate (Byrne and Avise, 2012). In many cases, mating by multiple males has been observed in the wild and in aquaria (e.g., Uchida et al., 1990; Henningsen et al., 2004b). The behavioral mating system may, however, differ from the genetic mating system, and this can be tracked under aquarium conditions, where samples may be more easily collected. An additional mating system, and one proving to be more common than previously thought, is that of automictic parthenogenesis, where the female is the sole genetic contributor. This system has been documented in several species of elasmobranchs (Chapman et al., 2007; Chapman et al., 2008; Feldheim et al., 2010, Henningsen, 2016; Robinson et al., 2011; Fields et al., 2015; Harmon et al., 2016). Additional research is needed, but it appears that the distinctive embryophagous and oophagous mode of development in *C. taurus* allows for a unique mating system, using a combination of behavioral polyandry and genetic monogamy (Chapman et al., 2013). Male dominance hierarchies have been documented in *C. taurus* in aquaria (Gordon, 1993; Henningsen et al., 2004b; Henningsen et al., 2008) and suggested for wild conspecifics (Lucifora et al., 2002). This hierarchy may serve to further control paternity. However, multi-paternity was recently confirmed in aquarium-bred *C. taurus* (Townsend et al., 2015).

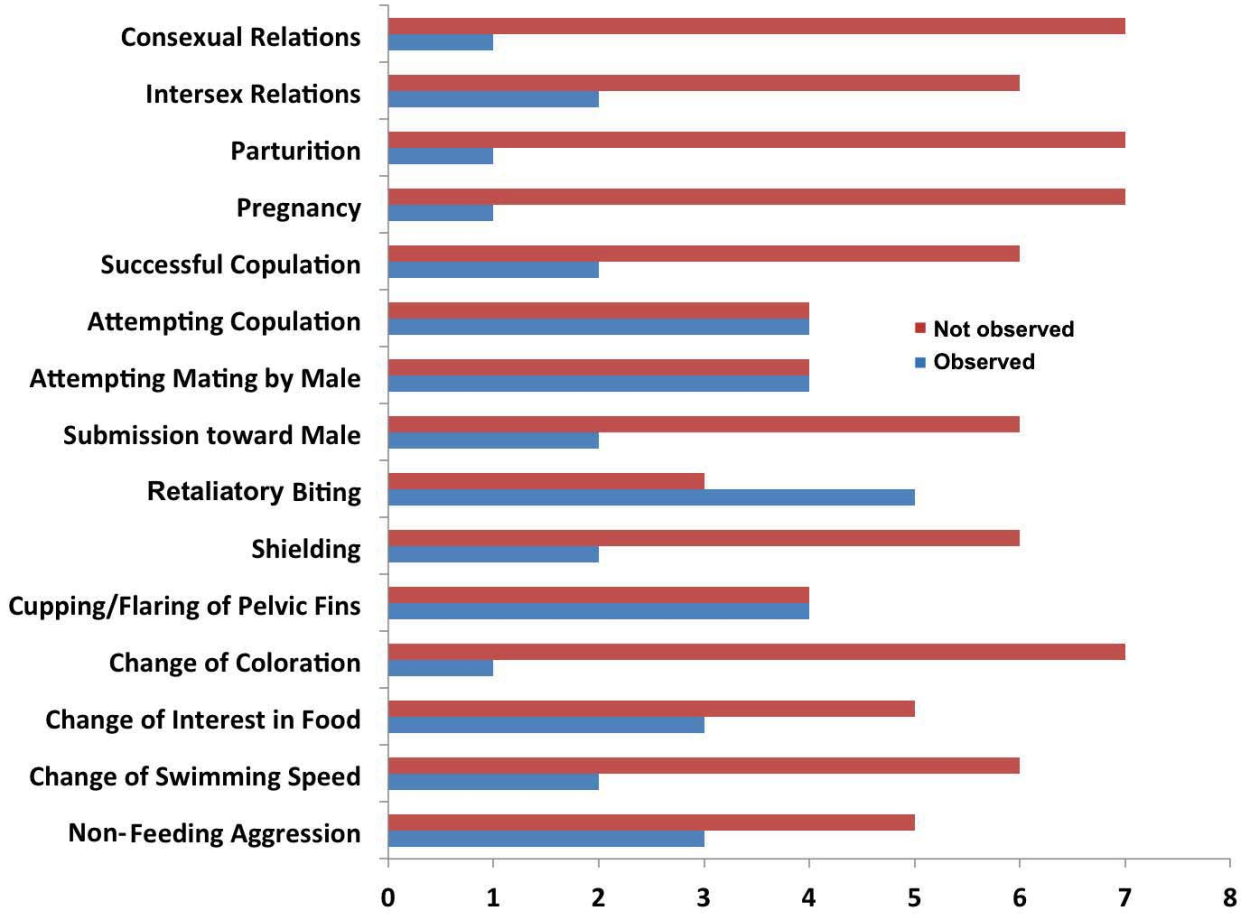

Figure 5. Reproductive behaviors observed (or not observed) in female aquarium sand tiger sharks, *Carcharias taurus* (Rafinesque, 1810), from nine AZA institutions.

COLLABORATION

It is essential that we, as stewards of living resources, are committed to and are able to manage these resources wisely. Perhaps one of the most important components of an animal management program is collaboration. It is important to initiate and facilitate collaboration for breeding programs as early as possible. All stakeholders should be involved in the collaboration from the onset, including aquarium and aquaculture (as appropriate) professionals, academic researchers, conservation groups, and local, state and federal governments. This type of focused, multi-disciplinary, collaboration increases the available knowledge base, facilitates funding, reduces the lag time to get a project started and allows stakeholders to better meet the breadth of project demands.

More recently, web-based collaborative groups have been formed to promote communication—e.g., Google Groups "SandTigerSSP". Formal efforts have been initiated by organizations, such as the South-East Zoo Alliance for Reproduction & Conservation (SEZARC), along with member institutions and others, to focus on artificial insemination and aquarium breeding of *C. taurus*. This effort has led to both aquarium staff and field researchers/officials to collaborate on the collection of blood and semen samples from *C. taurus*. Recently, representatives from 20 institutions attended a workshop where the maintenance of a sustainable aquarium population of *C. taurus* in the US was discussed (www3).

The primary aim of an aquarium *C. taurus* breeding program should be to provide specimens for public display and scientific research, thus allowing for continued scientific advancement, and an ongoing resource for educating the public about this species, its reproduction and relevance. It is unrealistic to expect that an aquarium elasmobranch breeding program could replenish (locally or globally) a depleted or extinct

population. However, the release of aquarium-bred specimens may be acceptable, as long as certain precautions are taken and providing there are no outstanding contraindications. Several aquarium specimens, including broadnose sevengill sharks, *Notorhynchus cepedianus* (Peron, 1807) (van Dykhuizen et al., 1998), *C. taurus* (Smale et al., 2012) and tiger sharks, *Galeocerdo cuvier* (Péron & Lesueur, 1822) (Marin-Osorno et al., this volume), have been released after years in captivity close to, or at, their capture position, and have survived and behaved in a manner consistent with wild conspecifics.

When contemplating release to the wild, it is important to consider the *Elasmobranch Plan of Action* (Smith et al., 2004), specifically: "*...Draft and adopt a re-introduction policy consistent with IUCN Re-introduction Specialist Group (www4) 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*..."

DISCUSSION AND SUMMARY

Building the framework

Although this chapter focused on aquarium reproduction of *C. taurus*, many of the concepts discussed are applicable to a range of species. Aquarium breeding programs, either small- or large-scale, can provide specimens for research and display purposes, thus reducing the need to collect animals from the wild. Research and display animals provide important biological information, allow us to increase our knowledge of husbandry practices and serve as educational tools for the general public (Henningsen et al., 2004b; Chapman and Chapman, this volume). Henningsen et al. (2004a) suggested several steps for the development of a successful aquarium breeding program for elasmobranchs and we have incorporated this information into a graphical framework (Figure 6). The key features identified by this assessment include robust collaboration, concerted management of the population (over all ontogenetic stages) and the sourcing of sustainable funding.

Another key element within the framework of an aquarium breeding program is the accuracy and maintenance of record keeping (including both successes and failures). Comprehensive records allow not only for the advancement of biological

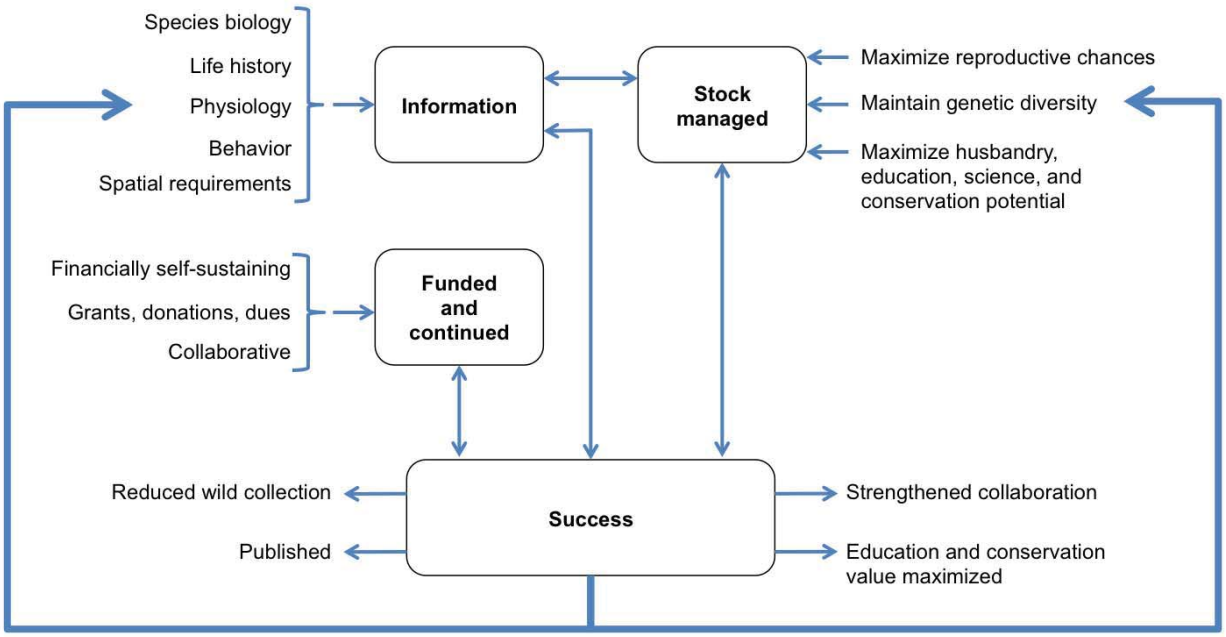

Figure 6. A process diagram outlining a framework for an aquarium elasmobranch breeding program.

and husbandry knowledge, but also for the replication of conditions. Similarly, detailed communication is needed for the accurate transfer of information. Collaboration is vital to increase the chance of success, and also to maintain funding and improve stock management. Funding can be sourced, as well as continued, through grants, donations and possibly dues to stakeholders within a breeding program. Robust stock management maximizes the chance of breeding success by placing adults of each sex into appropriate systems and conditions, and through the maintenance of genetic identity within the aquarium breeding program. The success of an aquarium breeding program is realized when the number of sharks collected from the wild is reduced, yet sufficient animal numbers are available for public display and research efforts, results are published, collaborations are strengthened, and educational and conservational opportunities are maximized.

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INTERNET SOURCES

www1 http://www.thebahamasweekly.com/publish/local/The_endangered_smalltooth_sawfish_gives_birth_at_ Atlantis22036.shtml

www2 https://ams.aza.org/iweb/upload/SharkSandTigerStudbook2014-6a44409f.pdf

www3 http://sezarc.org/updates/sand-tiger-shark-reproduction-workshop

www4 www.iucnsscrsg.org

www5 http://dx.doi.org/10.2305/IUCN.UK.2009-2.RLTS.T3854A10132481.en

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Chapter 38

Reproduction of the sand tiger shark, *Carcharias taurus* (Rafinesque, 1810), at UnderWater World SEA LIFE Mooloolaba from 1992 - 2012

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Abstract: The sand tiger shark, *Carcharias taurus* (Rafinesque, 1810), has been an important display species in public aquaria for decades. However, global attempts to reproduce *C. taurus* have met with limited success. Successful reproduction of *C. taurus* at UnderWater World SEA LIFE Mooloolaba appears to have been linked to its proximity to wild shark aggregation sites, ready access to healthy sexually mature specimens and to the replication of natural environmental conditions.

INTRODUCTION

The sand tiger shark, *Carcharias taurus* (Rafinesque, 1810), also known as the grey nurse shark in Australia and the spotted ragged-tooth shark in South Africa (Pollard et al., 1996; Last and Stevens, 2009), is one of three species belonging to the family Odontaspididae (Last and Stevens, 2009). *C. taurus* can be sighted on both the eastern and western coasts of Australia, with the East Coast population having a well-documented history of animals aggregating and breeding. The East Coast population covers a range of approximately 2,700 km (Figure 1a), and extends from Wolf Rock, Double Island Point (25° 55' S, 153° 12' E) in Queensland, to Narooma (36° 15' S, 150° 12' E) in Southern New South Wales (Cavanagh et al., 2003; Otway et al., 2003; Bansemer, 2009; Otway et al., 2009).

Globally, *C. taurus* is listed as "vulnerable" by the IUCN Red List (Pollard and Smith, 2009; IUCN Red List 2011). The Australian East Coast population of *C. taurus* is listed as "critically endangered" due to the combined effects of targeted spearfishing, incidental capture by commercial and recreational fishers, and beach protection shark meshing. On the West Coast of Australia the population is listed as "near threatened", due to the belief that the west coast has a more stable, non-targeted population.

Reproductive biology

C. taurus have a slow-growth development, become sexually mature later in life, have a low fecundity and a long life span (Department of the Environment, 2014). Sexual maturity in females occurs at 9 - 10 years of age and at a total length (TL) of 220 - 230 cm. Sexual maturity in males occurs at 6 - 7 years of age and at a TL of 190 - 195 cm (Bass et al., 1975; Gilmore et al., 1983; Branstetter and Musick, 1994; Lucifora et al., 2002; Goldman et al., 2006; Carlson et al., 2009). *C. taurus* are ovoviviparous. Unborn embryos feed on ova

produced by the female (oophagy), after the yolk sac has been absorbed, and then the pups employ intrauterine cannibalism (adelphophagy) as they continue to develop. Cannibalization of siblings results in a maximum of two viable pups per litter (one from each uterus). The pups are born at ~100 cm TL after 9 - 12 months gestation (Gilmore et al., 1983; Compagno, 2001; Pogonoski et al., 2002). Studies have found that female *C. taurus* have a biennial reproductive cycle (Bass et al., 1975; Lucifora et al., 2002; Dicken et al., 2006; Dicken et al., 2007). However, one study (Wolf Rock, East Coast of Australia) suggests that the reproductive cycle could be triennial in some cases (Bansemer and Bennett, 2009).

Attempted reproduction by *C. taurus* has been observed in aquaria around world. However, successful parturition has been limited to four aquaria: Sea World Durban, South Africa; The Scientific Centre Kuwait; Manly SEA LIFE Sanctuary, Australia; and UnderWater World SEA LIFE Mooloolaba, Australia. Literature on *C. taurus* breeding in aquaria is scant. Gordon (1993) has described pre-copulatory and copulatory behavior of *C. taurus* displayed at Manly SEA LIFE Sanctuary (formerly Manly Oceanworld). This chapter provides a detailed account of 20 years of *C. taurus* breeding at UnderWater World SEA LIFE Mooloolaba.

AQUARIUM CONDITIONS

UnderWater World SEA LIFE Mooloolaba, located on the Sunshine Coast, on the East Coast of Australia, opened to the public on 30 September 1989. Mooloolaba is within the mid- to northern range of the Australian East Coast population of *C. taurus* (Figure 1b). The aquarium maintains over 4,000 aquatic animals, displayed in 55 exhibits, including a 2,500 m^3 Oceanarium. The Oceanarium contains many large elasmobranch and teleost species, and, throughout its history, has intermittently maintained a breeding population of *C. taurus*.

Exhibit Dimensions

During the time that a breeding population of *C. taurus* was maintained at UnderWater World SEA LIFE Mooloolaba, the Oceanarium was divided into three separate areas: Pelagic Zone, Coral Zone and Cave Zone. The Pelagic Zone was the largest section, containing ~1,800 m^3 of natural seawater, within a space of dimensions 40 m long x 15 m wide x 3 m deep. The display contained concrete rock-work habitat around structural pillars and along the walls, which protruded no more than 30 cm into the water column. Rock structures, up to 1 m high, were scattered along the floor of the exhibit. This rock-work decor was not dissimilar to the rocky gutters that constitute the natural habitat of *C. taurus.* A walk-through

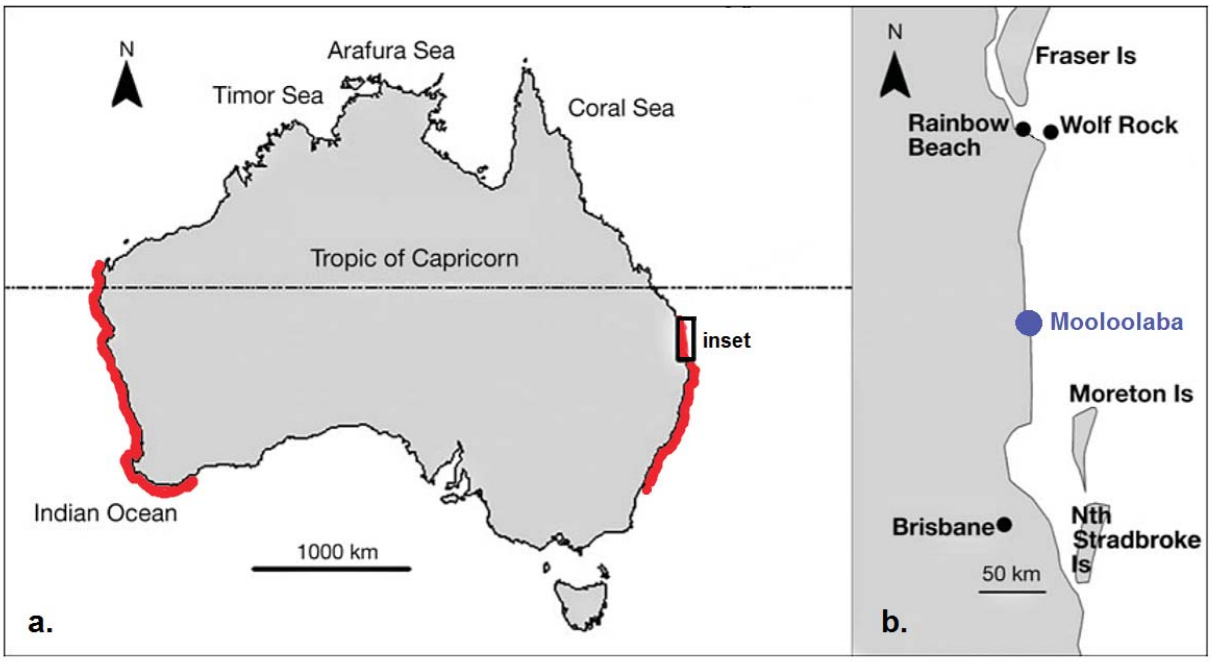

Figure 1. Australian distribution (a) of the sand tiger shark, *Carcharias taurus* (Rafinesque, 1810) (after Bansemer and Bennett, 2009).The location of specimen acquisition sites, Wolf Rock and North Stradbroke Island (Flat Rock), as well as the location of UnderWater World SEA LIFE Mooloolaba, are shown (b; inset).

tunnel ran the length of the exhibit and extended 1.25 m high into the water column. The tunnel created a partial division through the middle of the display resulting in two wide gutters. Elasmobranchs were able to swim though all areas of the Pelagic Zone without impediment.

Water supply and treatment

Good-quality natural seawater was supplied to the Oceanarium through an intake pipe and pump system located 150 m from the aquarium. The availability of makeup water was dependent on tidal fluctuations and poor weather could compromise water quality during the rainy season. In general, a water exchange of ~35%/day was achievable. Additional water treatment for the Oceanarium was provided by four gravity sand filters (each 7 m long x 5 m wide x 2.5 m deep). An ozone contact chamber was used to promote micro-flocculation of fine particulates before water entered the gravity sand filters.

Water quality

Water quality in the Oceanarium (2003 - 2011) was driven, in part, by ambient sea conditions in the nearby Mooloolaba Bay. Mean (± standard error; SE) salinity was 34.5 ± 0.1 g/L with a range of 26.7 - 38.9 g/L. Low salinity resulted from high rainfall during the rainy season (December to May), which typically influenced near-shore waters in Mooloolaba Bay. Mean pH was 7.8 ± 0.2 in the range 7.4 - 9.1. Dissolved oxygen ranged from 58.2 - 105.7% with a mean of 84.1 ± 0.4%. Mean water temperature was 23.8 ± 0.1°C in the range 18.2 - 29.3°C (Figure 2a). Mean annual minimum and maximum water temperature was 19.8 ± 0.3°C and 27.3 ± 0.4°C, respectively. During the summer of 2006, water temperature in the Oceanarium reached a record temperature of 30.0°C. Following the death of a female *C. taurus*, with post-mortem finding suggestive of heat stress, a chilling unit was added to the Oceanarium life support system to keep temperatures below 28.0°C during high summer.

Lighting

Daytime illumination of the Oceanarium was provided by 400 W / 10,000 K and 400 W / 14,000 K metal halides lights. Low illumination provided by blue fluorescent lighting was used to simulate moon-light during nighttime hours. Photoperiod

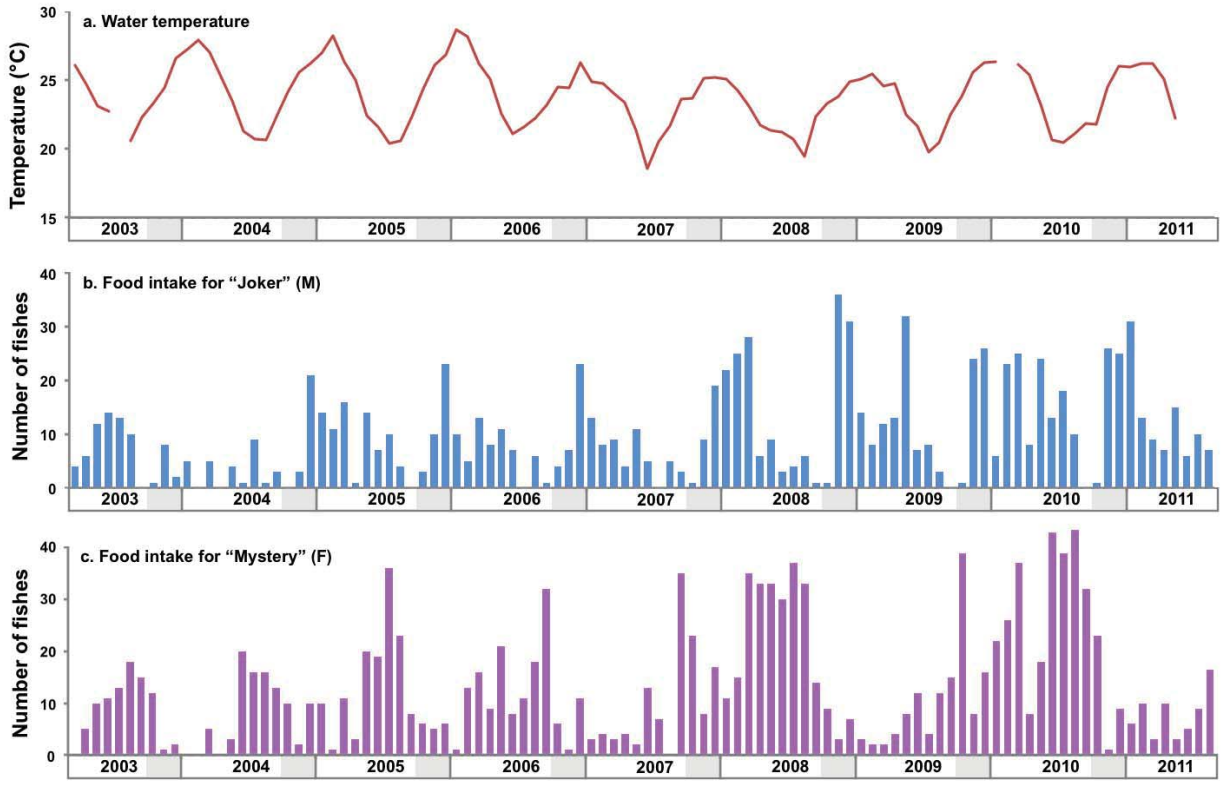

Figure 2. Feeding activity for two sand tiger sharks, *Carcharias taurus* (Rafinesque, 1810), maintained at UnderWater World SEA LIFE Mooloolaba (Queensland, Australia), showing: (a) average monthly water temperature, and monthly food intake for a (b) mature male and (c) mature female. Data was recorded from 2003 to 2011. The greyed area on the date bar indicates breeding season. Average mass of fed food fishes was ~0.5 kg.

during the winter was 12:12 light:dark, while in the summer it was 14:10 light:dark.

Nutrition

Large elasmobranchs within the Oceanarium were directly offered food by husbandry staff on SCUBA every second day. All elasmobranchs were hand fed a combination of flathead grey mullet, *Mugil cephalus* (Linnaeus, 1758), bluefish, *Pomatomus saltatrix* (Linnaeus, 1766), Australian bonito, *Sarda australis* (Macleay, 1881) and Australian salmon, *Arripis trutta* (Forster, 1801). Seasonal availability determined the species of fishes used as food, except for *M. cephalus*, which was provided year round. *C. taurus* would also occasionally prey, *ad libitum*, on other tank occupants, which included a variety of locally-caught teleost species.

ANIMAL ACQUISITION

Paper records were maintained prior to 2000 and the accuracy of recorded behavioral observations during this time is in question. However, cross-checking animal acquisition and specimen longevity data, indicate that these records, at least, were accurate. This chapter reports primarily on data from 2000 onwards, when electronic record-keeping was implemented, and only on animals maintained at UnderWater World SEA LIFE Mooloolaba long-term.

C. taurus were collected from the wild East Coast population, between Wolf Rock (Double Island Point) and Flat Rock (North Stradbroke Island). Anecdotal reports from aquarists working at the aquarium prior to 2000, indicate that as many as twenty *C. taurus* may have been maintained in the Oceanarium at any one time. Aquarists report that breeding and pupping occurred on a regular basis. In 2000, the population was broken up and many of the animals were relocated to other aquaria. Breeding and pupping frequency correspondingly diminished. By the end of 2000, three male and four female *C. taurus* were maintained in the Oceanarium.

REPRODUCTION BEHAVIOR

The behavior of *C. taurus* in the Oceanarium changed during the winter months, from late July to early August, when water temperatures dropped. When water temperatures reached 20°C, male *C. taurus* typically increased velocity and swam closer to the surface, at times lifting their rostrum out of the water. It was speculated that these behaviors signaled an innate drive to migrate to breeding grounds. Wild *C. taurus* on the East Coast of Australia travel north during the autumn - winter, then south during the spring - summer (Otway and Burke, 2004; Bansemer, 2009; Otway et al., 2009; Otway and Ellis, 2011), and Pollard et al. (1996) suggested that migratory movements could be linked to reproduction.

Precopulation

As water temperatures increased within the Oceanarium, female *C. taurus* frequently swam closer to the substrate, at times followed by male sharks. This behavior was described by Gordon (1993) as “tailing”, however it had previously been recorded between two males, rather than a male and female. The distance between female and male *C. taurus* typically shortened as water temperature reached 23°C. At times, the male sharks were observed “nosing” the females (as per Gordon, 1993). Female sharks would slow their velocity and swim very close to the substrate, almost touching the bottom of the tank, when males were in close proximity. Gordon (1993) described this behavior as “shielding”. On other occasions, female sharks would increase swimming speed, possibly in an attempt to evade males, with male sharks in a slow, but steady, pursuit. Male *C. taurus* regularly adjusted their position in the water column, and their swimming speed, depending on the location of females. Throughout breeding season, female *C. taurus* confined their resting swimming patterns to the widest and brightest areas of the Oceanarium, a behavior also noted by Gordon (1993).

Copulation

Gilmore et al. (1993) observed that *C. taurus* in the Atlantic breed synchronously each year in late winter - spring, after parturition off the eastern coast of Florida and in the northern Gulf of Mexico. This reported seasonal timing of reproduction is consistent with observed copulation behavior in the Oceanarium. Attempted copulation by *C. taurus* typically occurred when water temperatures reached 23 - 24°C, generally during late September to October. Copulation was observed in nearby wild populations of *C. taurus* during mid-October through late December (Bansemer and Bennett, 2009). Copulation attempts in the Oceanarium typically ceased during late October to early November, as water temperature rose above 24.5°C.

“Tailing” behavior was followed by a male *C. taurus* biting the pectoral fin and/or flank of a

female (as per Gordon, 1993). Male sharks would then attempt to rotate the female so they were inverted. If these attempts were successful, inverted females would enter dorsal recumbence and become docile. If a male *C. taurus* successfully grasped a female and inverted her, he would then attempt to insert a clasper into the cloaca of his intended mate. If successful, repetitive insertion of the penis was followed by the release of sperm in the form of a milky-white discharge, some of which could be seen leaking from the cloaca of the female. Once sperm discharge was complete, the male would release the female and both sharks would swim away. The claspers of the male would typically remain crossed for up to 30 min following attempted copulation.

The response of female *C. taurus* to copulation attempts was varied. The female shark either acquiesced to manipulation by the male, or proactively discouraged copulation by increasing swim speed, biting the male or other males within the vicinity (as per Gordon, 1993), or by wedging her body between rocky outcrops on or near the bottom. Intense periods of attempted copulations were observed during some breeding seasons, where, in some cases, multiple attempts were observed within a matter of hours. During other seasons, no attempted copulations were directly observed. However, fresh bite marks on both female and males sharks suggested that copulation attempts may have occurred overnight.

Some recorded observations suggested that a single female *C. taurus* may be more heavily targeted during a breeding season, and that this focus may shift to other females season by season. Should these observations represent some underlying pattern, which is speculative, it may be related to the biennial (or possibly triennial) reproductive strategy of female *C. taurus*.

Food intake

Food intake was tracked for all *C. taurus* within the Oceanarium. As individual prey items were not weighed, numbers of prey items ingested was used as an indicator of food ration. A sample of food items yielded a mean mass of ~0.5 kg.

The average monthly food intake for all (n = 3) male *C. taurus* was 9.7 ± 0.5 prey items, in the range 0 - 36. A typical annual cycle of food intake for a male *C. taurus*, "Joker", is shown in Figure 2b. Food intake appeared to decrease prior to breeding season, but this decline may also be related to changing water temperature (Figure 2a).

The average monthly food intake for all (n = 4) female *C. taurus* was 14.0 ± 0.8 prey items, in the range 0 - 66. There appeared to be a growth in food intake by mature female *C. taurus* over a biennial cycle during 2007 - 2008, and similarly during 2009 - 2010. A typical example is shown for a female *C. taurus*, "Mystery", in Figure 2c. A food intake increase over two years is similar to a pattern observed in a single female *C. taurus* made by Townsend and Gilchrist (this volume) and may be related to their biennial reproductive cycle. In general, food intake for female *C. taurus* increased over the winter and into the spring (May - October). Once breeding activity started, signaled by active copulation attempts, female *C. taurus* food intake typically decreased (Figure 2c). Female appetites would then remain suppressed until the following winter (May). Repeated copulation attempts, pregnancy or parturition appeared to have little additional effect on the appetite of female *C. taurus*.

Parturition

Female *C. taurus* have pups, at a maximum, once every 2 - 3 years (Bass et al., 1975; Lucifora et al., 2002; Dicken et al., 2006; 2007). Parturition events at UnderWater World SEA LIFE Mooloolaba were consistent with a female reproductive periodicity that skipped a year or more (Table 1 and Figure 3). However, seasonal timing of parturition (late spring - summer) in the Oceanarium, was different to that reported by Gilmore et al. (1993) for *C. taurus* in the Atlantic (late winter - spring). Parturition in the Oceanarium occurred primarily during the months of October and November, but were recorded as early as September.

Ultrasonography was not used to confirm or monitor pregnancies, as the risks to unborn pups and mother, resulting from catch and restraint, were deemed too great. As a proxy mechanism to assess female status, husbandry staff carefully monitored female *C. taurus* behavior during the spring (September - November). Food intake, copulation events and changes in girth were all carefully assessed. In the final few weeks before parturition, movement within the abdomen of the female was discernible. In addition, the female would occasionally "shudder" or "jerk". Parturition typically occurred in the early hours of the morning. A milky-white fluid was observed being expelled prior to pups being born.

Once born, pups were immediately removed from the Pelagic Zone of the Oceanarium and relocated to the Coral Zone, to eliminate the risk of predation

Table 1. Population of *Carcharias taurus* (Rafinesque, 1810), maintained at UnderWater World SEA LIFE Mooloolaba (Queensland, Australia), from 1998 - 2012, showing details of all recorded parturition events.

Name	ID #	Gender	Location acquired	Date acquired	Dam	Notes	Deceased	Years in Aquarium
Big Mumma	ST01	Female	Wild caught (Flat Rock)	13 Jul 1992	-	-	16 Feb 2006	13.6
Juliette	ST02	Female	Aquarium born	14 Jul 1992	Big Mumma	Deceased gravid	24 Nov 2004	12.4
Julie (Ali)	ST03	Female	Aquarium born	14 Jul 1992	Big Mumma	Transfer to Melbourne Aquarium 2 June 2000	30 Dec 2008	16.5
Joker	ST04	Male	Wild caught (Flat Rock)	19 Jul 1992	-	-	24 Oct 2012	20.3
Uncle Ray	ST05	Male	Wild caught (Flat Rock)	25 Jul 1992	-	-	27 Dec 2008	16.4
Fatty (Georgie)	ST06	Female	Aquarium born	08 Nov 1997	Big Mumma	Transfer to Melbourne Aquarium 5 October 2001	10 Jun 2009	11.6
Bent Spine	ST07	Female	Aquarium born	08 Nov 1997	Big Mumma	Miscarried 2006	07 Oct 2008	10.9
Romeo	ST08	Male	Wild caught (Flat Rock)	08 Apr 1998	-	-	12 Mar 2012	13.9
Mystery	ST09	Female	Wild caught (Wolf Rock)	1998	-	-	18 Nov 2011	13.6
Unnamed 17	ST10	Female	Aquarium born	Nov 1999	Big Mumma	Transfer to Melbourne Aquarium; Died during transfer	Mar 2000	0.3
Unnamed 18	ST11	Male	Aquarium born	Nov 1999	Big Mumma	Transfer to Melbourne Aquarium; Died during transfer	Mar 2000	0.3
Unnamed 21	ST12	-	Aquarium born	31 Aug 2000	Juliette	Born prematurely	31 Aug 2000	0
Unnamed 22	ST13	-	Aquarium born	31 Aug 2000	Juliette	Born prematurely	31 Aug 2000	0
Unnamed 23	ST14	Male	Aquarium born	22 Jun 2003	Juliette	Born prematurely	22 Jun 2003	0
Unnamed 24	ST15	Female	Aquarium born	06 Aug 2003	Juliette	Stillborn	06 Aug 2003	0
Unnamed 25	ST16	Male	Aquarium born	03 Oct 2004	Mystery	Stillborn	03 Oct 2004	0
Unnamed 26	ST17	Male	Aquarium born	03 Oct 2004	Mystery	Stillborn	03 Oct 2004	0
Unnamed 27	ST18	Male	Aquarium born	03 Oct 2004	Mystery	Stillborn	03 Oct 2004	0
Unnamed 19	ST19	-	Aquarium born	17 Sep 2005	Bent Spine	Stillborn	17 Sep 2005	0
Unnamed 20	ST20	-	Aquarium born	17 Sep 2005	Bent Spine	Stillborn	17 Sep 2005	0
Freckles	ST21	Female	Aquarium born	18 Sep 2009	Mystery	Transferred to Manly Sealife Sanctuary 13th March 2012	-	7.1 +
Unnamed 28	ST22	Female	Aquarium born	18 Sep 2009	Mystery	Stillborn	18 Sep 2009	0

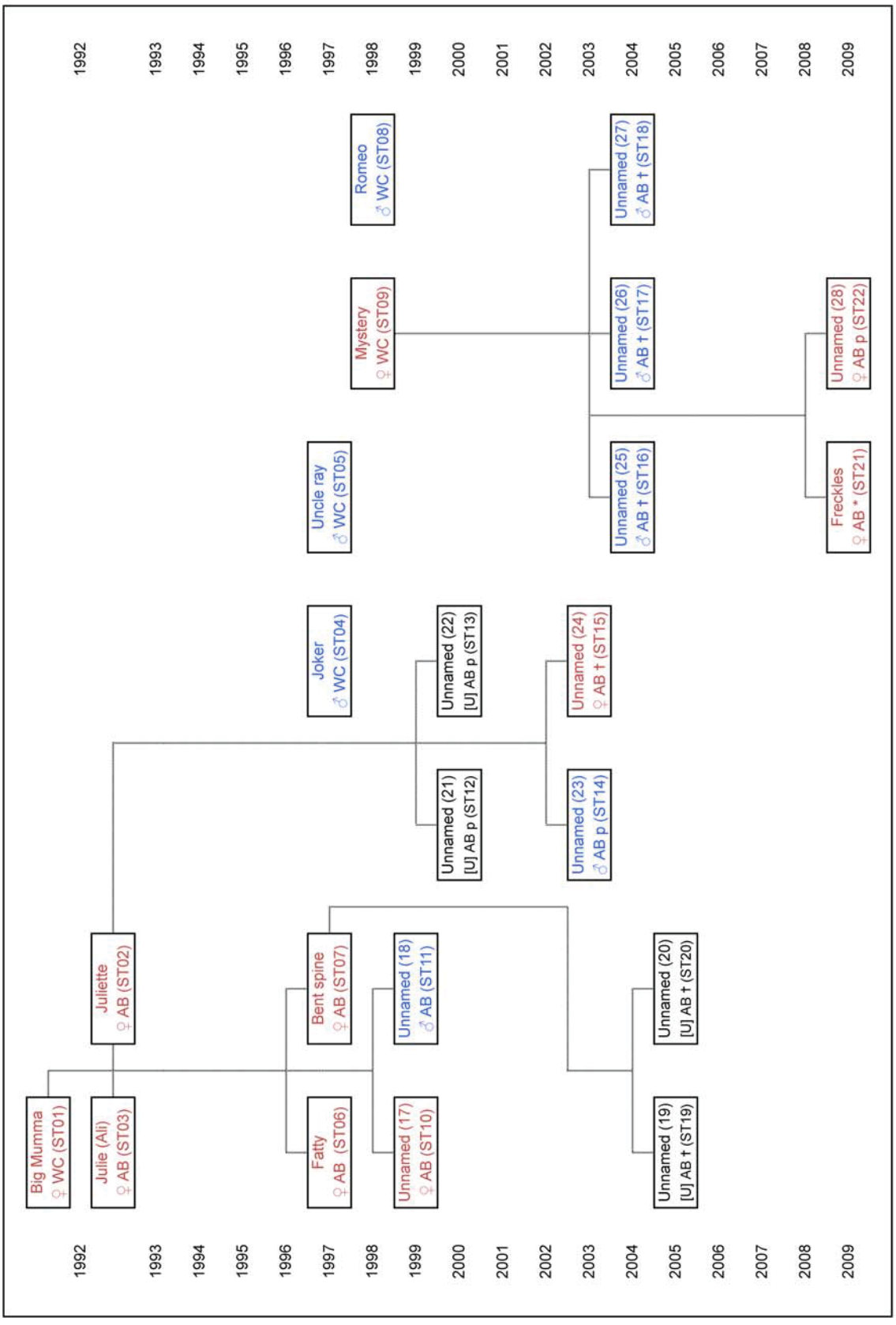

Figure 3. The genealogy of sand tiger sharks, *Carcharias taurus* (Rafinesque, 1810), maintained at Underwater World SEA LIFE Mooloolaba (Queensland, Australia), from 1998 - 2009. WC = wild caught. AB = aquarium bred. † = still born. p = premature. * = shark is alive at time of writing.

by larger sharks or grouper, *Epinephelus* spp. If the pups had difficulty remaining in the water column, husbandry staff on SCUBA would assist swimming until the animal was able to acquire the appropriate buoyancy.

REPRODUCTION EVENTS

Since 1992, eight parturition events have been recorded in the Oceanarium at UnderWater World SEA LIFE Mooloolaba. These events have resulted in a total of seventeen pups from four different females (dams). Two of these dams were born at the aquarium from a female ("Big Mumma") inseminated in the wild, in one case, and inseminated in the aquarium in another. The assumed aquarium insemination presupposes that female *C. taurus* cannot store sperm for 5 - 7 years postpartum. Of the seventeen pups, seven survived, four were underdeveloped (premature), and six were stillborn (Table 1 and Figure 3).

ST01 ("Big Mumma")
ST01, a gravid female *C. taurus*, was collected from the wild and gave birth the following day (14 July 1992) to two healthy female pups, ST02 and ST03. ST01 became gravid a second time and gave birth on 8 November 1997 to another two female pups, ST06 and ST07. ST07 was scoliotic between her first dorsal fin and the caudal peduncle. During November of 1999, ST01 gave birth one final time to two healthy pups, a female (ST10) and male (ST11). Both pups were transported to the Melbourne Aquarium during March 2000. Unfortunately, neither pup survived the transport.

During the unusually high summer temperatures of 2006, ST01 was observed swimming near the surface and ram ventilating, with her caudal fin at a lower than normal attitude. On the morning of 16 February 2006, the shark was found resting on the bottom of the Oceanarium with a depressed ventilation rate. The shark ultimately died and post mortem results suggested death related to extreme heat stress and senescence. The age of ST01 at death was estimated to be 32 years.

ST02 ("Juliette")
ST02 was conceived in the wild and born to ST01, the day following her wild acquisition. ST02 had two litters within the Oceanarium, which can only have resulted from insemination in the aquarium. The first litter was born on 31 August 2000, when ST02 was 8 years and 6 weeks of age. Both pups (ST12 and ST13) were premature and underdeveloped, at 70 - 80 cm TL. During 2003, ST02 had a second litter of pups consisting of an underdeveloped premature male (ST014), born 22 June 2003, and a stillborn female (ST015), born 6 August 2003. The cause of the split birth is unknown.

On 24 November 2004, ST02 was restrained and lifted out of the Oceanarium to allow for procedures related to reproduction research. ST02 suffered a hemorrhage from an enlarged ovary, which was ultimately mortal. At this time, a policy was implemented that resulted in the cessation of all research procedures on female *C. taurus* at the Aquarium.

ST07 ("Bent Spine")
ST07 was born to ST01 and is believed to be the result of insemination in the aquarium. ST07 was the only *C. taurus* in the collection to suffer from scoliosis. ST07 pupped for the first and only time, at an age of 7 years and 11 months, on 17 September 2005, giving birth to two perfectly formed stillborn pups (ST019 and ST20) of undetermined gender. During parturition, it was determined that ST07 was struggling to expel the pups. Husbandry staff on SCUBA helped remove the pups from the birth canal. It was speculated that scoliosis suffered by ST07 complicated parturition and that the pups may have survived had the birth canal not been obstructed. Husbandry records indicate that ST07 miscarried during 2006. However, no additional details were recorded and the report seems questionable given that only a year had passed since the previous birthing event.

On 5 October 2008, ST08 ("Romeo") attempted to copulate with ST07 within the confined space of a holding pool (3.30 m x 2.45 m x 1.25 m deep), which was directly connected to the Oceanarium. ST07 exited the holding pool, swam erratically and came to rest on the bottom of the Oceanarium. Blood was observed seeping from her left gill arch. Vitamin K was administered IM to staunch the hemorrhage. In addition, husbandry personnel on SCUBA provided swimming assistance to ST07. Due to her continued decline, ST07 was euthanized 56 h after the attempted copulation. Post mortem yielded no clear cause of death. It was speculated that death was related to injuries sustained during excessive copulation attempts throughout the 2008 mating season and, in particular, the attempted copulation in the holding pool of 5 October 2008. ST07 was 10 years and 10 months old at time of death.

ST09 ("Mystery")
ST09 was captured from the wild, in the autumn of 1998, at Wolf Rock, Double Island Point. ST09 had two litters during her 13 years at the aquarium. On 3 October 2004, ST09 gave birth to three stillborn male pups (ST16, ST17 and ST18). On 18 September 2009, ST09 gave birth a second time. The first pup, a 100 cm TL female (ST21 aka "Freckles"), was observed emerging tail-first and appeared to be 'trapped'. Husbandry personnel on SCUBA intervened and assisted parturition. The pup was in good health and swam away from the diver when released. A second female pup followed the first, but was stillborn and underdeveloped (ST22).

On 18 November 2011, ST09 died as a result of injuries sustained during copulation. At this time, the sex ratio of *C. taurus* in the Oceanarium was two males to one female. ST09 sustained numerous bites from multiple copulation attempts during the 2011 breeding season, despite her active attempts to avoid the male sharks. In response, husbandry personnel elevated the Oceanarium water temperature to prematurely terminate the 'breeding season', but to no avail. The Oceanarium was renovated in 2012 and a provision was made to allow for the separation of *C. taurus* should a similar situation of excessive copulation occur.

BUOYANCY CHALLENGES

Shortly after birth, ST21 ("Freckles") was relocated to the Coral Zone of the Oceanarium to protect her from predation. ST21 began feeding almost immediately, initially stick-fed, and then hand-fed, by husbandry staff on SCUBA. Food items included a combination of whole sand sillago, *Sillago ciliata* (Cuvier, 1829), yellowtail horse mackerel, *Trachurus novaezelandiae* (Richardson, 1843) and South American pilchard, *Sardinops sagax* (Jenyns, 1842), all ~15 cm TL. As ST21 grew, she was converted to pieces of, and then whole (30 - 35 cm TL), *M. cephalus*, *P. saltatrix* and *S. australis*.

ST21 had three compromised buoyancy events while in the Oceanarium. The first event occurred at 2 - 3 months of age. ST21 was first observed 'swimming', almost vertically, at the surface. Over ensuing weeks ST21 became increasingly inappetent and her activity levels diminished. Changes to diet, introduction of air near her mouth from a SCUBA tank and lifting her out of the water, all failed to change her behavior. A more invasive procedure was therefore employed. Two husbandry personnel on SCUBA caught and restrained ST21, and oriented her body so that she was facing toward the bottom of the Oceanarium. A third diver used a 60 mL syringe and plastic tubing to introduce 300 mL of air into the stomach of the shark. When released, ST21 was immediately neutrally buoyant and her feeding behavior improved.

The second compromised buoyancy event occurred six weeks after the first, with the same symptoms observed. ST21 was afforded time to correct her buoyancy naturally, but to no avail. The procedure described above was used again to correct her buoyancy, with the same seemingly successful result. Six weeks thereafter, a third compromised buoyancy event occurred. A more conservative approach to intervention was adopted. Compressed air was released near the mouth of the shark, *M. cephalus* inflated with air was fed to the shark and divers lifted the shark out of the water to encourage 'air gulping'. All efforts failed to correct the buoyancy challenge. On day four, ST21 was observed attempting to ingest air at the surface, without assistance, and her buoyancy temporarily improved. On day five, ST21 was discovered resting on the bottom of the Oceanarium. When approached by SCUBA divers, intending to provide assisted swimming, ST21 swam away. Throughout the balance of the day, her swimming behavior improved. During the evening of day six, ST21 was observed swimming to the surface and ingesting air. The shark regained neutral buoyancy and she began swimming and eating normally. No further buoyancy challenges were observed.

CONCLUSIONS

The *C. taurus* breeding program at UnderWater World SEA LIFE Mooloolaba has been the most successful to date. The aquarium has recorded the highest number of birthing events and the highest number of pups born, including four females sharks that reached maturity. Two females born in the aquarium produced second generation offspring, although all pups did not survive parturition. All *C. taurus* original founder stock were acquired locally and maintained in a minimalist environment with few obstacles to impede swimming patterns. The reasons for success are not completely understood, but may, in part, be attributed to: the provision of a habitat that mimics the natural environment of *C. taurus,* access to healthy sexually-mature specimens,

and natural lighting and water conditions, especially temperature, that replicate geographically consistent seasonal shifts. A sex ratio of three males to four females appeared to work effectively as a breeding population in the conditions described.

For future *C. taurus* breeding programs, the ability to isolate females to protect them from excessive copulation attempts is recommended. Similarly, the capacity to isolate newborn pups from predators is a recommended precaution.

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Chapter 39

Small-scale elasmobranch husbandry and life support systems for research environments

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Abstract: Elasmobranchs represent a diverse, evolutionarily important and ecologically vital group of animals. However, elasmobranchs can be technically challenging to study. Despite a keen research interest in this group, elasmobranchs are rarely considered viable laboratory animals. Reliable and effective small-scale aquarium systems, capable of supporting elasmobranchs, can be an invaluable resource for the study of chondrichthyan fishes and, indeed, for the study of vertebrate biology in general. Managed aquarium systems allow for superior monitoring and control of environmental conditions and provide an opportunity for constant animal surveillance. In addition, successful aquarium reproduction programs can provide a supply of study specimens, reducing the necessity to source animals from their natural range.

INTRODUCTION

Elasmobranchs exhibit a high degree of diversity in their biology, physiology, ecology and behavior, and are a widely distributed taxon. Yet, our understanding of some of the most basic characteristics of these animals remains limited by the numerous challenges associated with conducting research within their natural habitat. As an important adjunct to *in situ* research, laboratory and aquarium animals provide a significant resource for many studies. The ability to successfully maintain live elasmobranchs within, or near, laboratories is a necessary component for a range of behavioral, physiological, anatomical and reproductive studies (e.g., Pillans et al., 2005; Luer et al., 2007; Kajiura and Fitzgerald, 2009; Harahush et al., 2012). While procedures for collecting and transporting specimens for research purposes are relatively well understood, the technical rigors associated with maintaining healthy elasmobranchs in a controlled laboratory setting, longer-term, can present a challenge for researchers. Effective, small-scale elasmobranch holding and breeding systems represent a valuable alternative resource, capable of providing: (1) a reliable supply of healthy animals for laboratory research; (2) an environment that can be controlled and manipulated for experimentation; and (3) a reduced demand for the wild acquisition of study specimens.

Extensive information relating to best practices for the husbandry of large elasmobranchs, in large-scale facilities like public aquaria, is available. In addition, these large-scale facilities are generally well-resourced. However, small-scale elasmobranch research facilities are typically designed to meet a different set of needs, implying a range of different considerations. This chapter

addresses these considerations by providing details of a small-scale holding and breeding facility, which serves as a guide for investigators planning to conduct laboratory research on small elasmobranchs.

KEY CONSIDERATIONS

The key objectives of a small-scale elasmobranch aquarium research system and associated husbandry techniques are:

1. High-quality, ethical care for animals maintained for research purposes;
2. Successful maintenance of experimental animals long-term, with minimal mortality;
3. Increased precision and repeatability of research conditions;
4. Research conditions that represent realistic models of natural habitats; and
5. A capacity to breed healthy offspring, which may, in turn, be used for experimentation.

Planning

The current and future purpose(s) of a research facility (i.e., long-term housing, breeding, behavioral studies, etc.), along with the footprint of the facility, will dictate design specifications. When designing systems, flexibility should be incorporated wherever possible. Specifying equipment with a capacity to expand, building with redundancy, and ensuring the use of interchangeable and universal fittings will allow flexibility for maximum utility of the system as future demands dictate. Adequate and safe working space for holding tanks, life support systems (LSSs), makeup and wastewater storage, and laboratory and animal husbandry equipment is essential. LSSs should be specified to deliver water conditions that mimic the natural habitat of the species to be studied. The capacity to manipulate water parameters to accommodate experimental conditions is highly recommended.

The size, volume and number of tanks installed, will depend on the species, specimen numbers and sizes, and the developmental stage(s) of animals required for experimentation. The physiological and behavioral needs of species to be studied, such as swim-glide behavior, required turning radius (Porter et al., 2009) and space for propulsion from a resting position (aka take-off profile), must all be considered. When known, other behavioral factors (see Pratt and Carrier, 2001) should also be accommodated.

Regulations

All research facilities and experiments should be designed according to specific institutional, local and national regulations. For elasmobranch research facilities, regulations typically relate to the bio-security status of the facility (e.g., physical containment level, quarantine level, gene modification restrictions), animal ethics requirements, legislation that governs animal handling (e.g., collection, transportation, housing and breeding), and occupational health and safety (OH&S). A lack of knowledge or understanding of a particular regulation is not an acceptable excuse for non-compliance.

Water management

Facilities close to a reliable source of clean seawater may be able to pump or truck water to a reservoir adjacent to their living systems. Alternatively, artificial seawater can be manufactured on site by combining preconditioned freshwater and sea salt. A variety of seawater salt formulations are available commercially. If city supplied tap water is used for freshwater, caution must be exercised, as water treatment processes can introduce contaminants (e.g., chlorine, fluoride, chloramine, ammonia) that are toxic to elasmobranchs. The use of activated carbon, ion exchange resins, or even reverse osmosis filtration, may be required to prepare freshwater for seawater manufacture. Storage facilities for conditioned freshwater and newly produced seawater, will be required. Sufficient seawater, with appropriate water chemistry, must be stored on site to ensure use demands can be accommodated, even in the event of a complete system water exchange.

Water quality parameters should be carefully monitored and rigorously maintained to minimize stress on test subjects, and to ensure experimental accuracy and reproducibility. Dissolved oxygen, temperature, salinity, pH and nutrient concentrations should all be monitored and controlled by appropriate LSS components. Sub-optimal water quality is one of the leading factors affecting aquatic animal health (Garner, 2013). Furthermore abnormal fluctuations in water quality parameters can lead to unnatural behavior, abnormal physiology and compromised reproductive activity, potentially leading to skewed experimental results.

Redundancy should be built into aquarium LSSs. Appropriate back-up equipment and spares (e.g., pumps) should always be on hand, or quickly obtainable, as extended interruptions to water treat-

ment can prove fatal to test subjects. Multiple small pumps (as opposed to one large pump), arranged in an array, will enable a system to continue running should one of the pumps fail. Water filtration and circulation will still continue, albeit at a reduced level, while a repair can be undertaken.

Effluent water from a laboratory aquarium system must be disposed of in an appropriate manner. Many municipalities prohibit the disposal of seawater into the sanitary sewer. Water treated with chemicals will also be governed by specific legislation. In some cases, biosecurity measures will necessitate the storage and sterilization (using ozone, ultraviolet light, or chlorine) of water, before it is sent to waste.

Animal health

Maintaining animal health is especially important for research environments. Disease can spread rapidly through small, closed systems, potentially decimating an entire population of study animals. In addition, poor animal health will lead to inaccurate experimental results, and reduce longevity and breeding success. Preventing illness and injury in research animals should be of the highest priority, as treatment can be difficult, extensive, costly and disruptive to studies. Incoming animals should be quarantined in isolated systems to minimize the risk of disease introduction. Isolation tanks should also be set aside for the treatment of diseased animals. Rigorous hygiene protocols (e.g., dip buckets containing appropriate disinfectants for husbandry tools) should be employed to prevent cross-contamination between separate systems. In the event of disease outbreak, a local veterinarian familiar with fish health should be consulted. Disease etiology, or the cause of trauma, should be clearly identified so that similar challenges can be prevented in the future.

Sourcing and Breeding

Research animals should be sustainably sourced under permit or supplied by reputable commercial collectors. If animals are maintained for breeding purposes females should outnumber males, maximizing offspring and reducing harmful or even fatal intraspecific aggression and mating trauma (Chapman and Chapman, unpublished results).

Deaccession

Before acquiring an elasmobranch species for research a clear animal deaccession plan must be determined. If animals are used for isolated breeding events or for non-terminal experimentation, it is unethical to simply leave them in a research facility for the remainder of their life. Animal deaccession or euthanasia plans should be based on institutional policies, in addition to local and national legislation. In general, euthanasia should be the last resort as a deaccession strategy (refer Murray, this volume). Animal reintroduction to the wild is discouraged by the International Union for the Conservation of Nature (IUCN), except in exceptional circumstances (www1). Even if reintroduction is an acceptable strategy, special permits, health certificates, and/or tagging and monitoring plans may be required before an animal can be released back into the wild. The location where wild animals were acquired should be carefully recorded, preferably with GPS, to allow for release in the same location, helping avoid disruption to distinct animal populations or subgroups.

Animal donation to other research facilities or a public aquarium can be a viable option, however, plans should be comprehensively discussed and agreed upon prior to animal acquisition. Regular communication with the receiving facility should be maintained, as animal populations can quickly change and may compromise an instiution's capacity to accept donated specimens.

Experimental conditions may also affect the appropriateness of a test subject for release or donation. If animals have been genetically modified, undergone drug therapy, or have interbred (e.g., between animals originating from separate and isolated populations), then it is unlikely that they will be acceptable for release to the wild or for donation to a public aquarium. If no other options are available, animals will have to be euthanized. If animal termination is the only option, then appropriately prepared samples should be donated to other research projects, wherever possible, to maximize the value of the animal and reduce the demand on wild populations.

CASE STUDY

A breeding and holding facility for brownbanded bamboosharks, *Chiloscyllium punctatum* (Müller & Henle, 1838), was constructed at The University of Queensland, Brisbane, Australia, for the purpose of establishing a reliable source of embryonic and juvenile sharks for a developmental study. To accommodate the number of animals required for the project, six mature adult *C. punctatum* were acquired to use as brood stock. A sex ratio of two males to four females was cho-

sen to maximize reproductive activity and ensure adequate numbers of viable embryos (based on Harahush et al., 2007). Adult animals were donated by Sea World Australia (Southport, Queensland, Australia). It was agreed that the animals would be returned to Sea World when they were no longer required. The sharks were maintained in three 10 m^3 round polyethylene tanks, built on a concrete platform within a well-ventilated enclosure. The enclosure had a Plexiglas roof, creating a stable environment with natural lighting. Each of the three tanks was used for a specific purpose: tank #1 for breeding; tank #2 to hold neonates; and tank #3 for quarantine/isolation.

Egg-holding system

Egg cases were removed from the breeding tank on the day of deposition, to avoid predation. Individual eggs were suspended in small glass aquaria, on a separate system, for the duration of embryogenesis (as per Harahush et al., 2007). The egg-holding system was contained in a temperature-controlled room. Artificial seawater for the system was fabricated from reverse osmosis (RO) freshwater and sea salt. The LSS for the egg-holding system was a simple mechanical filter. Gas exchange was provided using air-stones fed by an air compressor. Water quality parameters were maintained to match those of the breeding system (tank #1).

Breeding and neonate system

The breeding tank (#1) and neonate tank (#2) were linked to a single LSS (Figure 1). Each tank contained a surface skimmer and two suction drains at the bottom. The surface skimmer and one suction drain fed a protein skimmer (PPS 5 Foam Fractionator, Aquasonic Pty. Ltd., Wauchope, Australia) via a centrifugal pump (Onga 414 series, Pentair, Northgate, Australia) at a flow rate of 9 m^3/h.

A second treatment loop fed water from a separate bottom drain to both a 10 kW titanium heater/chiller heat exchanger (Enviro Filtration Systems, Sunshine Coast, Australia) and a biological filter via a second centrifugal pump (Onga 414 series, Pentair, Northgate, Australia). The biological filter/degas towers were elevated above the tanks on a cement block platform, and consisted of a circular plastic container (1.0 m diameter x 1.5 m

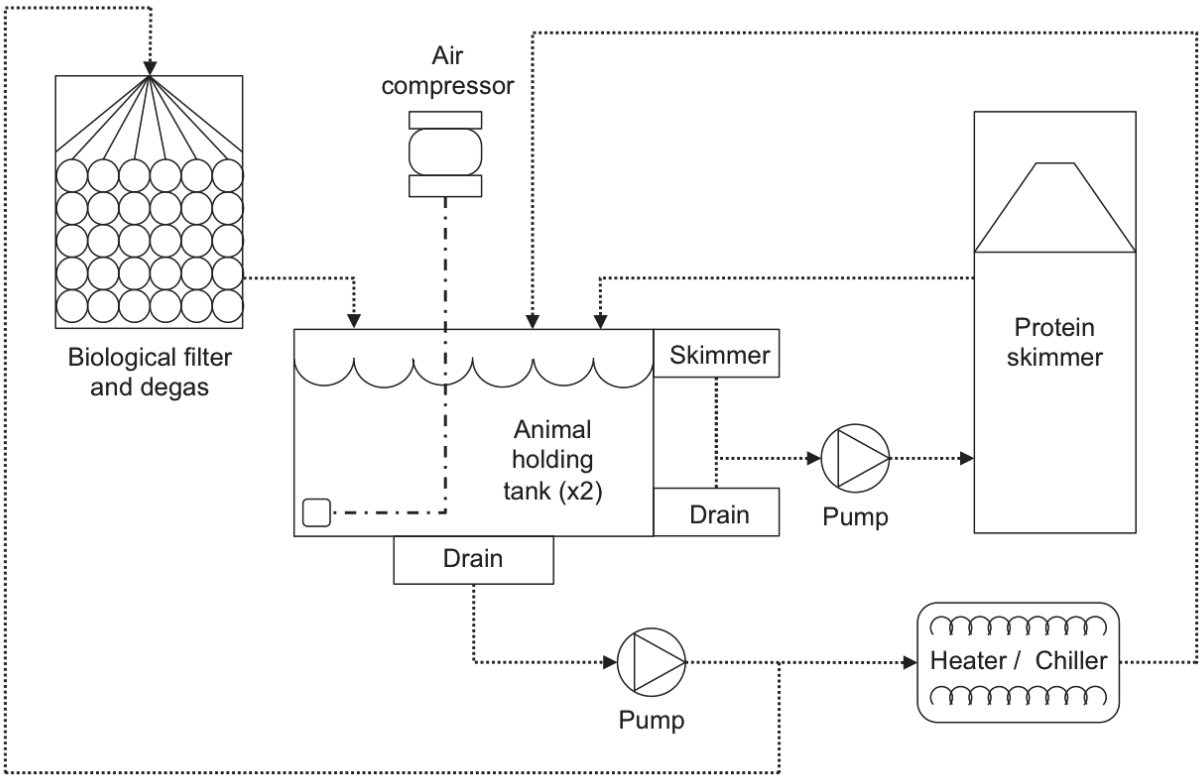

Figure 1. Schematic diagram of a small-scale life support system designed for the long-term maintenance of neonatal and breeding brownbanded bamboosharks, *Chiloscyllium punctatum* (Müller & Henle, 1838).

high) containing bioballs (Ovi-Flow II Biological Media, Aquasonic, Wauchope, Australia). The biological filter was seeded using Bio Culture Concentrate and Bio Start (Aquasonic, Wauchope, Australia). A polymer rack was added to the top of the biological filter, in which custom cut foam pads (Super Mat, Aquasonic, Wauchope, Australia) were added to serve as a mechanical filter. The biological filters were modularized to allow for the addition or removal of units to reduce or augment capacity as required. Water was returned to the breeding and neonate tanks from the biological filter/degas towers via gravity. Water was allowed to cascade into the tanks to promote gas exchange and water circulation. Volumetric turnover time for the entire system was 132 min.

Quarantine/isolation system

The quarantine/isolation tank (#3) was equipped with its own LSS (Figure 2), allowing for the complete separation of animals in the event of a health issue. The quarantine/isolation tank was also used to separate males from females, when necessary, to control breeding. A biological filter was not included in the quarantine/isolation tank LSS because frequent fluctuations in biomass were anticipated and nitrifying bacteria populations would not have been able to accommodate these rapid changes in biomass.

The surface skimmer, and a bottom drain, fed a protein skimmer (PPS 4 Foam Fractionator, Aquasonic Pty. Ltd., Wauchope, Australia) and a side-stream 50-W ultraviolet sterilizer (Smart High Output UV Sterilizer, Emperor Aquatics, Pottstown, USA), via a magnetic drive pump (Uno Magnetic Drive Pump, Aquasonic, Wauchope, Australia) at a flow rate of 6 m^3/h.

A second treatment loop fed water from a separate bottom drain to both a 5 kW titanium heat exchanger (Enviro Filtration Systems, Sunshine Coast, Australia) and a side-stream pleated-media mechanical filter (C50 TCF Cartridge Filter, Aquasonic, Wauchope, Australia) via a centrifugal pump (Onga Hi Flo Centrifugal, Pentair, Northgate, Australia) with basket pre-filter. Volumetric turnover time for the system was 120 min. Protein skimmer dwell time was two minutes. The ultraviolet sterilizer provided both bacterial and algal control.

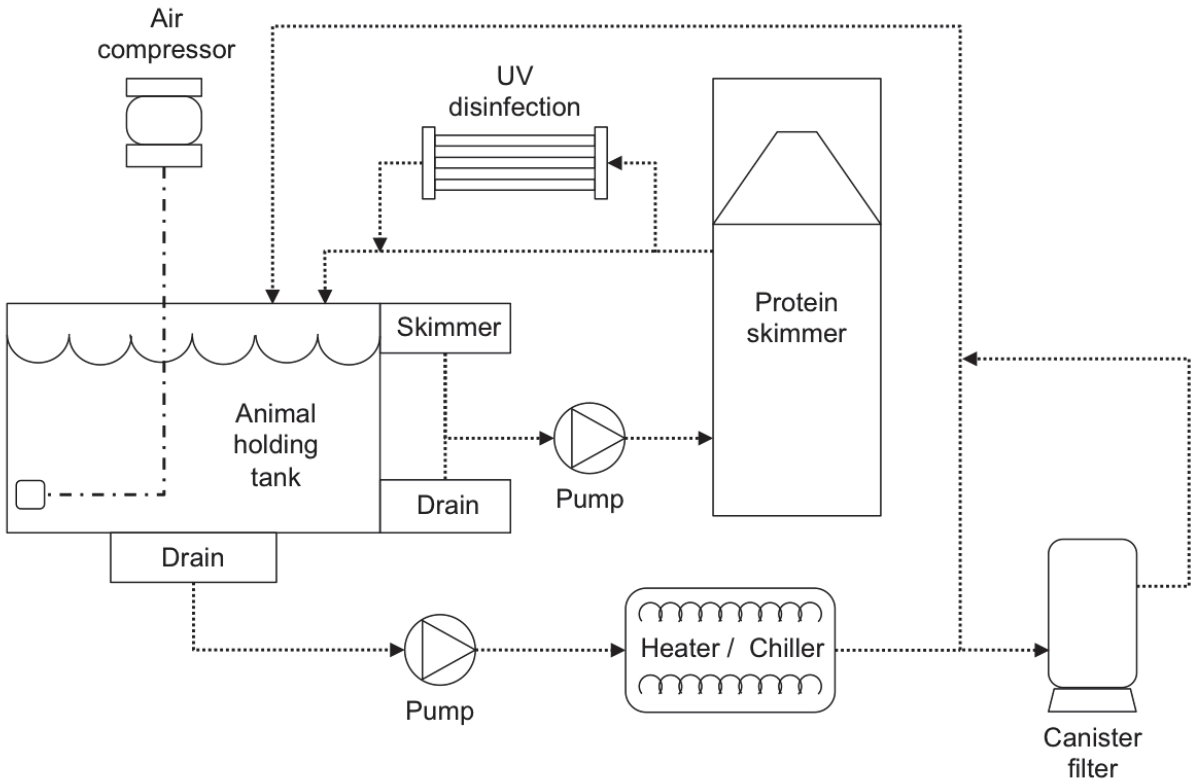

Figure 2. Schematic diagram of a small-scale life support system designed for the quarantine/isolation of brownbanded bamboosharks, *Chiloscyllium punctatum* (Müller & Henle, 1838).

System maintenance

A contractor collected seawater for all systems (with the exception of the egg-holding system) from Moreton Bay (27° 27.2'S, 153° 11.4'E). Seawater exchanges (20%) were performed monthly, when new water was delivered. Additional exchanges were occasionally performed, as dictated by water quality.

All suction points within the tanks were fitted with guards to prevent blockage. Air was continuously provided to the tanks using air-stones (two per tank) fed by an air compressor (Kamair Diaphragm Air Compressor, Aquasonic, Wauchope, Australia). All tanks were fitted with secured, flexible netting covers to prevent animal escape. A natural photoperiod was achieved by using a transparent roof above the tanks. No additional lighting sources were necessary. The Super Mat mechanical filter media was cleaned weekly and tanks were scrubbed and vacuumed as necessary to remove excess detritus and algae.

Anecdotal evidence suggests that physical environment can influence egg-laying success. *C. punctatum* have been observed preferentially selecting locations with rocks or corals to deposit their eggs (Harahush et al., 2007). Therefore, natural rock and small coral bomboras were introduced into the breeding tank to provide cover for the sharks and locations for egg attachment. No other substrate was added.

Water quality parameters were analyzed and recorded daily. Temperature, salinity, pH and dissolved oxygen were measured using a multisensor (TPS WP-90 probe, TPS Pty. Ltd., Brisbane, Queensland, Australia). Ammonia, nitrite, nitrate and calcium analyses were performed weekly using colorimetric text kits (API, Chalfont, USA). Water temperature was adjusted, as required, to mimic the average temperature profiles of Moreton Bay, the local natural habitat of *C. punctatum*. Salinity, pH and calcium were maintained in the ranges 32 - 36 g/L, 7.9 - 8.4 and 350 - 500 mg/L, respectively. Oxygen was maintained at >80% saturation. Nitrate, nitrite and ammonia were maintained at < 50 mg/L, < 0.1 mg/L and < 0.1 mg/L, respectively.

Animal feeding rates, observations of breeding activity, egg deposition dates and any other animal health observations and treatments were recorded.

CONCLUSIONS

Successful long-term maintenance of smaller elasmobranch species, in a controlled environment, provides a great, but relatively underused opportunity for research. Most references to LSSs designed to maintain elasmobranchs reflect the practices of large-scale commercial aquaria (Smale et al., 2004). In general, there is a paucity of information on small-scale LSSs for elasmobranch research facilities. We have addressed much of this knowledge gap by giving an overview of the key considerations for small-scale elasmobranch facilities and detailing an example system.

Designing, constructing and managing small-scale elasmobranch facilities, as well as collecting, transporting and maintaining healthy specimens for extended periods, is a readily achievable goal for any research laboratory. The primary limits to establishing a small-scale elasmobranch system include: space, a reliable water supply, capital costs to build and maintain the facility, and technical expertise. Careful research and planning allow these limitations to be addressed and managed so that a healthy population of test elasmobranchs can be maintained long-term.

ACKNOWLEDGEMENTS

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Chapter 40

Aquarium reproduction, growth and husbandry of the Pacific angelshark, *Squatina californica*

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Abstract: Seven Pacific angelsharks, *Squatina californica* (Ayres, 1859), were born at the Aquarium of the Bay in San Francisco (California, USA) during May and July of 2009. This event marked the second successful parturition of *S. californica* in aquaria, providing an opportunity to study the early stages of growth in this species and to document husbandry practices employed for neonates. Mean (± standard error; SE) total length (TL) at birth was 22.4 ± 0.2 cm, increasing to 43.2 ± 0.6 cm after a year and 55.9 ± 0.5 cm after two years. Mean (± SE) body mass (BM) was 122.4 ± 3.6 g, 812 ± 51.8 g and 1,670 ± 61.3 g, at birth, after one year and after two years, respectively. Mean (± SE) monthly food consumption for the sharks was 5.0 ± 2.8 g, 12.3 ± 5.3 g, and 16.8 ± 5.9 g at one month, one year and two years of age, respectively. These food rations corresponded to a mean (± SE) monthly %BM consumption of 3.5 ± 1.8 %BM, 1.6 ± 0.6 %BM and 1.0 ± 0.3 %BM, at one month, one year and two years of age, respectively. Mean food intake increased rapidly over the first 100 days postpartum and continued to increase, at a reduced rate, thereafter. TL and BM of the sharks increased more quickly over the first year when compared to increases over the second year. No significant difference was observed between male and female *S. californica* growth rates or food consumption rates.

INTRODUCTION

The Pacific angelshark, *Squatina californica* (Ayres, 1859), is a flattened, ray-like shark found off the continental shelf of the eastern Pacific Ocean. *S. californica* ranges from southeastern Alaska to the Gulf of California in North America, and is found off the coasts of Ecuador, Peru and Chile in South America. *S. californica* typically inhabits shallow bays, estuaries, submarine

canyons, and rocky reef or kelp forest perimeters, at depths of 3 - 100 m, although they can be found at depths of up to 200 m (Ebert, 2003; Compagno et al., 2005). As with other angel shark species, the *S. californica* is a cryptically colored, benthic species that partially buries itself in soft mud or sand to ambush prey (Ebert, 2003; Compagno et al., 2005). *S. californica* attains sexually maturity at ~90 - 100 cm total length (TL) (Natanson and Cailliet, 1986; Ebert, 2003; Compagno et al., 2005).

S. californica has a well-established gestation period of 9 - 10 months, with parturition off the coast of California between March and June (Cailliet et al., 1992). This species is ovoviviparous, with developing young nourished by an external yolk sac. As with many other shark species, a combination of late age-at-maturity, a long gestation period and low fecundity exposes *S. californica* to a slow recovery from exploitation (Hoenig and Gruber, 1990; Cortés, 2000; Cailliet et al., 2006). It has been well documented that *S. californica* stock collapsed as a result of a rapid increase in fishing pressure and a lack of information about species life history parameters (Cailliet et al., 1992; Richards, 2001). The commercial fishery for *S. californica* in the United States (US) ended in the early 1990s, when gill and trammel netting were banned in California state waters (Richards, 2001). However, this species is still targeted in Mexican waters, is taken as incidental by-catch by some bottom trawl fishers, and is subject to fishing pressure from recreational and spear fishers in the USA (Richards, 2001; Ramirez-Amaro et al., 2013).

S. californica is listed as “near threatened” by the IUCN Red List, based on continued exploitation of the species and a lack of international conservation measures (Richards, 2001; Compagno et al., 2005). Although tagging and tracking studies have taken place in Bodega Bay and Santa Catalina Island, California, a comprehensive population study has not been conducted since 1992. Growing evidence suggests that genetically isolated sub-populations may exist along the Pacific coastline (Gaida, 1997; Carter et al., 2010; Ramirez-Amaro et al., 2013), which may have divergent life history traits and therefore require specific management programs (Gaida, 1997; Ramirez-Amaro et al., 2013).

The Aquarium of the Bay (San Francisco, California, USA) is one of the few institutions that has successfully maintained *S. californica* long-term (Grassmann et al., 2012). In 2007, the Aquarium of the Bay was the first institution to report the birth of *S. californica*. The pups were premature and did not survive more than a few months. Between May and July of 2009, however, seven healthy full-term *S. californica* pups were born in the Near Shore Tunnel exhibit at the Aquarium. This event marked the second reported case of *S. californica* breeding in aquaria (Natanson and Cailliet, 1986; Cailliet et al., 1992; Schaadt and Landesman, 1997). The birth of the sharks presented a unique opportunity to gather detailed information on early developmental growth, ontogenetic changes and successful animal care practices for neonates of the species.

MATERIALS, METHODS and RESULTS

Collection and transport

The Aquarium of the Bay maintained three adult *S. californica*, one sexually-mature male and two sexually-mature females. The sharks were collected from Bodega Bay by staff biologists, on SCUBA, in September 2003 (male #SC02), November 2005 (female #SC03) and October 2007 (female #SC04). Following acquisition, each shark was transported to the Aquarium in an oxygenated transport container and acclimatized to display conditions before being released into the Near Shore Tunnel exhibit (volume = 1,320 m^3). The exhibit was supplied by filtered, temperature-controlled water from San Francisco Bay on a flow-through system.

Parturition

During a routine maintenance dive on 21 May 2009, four newly born viable *S. californica* pups were discovered, in addition to a stillborn pup. Two additional viable pups were found 16 days later (06 June 2009), and an eighth viable pup was discovered a further 35 days thereafter (11 July 2009). The viable pups comprised three males and four females. Following the discovery of each pup, the cloacae of the two adult females were examined for redness and swelling to determine maternity, which suggested that all young were produced by the same female (#SC04). For further confirmation of maternity, and to establish if copulation occurred within the exhibit, fin clips from the stillborn shark were sent for genetic analysis. The techniques employed for loci-amplification were similar to those used in genetic studies of other shark species (i.e., bluntnose sixgill shark, *Hexanchus griseus* (Bonnaterre, 1788) and broadnose sevengill shark, *Notorynchus cepedianus* (Péron, 1807)),

although primers specific to *S. californica* (Larson et al., 2010) had not yet been developed. Genetic analysis suggested that the premature neonates from the 2007 litter and the stillborn pup from the 2009 litter were from different females (high probability #SC03 and #SC04, respectively), and that all pups shared genetic likeness with the same male (#SC02).

The viable pups were measured and weighed, before being placed into three separate 0.38 m^3 rectangular holding tanks in the Quarantine Area of the aquarium. Tanks were supplied by filtered, temperature-controlled water from San Francisco Bay, on a flow-through system. Water quality parameters, including temperature, pH and dissolved oxygen, were routinely tested and recorded twice a week. Sand substrate was added to the holding tanks and artificial light sources were set on timers to mimic natural, seasonal photoperiods. Each tank was covered with plastic mesh to partially attenuate the light source.

Food and feeding

The neonate *S. californica* were offered a variety of food items six days per week and fasted one day per week. As the pups developed, food was withheld on two non-contiguous days per week. Diet consisted of local species, including: capelin, *Mallotus villosus* (Müller, 1776), South American pilchard, *Sardinops sagax* (Jenyns, 1842), Pacific herring, *Clupea pallasii* (Valenciennes, 1847), Atlantic silversides, *Menidia menidia* (Linnaeus, 1766), chub mackerel, *Scomber japonicus* (Houttuyn, 1782), and grass shrimp, *Crangon franciscorum* (Stimpson, 1856). Each food item was weighed to the nearest 0.1 g, prior to feeding. Food was supplemented with vitamins (Vita-Zu Shark/Ray tablets, Mazuri®, Purina Mills LLC, Nutrition International LLC, Richmond, USA) once a week.

Food was offered to the sharks on a monofilament line attached to a clear PVC feeding stick. As the size and mass of food items increased, the monofilament line was replaced with a small cable tie. Food was slowly moved toward the shark and then waved laterally ~5 - 10 cm above its head (adapted from Fouts and Nelson, 1999). Initially, the neonates were offered food for two minutes, allowing time for the sharks to detect and strike at the prey item. As the sharks increasingly responded to offered food, and continued to gain weight, the frequency and duration of feedings was decreased. Small schools of live juvenile Californian anchovy, *Engraulis mordax* (Girard, 1854), were frequently released into the holding tank to determine if the sharks would actively hunt for the live fish. When live food was offered, active predation was both directly observed and indicated by a declining number of *E. mordax* over time. Behavior (e.g., reaction to food, body position during a strike attack, post-attack reaction) was carefully monitored and recorded during each feeding session and used as a qualitative measure of shark progress and health status.

Allometry

Data from the seven pups was used to determine von Bertalanffy growth function (VBGF) parameters (von Bertalanffy, 1938), for theoretical maximum length (L^{inf}) and growth rate constant (*k*), in order to generate *S. californica* size-at-age growth curves. Initial attempts to determine VBGF parameters and model growth curves were inconclusive, so growth data was included from the recapture of wild sub-adult and adult *S. californica* from previous studies (Natanson and Cailliet, 1990; Cailliet et al., 1992; Schaadt and Landesman, 1997). Once the data sets were consolidated, a better estimate for growth over time was achieved and theoretical growth curves were generated using three different demographic models: (1) Fabens (1965); (2) Gulland and Holt (1959); and (3) GROTAG, or maximum likelihood model (Francis, 1988) (Figure 1).

Previously published allometric results for both wild and aquarium *S. californica* (Natanson and Cailliet, 1990; Cailliet et al., 1992; Schaadt and Landesman, 1997) were compared to those from specimens at the Aquarium of the Bay. Allometry suggested that the *S. californica* born at the Aquarium were smaller than newborns in the wild (Ebert, 2003; Compagno et al., 2005). However, these differences may not be significant, as the sample size of the study group was small.

Morphometrics

The neonate *S. californica* were measured weekly for their first six months, and then every second week for the subsequent 18 months. Once the sharks reached two years of age, they were measured on a monthly basis in conjunction with visual examinations for wounds or external parasites. Measurement frequency then dropped to once every other month when they reached three years of age. TL, pre-caudal length (PCL), and disc width (DW) were measured to the nearest 0.2 cm, and body mass (BM) was measured to the nearest gram (g). TL was measured from the tip of the rostrum to the trailing edge of the lower lobe of the caudal fin, as

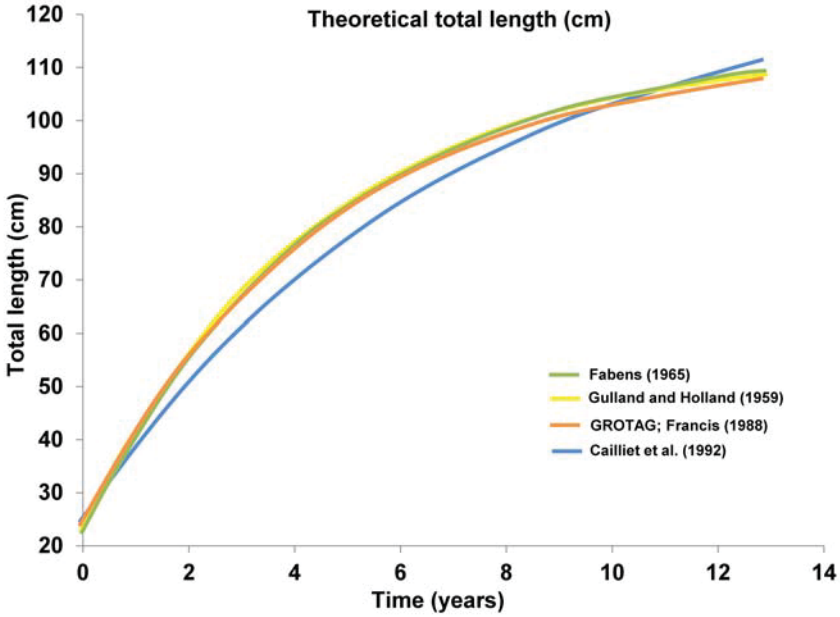

Figure 1. Theoretical growth curves for Pacific angelshark, *Squatina californica* (Ayres, 1859), based on three different demographic models: Fabens (1965); Gulland and Holt (1959); and GROTAG, or maximum likelihood model (Francis, 1988). Data from seven *S. californica* born and maintained at the Aquarium of the Bay (California, USA), as well as growth data from previous studies (Natanson and Cailliet, 1990; Cailliet et al., 1992; Schaadt and Landesman, 1997), was used to generate the von Bertalanffy (1938) growth function and thereafter the theoretical curves. A theoretical growth curve from Cailliet et al. (1992) has been included for comparison.

described by Cailliet et al. (1992). Food consumed as a percent of body mass (%BM) was calculated by dividing monthly food consumption (g) by shark BM, and multiplying the result by 100.

TL and BM for the seven sharks immediately postpartum, after a year and following two years, are reported in Table 1. Growth rates and percentage growth rates are also shown for both TL and BM. A curve for mean TL and BM over time, is shown in Figures 2 and 3, respectively, and a length to weight scattergram is shown in Figure 4. Growth rates of *S. californica* in the Aquarium appeared to be slightly faster during the first two years than previously observed for wild conspecifics (Figure 2).

Mean (± SE) monthly food consumption was 5.0 ± 2.8 g, 12.3 ± 5.3 g, and 16.8 ± 5.9 g at one month, one year and two years of age, respectively (Figure 5). These intakes corresponded to a mean (± SE) monthly %BM consumption of 3.5 ± 1.8 %BM, 1.6 ± 0.6 %BM and 1.0 ± 0.3 %BM, at one month, one year and two years of age, respectively (Figure 6). Mean food intake increased rapidly over the first 100 days postpartum and continued to increase, at a reduced rate, thereafter (Figure 5).

Correspondingly, TL and BM increased more rapidly over the first year, when compared to increases over the second year (Table 1). *S. californica* mean monthly food consumption as %BM peaked at 5.6 ± 1.0, when the sharks were approximately three months of age, and then declined steadily over the rest of the two-year period. No significant difference was observed between male and female *S. californica* growth rates or food consumption rates.

Animal health

As the *S. californica* neonates grew, it became apparent that overcrowding was causing stress to the sharks. As a result, the sharks were moved to a single, larger, round holding tank (volume = 4.2 m^3). Although individual sharks could be identified using coloration, patterning and size, each specimen was tagged with spaghetti and disc tags (Floy Tags, Seattle, Washington, USA) for easier identification. Unfortunately, the tags were an irritant to the sharks. Unusual behavior, symptomatic of the irritation included: inappetence, a reluctance to bury in the substrate and sustained periods of swimming in the water column. The external tags were removed and replaced with internally implanted passive integrated transponder (PIT) tags. Behavioral

Table 1. Total lengths (TL) and body masses (BM) of seven Pacific angelsharks, *Squatina californica* (Ayres, 1859), born and maintained at the Aquarium of the Bay, California, USA. Measurements were taken immediately after birth, after a year and following two years. M = male; F = female.

ID #	Gender	TL at birth (cm)	TL year 1 (cm)	TL growth rate year 1 (cm/year)	TL growth rate year 1 (%)	TL year 2 (cm)	TL growth rate year 2 (cm/year)	TL growth rate year 2 (%)
1301	M	21.9	43.6	21.7	99.09	56.4	12.8	29.36
1302	M	22.1	40.6	18.5	83.71	55.2	14.6	35.96
1303	F	23.0	41.8	18.8	81.74	54.0	12.2	29.19
1304	F	22.3	43.4	21.1	94.62	57.6	14.2	32.72
1305	M	23.0	45.2	22.2	96.52	55.4	10.2	22.57
1306	F	22.8	44.6	21.8	95.61	55.2	10.6	23.77
1307	F	22.0	43.0	21.0	95.45	57.8	14.8	34.42
Mean ± SE		22.4 ± 0.2	43.2 ± 0.6	20.7 ± 0.6	92.4 ± 2.6	55.9 ± 0.5	12.8 ± 0.7	29.7 ± 1.9

ID #	Gender	BM at birth (g)	BM year 1 (g)	BM growth rate year 1 (g/year)	BM growth rate year 1 (%)	BM year 2 (g)	BM growth rate year 2 (g/year)	BM growth rate year 2 (%)
1301	M	120	890	770	641.67	1,840	950	106.74
1302	M	120	635	515	429.17	1,640	1,005	158.27
1303	F	120	695	575	479.17	1,570	875	125.90
1304	F	115	740	625	543.48	1,840	1,100	148.65
1305	M	143	880	737	515.38	1,680	800	90.91
1306	F	123	1,040	917	745.53	1,380	340	32.69
1307	F	116	810	694	598.28	1,740	930	114.81
Mean ± SE		122.4 ± 3.6	812.9 ± 51.8	690.4 ± 50.8	564.7 ± 40.3	1,670 ± 61.3	857.1 ± 93.9	111.1 ± 15.8

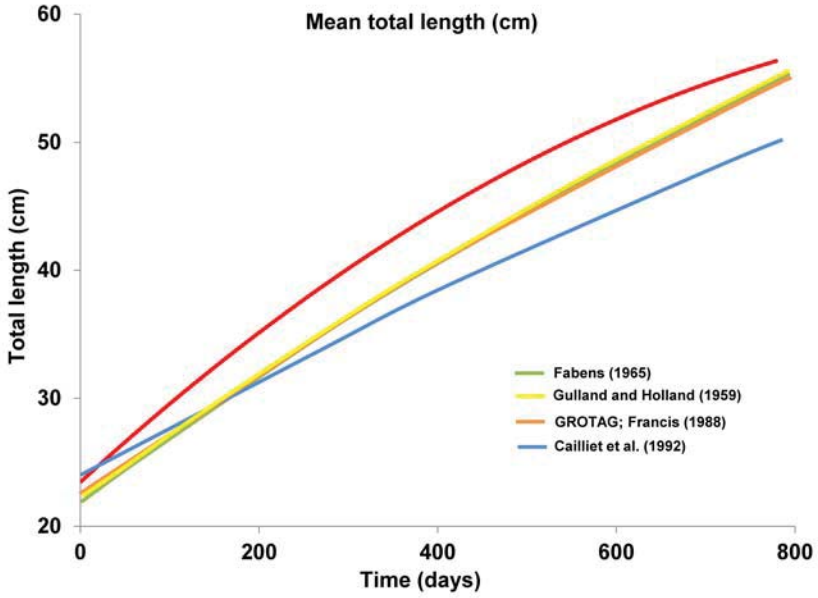

Figure 2. Mean total length (TL) over time (red line) of Pacific angelsharks, *Squatina californica* (Ayres, 1859), born and maintained at the Aquarium of the Bay (California, USA). Theoretical growth curves generated using data from the sharks have been included. A theoretical growth curve from Cailliet et al. (1992) has also been included for comparison.

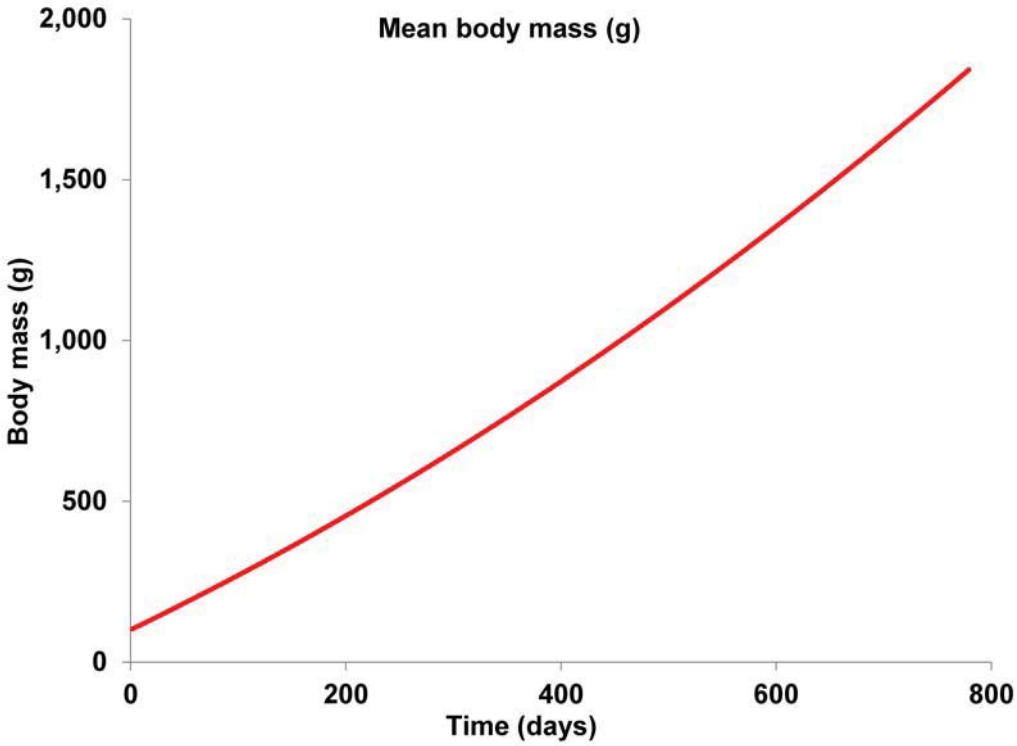

Figure 3. Mean body mass (BM) over time of seven Pacific angelsharks, *Squatina californica* (Ayres, 1859), born and maintained at the Aquarium of the Bay (California, USA.).

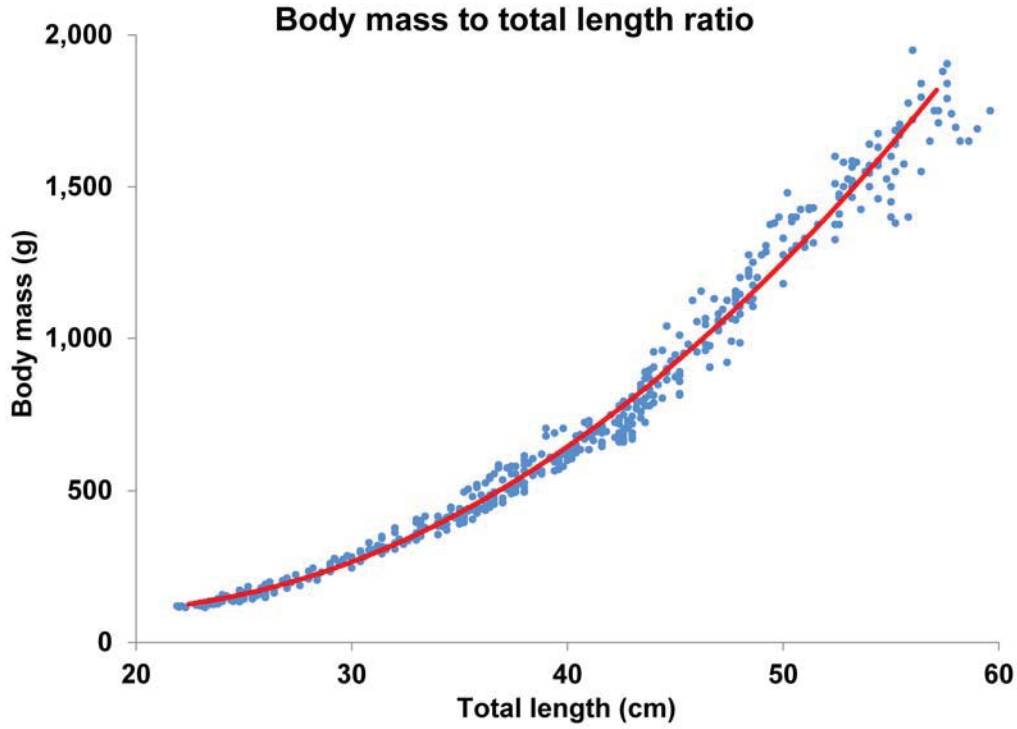

Figure 4. Body mass to length ratio of seven Pacific angelsharks, *Squatina californica* (Ayres, 1859), born and maintained at the Aquarium of the Bay (California, USA.).

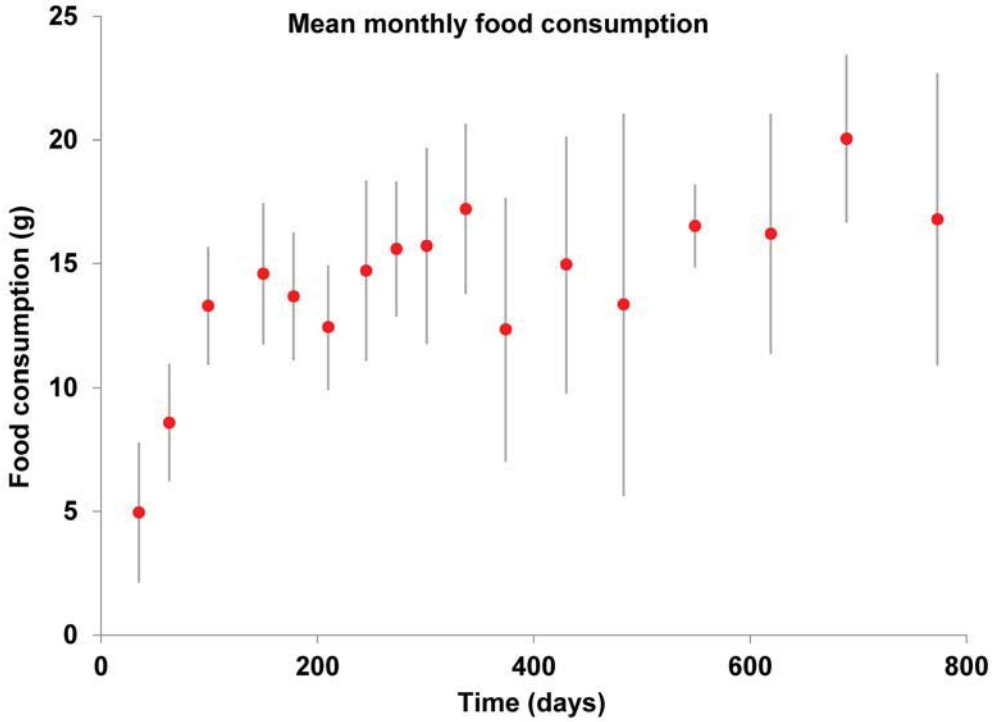

Figure 5. Mean monthly food consumption of seven Pacific angelsharks, *Squatina californica* (Ayres, 1859), born and maintained at the Aquarium of the Bay (California, USA.). Grey bars represent standard error.

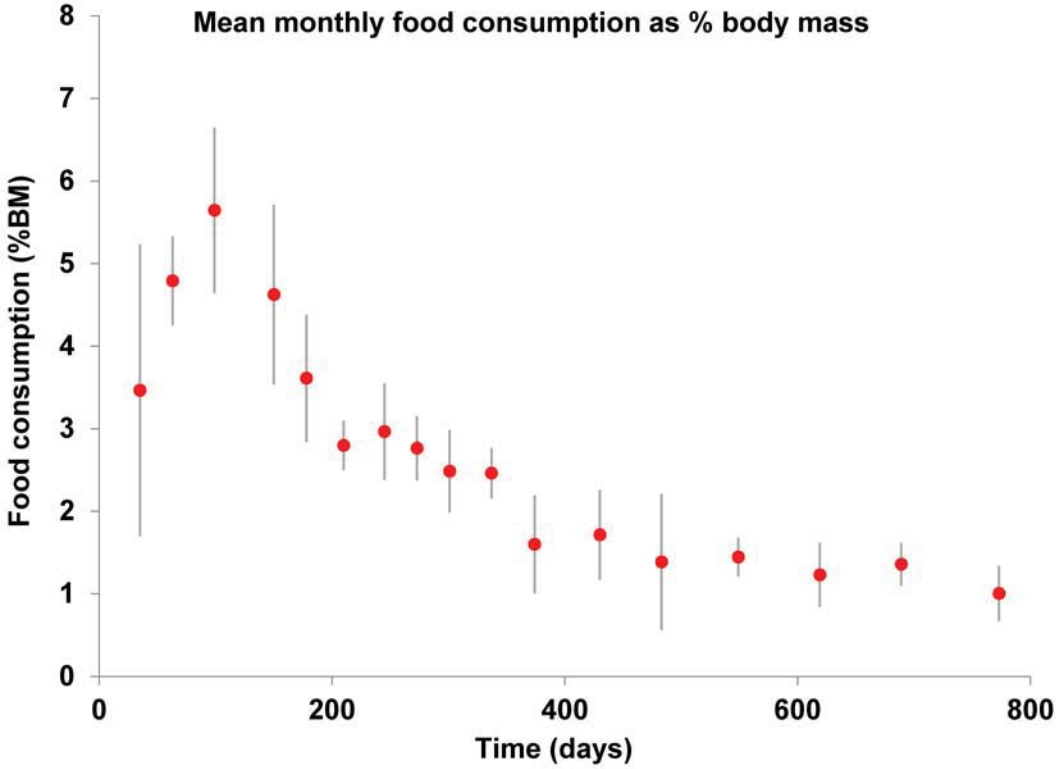

Figure 6. Mean monthly food consumption as a percentage of body mass of seven Pacific angelsharks, *Squatina californica* (Ayres, 1859), born and maintained at the Aquarium of the Bay (California, USA.). Grey bars represent standard error.

signs of stress were no longer observed and consumption rates immediately returned to normal. The *S. californica* were administered florfenicol (as per Noga, 2010) at 30 mg/kg intramuscularly (IM) to treat aggravated lesions resulting from the external tags. No contraindications were observed and the lesions healed well.

Contact abrasions on the pectoral girdle of four of the *S. californica* appeared periodically. Maintaining an evenly-spread deep bed of sand substrate typically alleviated these lesions. The frequency and severity of contact abrasions increased with growth and weight gain.

The young *S. californica* were infected with flatworms (class: Monogenea) on two occasions during their first three years. These outbreaks were successfully treated with three successive immersion treatments of praziquantel (as per Noga, 2010) dosed at 20 mg/L for 1.5 hours every other day (EOD). The *S. californica* showed no adverse reaction to the drug, but did display heightened activity immediately following the addition of praziquantel to the water. In addition, the sharks generally avoided food for the duration of the treatment.

During the winter of 2011 - 2012, a period of unseasonable weather resulted in unusually low water temperatures. During this time, one of the male *S. californica* pups (#1301) became inappetent and displayed an unusually dark coloration. After four weeks of refusing to eat, a weekly assisted feeding regime was implemented. The shark did not respond well to assisted feeding and, despite conscientious efforts, died seven weeks thereafter. An additional two pups (male #1305 and female #1306) displayed the same symptoms and died within three months. A post mortem was conducted on all specimens. Histology revealed that all three sharks suffered from viral meningitis, which was the likely precursor to inappetence and their ultimate death.

DISCUSSION

Despite thorough investigation, no evidence of sperm storage has been documented for *S. californica* (Natanson et al., 1984; Natanson and Cailliet, 1986; Gaida, 1997), suggesting that the sharks born at the Aquarium of the Bay were the result of mating within the exhibit. Preliminary genetic testing supported the conclusion that sharks #SC02 and #SC04 were the sire and dam, respectively. Redness and swelling around the cloaca of shark #SC04, after the pups had been discovered, corroborated this conclusion. Both sharks were acquired at least 12 months prior to pupping (70 months for the male shark #SC02 and 19 months for the female shark #SC04), consistent with the conclusion that both copulation and fertilization took place in the Aquarium.

The growth rate of *S. californica* within the aquarium was more rapid and variable than previously documented for wild conspecifics and other specimens maintained in aquaria (Natanson and Cailliet, 1986; Cailliet et al., 1992; Schaadt and Landesman, 1997). Variable growth rates could be the result of environmental conditions and/or nutrition. *S. californica* food consumption rates temporarily decreased in response to significant changes in water quality (e.g., shifts in temperature, elevated nutrient levels), habitat, and animal health status, as well as handling for morphometric measurements and medical procedures. Similar changes in feeding behavior and food consumption rates have been documented in other fish species when subjected to changing environmental conditions and other extrinsic factors (Murru, 1990; Spotte, 1992).

Documented *S. californica* life history and demographic information has been collected from sharks caught in Southern California, near the Channel Islands and Santa Barbara, or in the Gulf of California. *S. californica* used in this study were sourced from Bodega Bay, California, where population demographics remain unevaluated (Natanson and Cailliet, 1986; Cailliet et al., 1992; Gaida, 1995; Bizzarro et al., 2009; Carter et al., 2010). Evidence suggests that *S. californica* along the West Coast of the USA and Mexico may belong to two genetically isolated subpopulations, with divergent life history traits (Gaida, 1997; Ramirez-Amaro et al., 2013). The husbandry team at the Aquarium of the Bay will continue to collect and record data from *S. californica* sourced from Bodega Bay and born at Aquarium. It is hoped that this information will supplement the knowledge base about this intriguing and conservation-dependent species.

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Chapter 41

Reproduction and husbandry of zebra sharks, *Stegostoma fasciatum*, in aquaria

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Abstract: Zebra sharks, *Stegostoma fasciatum* (Hermann, 1783), are becoming more popular in public aquaria due to their large size and engaging appearance. Many institutions have successfully bred *S. fasciatum*. In an effort to manage the *S. fasciatum* population and increase reproduction, studbooks were created in both Europe and North America. Mean size of reproducing female (n = 10) and male (n = 9) *S. fasciatum* was 213 ± 9.8 cm total length (TL) (range: 183 - 235 cm TL) and 208 ± 12.2 cm TL (range: 188 - 250 cm TL), respectively. The body mass (BM) of reproducing female (n = 10) and male (n = 8) *S. fasciatum* was 47.9 ± 12.3 kg BM (range: 25.6 - 65.0 kg BM) and 33.8 ± 3.4 kg BM (range: 28.6 - 39.8 kg BM), respectively. Mean size at birth was 28.7 ± 1.8 cm TL (n = 109) in the range of 15 - 32 cm TL. Mean BM of newborns was 28.7 ± 1.8 cm (n = 109) in the range of 50 to 130 g. Preliminary results suggest that *S. fasciatum* incubation period was temperature dependent, with higher temperatures resulting in a shorter incubation time. Sharing collective knowledge about *S. fasciatum* through studbooks, and between aquaria in general, will lead to improved husbandry practices and help the management of a healthy, sustainable and genetically-robust population for continued public exhibition.

INTRODUCTION

The zebra shark, *Stegostoma fasciatum* (Hermann, 1783), is a tropical, benthic elasmobranch found in the shallow coastal waters of the Indo-West Pacific (Red Sea and East Africa to New Caledonia, Japan, and Australia). They preferentially inhabit areas with sandy substrate that are in close proximity to coral reefs, at depths of less than 62 m (Van der Elst, 1993; Compagno, 2002).

Adult *S. fasciatum* have five distinctive longitudinal ridges on a stout, cylindrical body. The central ridge follows the dorsal midline, and two dorso-lateral ridges are present on either side of the body. *S. fasciatum* attains a total length (TL) of up to 3.5 m, although sharks greater than 2.5 m TL are rare in the wild (or aquaria) and the long caudal fin comprises nearly half their TL (Compagno, 2002). Juvenile *S. fasciatum* are uniquely colored with yellow/white vertical bands and spots on a dark brown body. Small brown spots on a light colored background gradually replace juvenile coloration as the shark grows to 50 - 90 cm TL (Compagno, 2002). *S. fasciatum* are an oviparous species, laying large (17 cm long x 8 cm wide x ~5 cm thick) dark egg cases with

fine lateral tufts of hair-like fibers that serve to anchor them to the substrate (Compagno, 2002).

Targeted and non-targeted fishing has led to *S. fasciatum* being classified by the International Union for Conservation of Nature (IUCN) as "vulnerable", with a declining population trend throughout most of their range (Pillans and Simpfendorfer, 2003), although they are listed as "least concern" in Australia (Cavanagh et al., 2003). Limited gene flow exists between populations classified as "vulnerable" and "least concern" (Dudgeon et al., 2009).

Studbooks

A studbook provides a repository of details for individuals within, and an overview of the dynamics of, a metapopulation of a specific taxon under human care. Studbooks have been used to manage mammal and bird populations in zoological parks for decades, but have only been used for elasmobranchs in very recent years. Studbooks have been established for *S. fasciatum* in two regions: Europe (including the Middle East) in 2007, through the European Association of Zoos and Aquaria (EAZA), and North America (including an aquarium in China) in 2010, through the Association of Zoos and Aquariums (AZA). In 2013, the living metapopulation of *S. fasciatum* covered by the EAZA studbook consisted of 29 males and 40 females, while 41 males and 52 females were managed within the AZA studbook. In 2011, a species survival plan (SSP) was created, based on the genetic analysis of *S. fasciatum* managed within the AZA studbook. The SSP provides animal placement and breeding recommendations based on maintaining a robust genetic stock across the entire studbook population.

According to the EAZA and AZA studbooks, *S. fasciatum* was first displayed in an aquarium in 1988 and reproduction was first recorded in 1999, when a female pup hatched at the Henry Doorly Zoo in Omaha (Nebraska, USA).

The survey

To illuminate factors that influence the successful breeding and rearing of *S. fasciatum*, a global survey (hereafter "survey") of all aquaria (n = 11) was conducted during 2013. Facilities with successful reproduction collectively held 24 *S. fasciatum* (both genders), producing a total of 154 offspring. The survey collected data on both adults and neonates, including morphometrics, egg production rates and incubation times, as well as information about aquarium dimensions, water quality parameters, diet and feeding techniques.

REPRODUCTION

Sexual maturity

According to the survey, the mean size of reproducing female (n = 10) and male (n = 9) *S. fasciatum* was 213 ± 9.8 cm TL (range: 183 - 235 cm TL) and 208 ± 12.2 cm TL (range: 188 - 250 cm TL), respectively. The body mass of reproducing female (n = 10) and male (n = 8) *S. fasciatum* was 47.9 ± 12.3 kg BM (range: 25.6 - 65.0 kg BM) and 33.8 ± 3.4 kg BM (range: 28.6 - 39.8 kg BM), respectively. Data was not available for all reproducing individuals within AZA and EAZA aquaria. There was some evidence to suggest that animals weighing above 50 kg had a lower fertility rate, although the sample was of insufficient size to allow for statistical verification.

In the wild, male and female *S. fasciatum* attain sexual maturity at 150 cm TL and 170 cm TL, respectively (Van der Elst, 1993; Compagno, 2002). A male and female *S. fasciatum* were acquired by the Shedd Aquarium (Chicago, Illinois, USA) when they were approximately six months of age (based on morphometric data taken from specimens of known age). The male sired its first offspring at seven and a half years of age, when it had attained 213 cm TL and 37.7 kg BM. The female produced her first offspring at six years of age, at which time she was 214 cm TL and 48.8 kg BM.

Copulation

According to Kunze and Simmons (2004), pre-copulatory behavior by *S. fasciatum* consisted of the male following the female and biting at her caudal and pectoral fins, for periods lasting up to several hours, while the female either continued to swim or lay on the bottom of the exhibit. While male *S. fasciatum* primarily targeted the distal caudal fin and the trailing margins of the pectoral fins, they were also observed biting the first and second dorsal fins of females.

Pre-copulatory behavior occasionally led to mating. During copulation, male *S. fasciatum* have been observed curling their body around the female and inserting one clasper into her cloaca. Copulation was observed to last for 2 - 5 minutes (Bone, personal communication).

Husbandry staff at Reef HQ (Queensland, Australia) repeatedly observed a male *S. fasciatum* gulping water prior to attempting copulation, causing the abdomen to become distended. The abdomen of the male remained swollen for up to an hour after copulation attempts.

This behavior was only ever observed in conjunction with attempted copulation (Bone, personal communication).

Successful reproduction in *S. fasciatum* occurred when females were in equal, or greater numbers, than males. While some facilities reported that breeding activity only occurred during the natural mating season (Bone, personal communication), other facilities reported continuous mating activity throughout the year. In some cases, year-round mating resulted in substantial chronic fin wounds on females. Intraspecific aggression between males was also documented (Brunnschweiler and Pratt, 2008). Mating and agonistic behavior occasionally resulted in lesions or more traumatic injuries such as crushed caudal vertebrae. Since septicemia and even mortality can occur from injuries aggravated by repeated copulation attempts, the capapcity to separate individual *S. fasciatum* as a collection management tool is recommended (Christopher and Thomas, 2009).

Parthenogenesis and sperm storage

Robinson et al. (2011; this volume) was the first to document parthenogenesis in a female-only population of *S. fasciatum*. A female within the population produced 15 offspring over a period of four consecutive years. Parthenogenesis was confirmed using DNA analysis on three of the pups. Parthenogenesis in *S. fasciatum* has also been reported at Moody Gardens (Galveston, Texas, USA) and the Aquarium of the Americas (Long Beach, California, USA) (Dubach, personal communication).

S. fasciatum may also be able to store sperm. Female *S. fasciatum* at the Shedd Aquarium were exposed to a male for only one week a year, yet the females produced fertile eggs year-round. The period when the male *S. fasciatum* was introduced to the females varied every year. The maximum duration of viable sperm storage in *S. fasciatum* is unknown.

Parthenogenesis and sperm storage complicates the genetic management of the *S. fasciatum* metapopulation in aquaria, placing further reliance on genetic analysis technology for the determination of offspring paternity.

Artificial insemination

Artificial insemination (AI) has rarely been used with elasmobranchs, but represents a powerful tool for maintaining genetic variance within aquaria metapopulations without the need to transport the animals themselves. To date, successful AI has been achieved in two elasmobranchs, the clearnose skate, *Raja eglanteria* (Bosc, 1800) (Luer et al., 2007), and the whitespotted bambooshark, *Chiloscyllium plagiosum* (Bennett, 1830) (Motoyasu et al., 2005).

AI of *S. fasciatum* was first attempted in 2012 at Burgers' Zoo (Arnhem, Netherlands). A tom-cat 3½" closed end catheter (Jørgen Kruuse A/S, DK-5550 Langeskov, Denmark) was inserted 10 cm into the left seminal vesicle of a nine-year old male, via the urogenital papilla at the base of the clasper. Semen (5 mL) was extracted, confirmed to be active using microscopy, and diluted four times using sterilized seawater taken from the exhibit (salinity 33.0 g/L). A second tom-cat 3½" closed end catheter was then inserted 5 cm into the uterus of a nine-year old female, via the cloaca, to inject the diluted semen. The female had a two-year history of producing egg cases without yolk and, despite the attempted AI, continued to produce empty eggs. The reasons for her apparent infertility are not known.

More recently, AI was successfully employed to impregnate a female *S. fasciatum* at the Aquarium of the Pacific (Long Beach, California, USA). The procedure was confirmed to be successful using genetic testing (Adams, personal communication).

Egg laying

Sexually-mature *S. fasciatum* typically produce 20 - 50 eggs per year, although there is a great deal of variability with some individuals producing upwards of 100 eggs annually. Egg laying behavior in *S. fasciatum* has been described by Kunze and Simmons (2004). Egg tendrils would protrude from the cloaca of a female, in advance of laying, and her swimming behavior would change. The female would slowly circle vertical structures until the egg tendrils became entangled and the egg was pulled from the oviduct, at which point the female resumed normal swimming behavior.

Female *S. fasciatum* at the Shedd Aquarium would lay up to seven eggs during one oviposition event, although 4 - 5 eggs were more typical. Eggs were laid at 6 - 8 day intervals throughout a period of 4 - 5 months (Christopher and Thomas, 2009). The survey indicated that others observed egg-laying periods of 1 - 6 months, although 2 - 4 month egg-laying periods were more frequently noted. In general, egg-laying was not seasonally dependent, with oviposition occurring throughout

the year. However egg-laying by *S. fasciatum* at Reef HQ (Townsville, Queensland, Australia) only occurred during September - December, coinciding with breeding season observed in the local wild population (Bone, personal communication). This adherence to seasonal oviposition may be related to a semi-open life support system and 'flow-through' water supply, resulting in water temperature fluctuations closely tracking seasonal changes. Mean water temperature at Reef HQ was of 26.0°C, with a range of 21.0 - 31.0°C.

Embryo care

Once eggs had been laid by *S. fasciatum* at the Shedd Aquarium, the eggs were removed from the breeding aquarium to prevent predation of the eggs by other tank-mates and to allow for monitoring of embryo development. The eggs were transferred to grow-out aquaria with similar water quality parameters as the breeding aquarium. If there was a water quality differential, the eggs were gradually acclimatized to the new parameters. Eggs were kept submerged throughout the transfer process whenever possible. A high-powered underwater light was used to examine (or 'candle') the egg to establish the presence of a yolk. Yolkless eggs were occasionally laid in the same clutch as viable eggs. Egg tendrils were removed by gently peeling them away from the sides of the egg to prevent neonate entanglement on hatching, a risk previously observed in aquaria.

A variety of techniques were employed by surveyed institutions to brood the eggs. Some facilities placed the eggs on the floor of a grow-out aquarium. Others elevated the eggs on a piece of grating to encourage water flow around the entire embryo. Some institutions placed eggs in baskets floating just underneath the surface of the water. Other institutions suspended the eggs below the surface of the water using monofilament fishing line, cable ties, or other similar material, which were threaded through the tapered ends of the eggs and secured to a PVC pipe laid across the top of the grow-out aquarium. All survey respondents considered that adequate water flow around the eggs was essential to healthy embryo development.

Rigorous record keeping was a key part of the *S. fasciatum* breeding program at the Shedd Aquarium. Details of oviposition date and time, as well as details of dam and sire (if known), were all recorded. A copy of this information was attached to a label on the corresponding holding basket or grow-out aquarium where the eggs were maintained, or to the eggs themselves if they were suspended mid-water using monofilament line. Detailed records aided the prediction of hatching dates and were key to monitoring and interpreting embryo development.

At the Shedd Aquarium, eggs were 'candled' or examined with ultrasound at least once every other week, to monitor embryonic viability and development. Direct observation of active embryos further confirmed viability. Embryos less than 30 days old were typically difficult to interpret, but a well-defined uniform oval yolk was considered promising. Infertile eggs did not contain a discrete yolk (Poll, personal communication) and began to deteriorate ~4 - 6 weeks after oviposition. Infertile eggs were immediately removed as their decomposition could potentially compromise other developing eggs in close proximity.

Hatching

Incubation data was collected from eight separate aquarium facilities. Mean (n = 72) incubation time was 152 ± 16 days, in the range of 99 - 243 days. Eggs were incubated at 23.5 - 30.0°C. Median temperature was compared to average incubation time (Table 1). Preliminary results suggest that *S. fasciatum* incubation period was temperature dependent, with higher temperatures resulting in a shorter incubation time.

Various survey respondents indicated that hatching pups would occasionally require manual assistance to successfully emerge from the egg case. Kunze and Simmons (2004) described *S. fasciatum* neonates failing to emerge and dying within the egg case. If neonates at the Shedd Aquarium did not hatch within four weeks of completely internalizing their yolk, the egg case was manually opened. Pups that hatched with some external yolk remaining would often refuse to eat until the entire yolk had been consumed. However, food was still offered to the pups on a daily basis and was occasionally eaten.

Newborn *S. fasciatum* were measured and assessed by many survey respondents. Mean neonate size at birth was 28.7 ± 1.8 cm TL (n = 109) in the range of 15 - 32 cm TL. Mean BM of newborns was 28.7 ± 1.8 cm (n = 109) in the range of 50 to 130 g. Incubation temperature did not appear to influence birth size or weight.

Table 1. Zebra shark, *Stegostoma fasciatum* (Hermann, 1783), egg incubation times and temperatures in surveyed aquaria.

Median temperature (°C)	Incubation period Mean (days)	Incubation period Range (days)	Number of animals
25.0	157.4 ± 13.1	135 - 176	n = 5
25.5	160.4 ± 8.2	99 - 243	n = 51
26.5	124.2 ± 2.3	123 - 133	n = 9
28.3	119.9 ± 7.5	113 - 139	n = 7

Survival rates

As of September 2013, the historical *S. fasciatum* metapopulation was 355, consisting of 115 (51.64.0; males.females.unknowns) sharks at 40 EAZA institutions, and 240 (105.134.1) sharks at 38 AZA institutions. Of these sharks, 25 (4.21.0) were the result of breeding in four EAZA aquaria, and 144 (63.81.0) were the result of breeding in 11 AZA aquaria.

Although *S. fasciatum* have successfully bred in several facilities throughout the world, the mortality rate of hatchlings is high. The average mortality within the first year of life, as reported in the AZA SSP, was 53% for males and 59% for females (Marti and Watson, 2011). Of offspring born in studbook-managed aquaria, only 20 (3.17.0) and 38 (17.21.0) remain alive in EAZA and AZA institutions, respectively. These numbers equate to a survival rate of 84% and 26% for EAZA and AZA aquaria, respectively. The reported survival rate for EAZA aquaria might be an overestimate, as data for all juveniles was not available.

HUSBANDRY OF JUVENILES

Environment and water quality

S. fasciatum should be maintained in aquaria that provide sufficient swimming space, without obstructions (Kunze and Simmons, 2004). Mean enclosure volume for the rearing of neonates, at surveyed facilities, was 1,096 ± 551 L (n = 7) and ranged from 280 - 2,510 L. Enclosures had an average length or diameter of 1.5 m and an average depth of 0.6 m.

Aragonite or coral sand substrate was generally used on the floor of at least half of the rearing enclosure. When substrate was not present, or provided in insufficient quantities, *S. fasciatum* neonates were prone to dermatitis or pressure sores on the ventrum (Christopher and Thomas, 2009).

Kunze and Simmons (2004) recommend that newly hatched neonate *S. fasciatum* should be maintained in enclosures by themselves, or with other hatchlings. When maintained with larger conspecifics, the pups were susceptible to intraspecific aggression.

Water quality parameters in surveyed institutions where *S. fasciatum* neonates were successfully reared were as follows: temperature, salinity and pH in the ranges 24.3 - 26.4°C, 31.0 - 33.7 g/L and 7.9 - 8.2, respectively. Ammonia (NH_3) and nitrite (NO_2^-) were maintained below 0.1 mg/L, and mean nitrate (NO_3^-) was 20.6 mg/L.

Food and feeding

It was not unusual for *S. fasciatum* neonates at the Shedd Aquarium to retain a remnant yolk sack on the ventrum for some days after hatching. Despite this source of nutrition, neonates generally began feeding within 48 hours of emerging from the egg (Christopher and Thomas, 2009). Kunze and Simmons (2004) initially fed pups by positioning a food item directly under the mouth of the shark using a feeding stick, to encourage a feeding response. Pups were offered bite-sized pieces of food, weighing 0.5 - 2.0 g each, for a total ration of 1.5 - 4.0% BM/day.

At the Shedd Aquarium, food was initially offered 3 - 4 times a day, then feeding frequency was gradually reduced as pups approached ~50 cm TL, or around two months of age. *S. fasciatum* grew rapidly, so food intake was substantial and needed to be carefully monitored so an appropriate food ration could be maintained. Adjustments to the food ration were made on a weekly basis in line with specimen BM. On average, food ration was increased by 1.0 g/week, but routine weighing was used to confirm adjustments to the ration.

When devising a dietary plan for any species, the diet of wild conspecifics should be referenced. Cortés (1999) analyzed the stomach contents of *S. fasciatum* (n = 6) and found mollusks (excluding squid) only. Compagno (2002), however, suggested that while *S. fasciatum* feed primarily on mollusks (gastropods and bivalves), they might also opportunistically prey on crustaceans (crabs and shrimp), small bony fishes and possibly sea snakes.

According to survey respondents, a variety of food items were typically offered to *S. fasciatum* neonates. Squid (*Loligo* spp.), with the 'pen' removed, and various species of shrimp were the most frequently offered foods (n = 7), while clams were also frequently offered (n = 4). Once pups were a little older, a variety of fishes and invertebrates were the dietary staple. At the Shedd Aquarium, larger fishes such as little tunny, *Euthynnus alletteratus* (Rafinesque, 1810), mackerel, *Scomber* spp. (Linneaus, 1758), and Atlantic herring, *Clupea harengus (*Linnaeus, 1758), were presented to neonates as fillets without skin or bones. Smaller fishes, such as Atlantic silversides, *Menidia menidia* (Linnaeus, 1766) and capelin, *Mallotus villosus* (Müller, 1776), were presented in thin slices. Food was soaked in a liquid vitamin supplement (e.g., Vita Fish™, Marine Enterprises International, USA) for 10 minutes prior to feeding.

One of the most frequent challenges rearing *S. fasciatum* pups is periodic inappetence. Inanition or inadequate feeding response has been documented in the AZA studbook as a common cause of death among neonates (n = 16). Assisted feeding of neonates may be necessary to meet their metabolic demands. Animal health staff at the Shedd Aquarium developed various assist-feeding diets for juvenile *S. fasciatum*. A fish-based gruel was developed initially, but resulted in gastrointestinal challenges for some juveniles. It was concluded that *Scomber* spp. was difficult to digest, based on an analysis of undigested food aspirated from the stomach. The gruel recipe was therefore modified to consist solely of clam, blended with water (purified by reverse osmosis). Vitamin supplementation and/or medications were occasionally added to the diet, depending on the status of the pup.

HUSBANDRY OF ADULTS

Environment and water quality

A formula to calculate appropriate aquarium size for pelagic sharks was developed and reported by Choromanski (2004). A similar formula has not yet been established for benthic shark species. However, as a guideline, mean exhibit dimensions, reported by survey respondents who have successfully maintained breeding *S. fasciatum*, were as follows: 36.9 ± 13.8 m long (range: 17 - 82 m) x 15.8 ± 6.5 m wide (range: 7.6 - 30 m) x 7.0 ± 1.9 m deep (range: 4.5 -11 m).

In general, water quality is an important factor contributing to the successful care of elasmobranchs in aquaria and this subject has been thoroughly addressed by Mohan and Aiken (2004). The water parameters reported for adult *S. fasciatum* by survey respondents were the same as those reported for neonates (see above). Maintaining *S. fasciatum* in water quality outside these ranges may be possible, but could affect breeding activity.

Food and feeding

In general, adult *S. fasciatum* were individually fed at surveyed institutions and broadcast feeding was rare. Snowden (2008) suggests target training and target feeding as a useful tool to aid monitoring of food and vitamin intake. Feeding frequency depended on the size and specific needs of individual sharks. *S. fasciatum* of 2 - 10 kg BM were generally fed 1 - 2 times per day, while sharks of >10 kg BM were fed 3 - 7 times per week. Providing multiple feeding sessions, with a smaller food ration per session, was considered a better strategy than fewer feeding sessions with a larger food ration. This approach was even more important for neonates or for older animals being reinforced with food during training sessions. Regardless of the strategy employed, deliver of the weekly food ration was considered of paramount importance.

According to survey respondents adult *S. fasciatum* were fed a mean ration of 5% BM/week. This food ration is in agreement with Janse et al.

(2004), who advised a feeding ration of 4 to 6% BM/week for benthic sharks. Forage was varied, but, in general, included 50% fatty foods (~150 kcal/100 g) and 50% lean foods (~90 kcal/100 g), or a mean of 120 kcal/100 g. By extrapolation, at a food ration of 5% BM/week, the weekly calorie intake for *S. fasciatum* at surveyed institutions was ~60 kcal/kg BM/week.

Vitamins

Good nutrition is critical to the successful maintenance of healthy elasmobranchs (for an overview of elasmobranch nutrition, refer to Janse et al., 2004). Most diets for sharks in aquaria consist of frozen food, which can lose vitamin integrity during freezing, storage and thawing (De Silva and Anderson, 1995). A reduced intake of essential nutrients and vitamins may impact the capacity of *S. fasciatum* to successfully breed. An essential component of a nutrition plan for an elasmobranch collection is vitamin supplementation. A survey of public aquaria (n = 17) maintaining *S. fasciatum* was conducted in 2007. The survey revealed that a variety of vitamin supplements were added to shark feeding rations, including Vita-Zu Shark/Ray II Tablet 1.5 g # 5MD8 (Land O' Lakes Inc., Arden Hills, Minnesota, USA) (n = 5); Twilmij multivitamin (Twilmij B.V., 3776 LZ Stroe, The Netherlands) (n = 2); Aquavits (International Zoo Veterinary Group LLP, Station House, Keighley, BD21 4NQ) (n = 5); Aquaminivits (International Zoo Veterinary Group LLP, Station House, Keighley, BD21 4NQ) (n = 1); and Fishvits® Color-Marine® (Zoolife International Ltd., London SW1W 9PP) (n = 3). Only one aquarium did not use vitamin supplements. A comparison of relative supplementation levels used at different aquaria has been summarized in Figure 1. The composition of Zoolife International Ltd. products could not be obtained and were excluded from Figure 1.

MEDICAL CONSIDERATIONS

Health challenges presented by *S. fasciatum* at surveyed aquaria were classified by locus of pathology (Table 2) and etiological agent (Table 3). As some health challenges were restricted to a specific age class, findings were divided into: (1) neonates, 0 - 2 months old; (2) juveniles, 2 months - 2 years old; and (3) adults, >2 years old. Many pathologies remained unidentified (22% of the locus of pathology and 23% of the ethological agents), suggesting an important area for future investigation. The majority of pathologies were focused in the liver or the bloodstream. Liver challenges included: hepatic atrophy (n = 2), hepatitis with lesions and hemorrhages (n = 8), depleted hepatic storage (n = 2), fatty liver syndrome (n = 1) and scuticociliatosis (n = 2) (Stidworthy et al., 2013). Sepsis was the primary pathology associated with blood (n = 16), although of undetermined origin. Two health challenges presented in the blood as

Table 2. Health challenges identified in zebra sharks, *Stegostoma fasciatum* (Hermann, 1783), showing the locus of pathology. These findings were based on 104 necropsies of specimens within AZA and EAZA institutions.

Locus of pathology	Neonate (< 2 months)	Juvenile (2 month - 2 year)	Adult (> 2 year)
Blood		6	12
Liver		5	10
Intestine		5	5
Skin	3		6
Brain		3	6
Gills	2	3	1
Spleen		1	3
Reproductive system			4
Kidney		1	
Other	8	9	8
Unknown	2	8	13

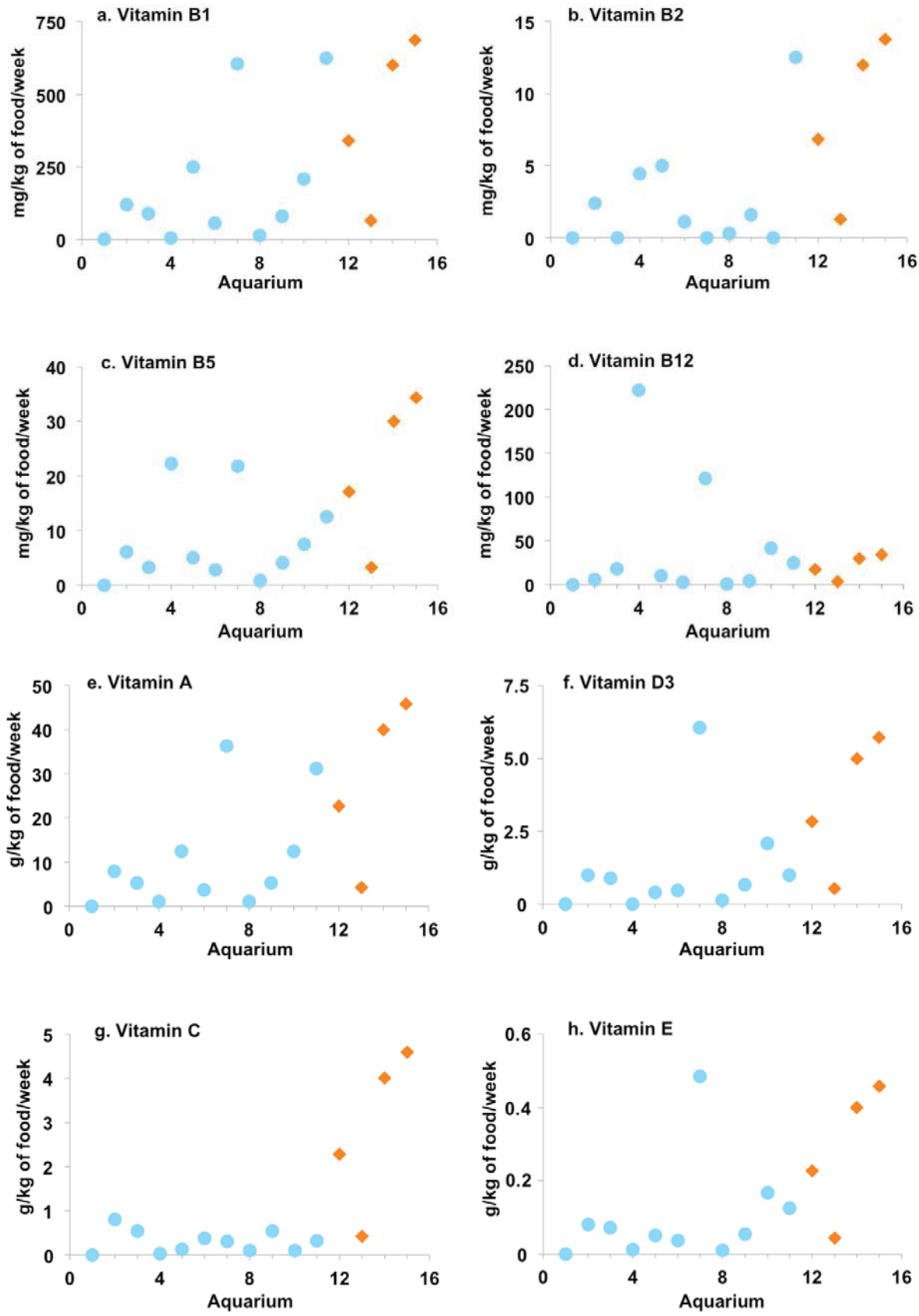

Figure 1. An analysis of vitamin supplements used in aquaria that maintains zebra sharks, *Stegostoma fasciatum* (Hermann, 1783), showing facilities where successful breeding has (blue circle) or has not (red diamond) occurred.

Table 3. Health challenges identified in zebra sharks, *Stegostoma fasciatum* (Hermann, 1783), showing the suspected etiological agent. These findings were based on 104 necropsies of specimens within AZA and EAZA institutions.

Etiological agent	Neonate (< 2 months)	Juvenile (2 month - 2 year)	Adult (> 2 year)
Bacteria	3	7	25
Trauma	2	4	10
Nutritional deficiency	5	6	3
Parasite		4	3
Transport complications	1	4	
Water quality	1	1	2
Virus			2
Toxin		1	
Other	2	2	1
Unknown	2	8	14

erythrocyte inclusion bodies of possible viral origin. Other necropsy results suggested the presence of viral agents, but were not confirmed. 'Egg binding', where the eggs had become necrotic and caused systemic infection, resulted in a number (n = 4) of mortalities.

The most frequent etiological agents were bacterial (n = 35), some form of trauma (n = 16) or nutritional deficiency (n = 16). Two cases of trauma were the result of hook wounds resulting from wild acquisition. Other cases of trauma, in adults, were the result of mating wounds, which became septic (n = 4).

Increased mating frequency in aquaria could result in chronic wounds to the dorsal, pectoral and distal caudal fins, as well as the claspers. When severe wounds from mating were observed, animals were separated and wounds treated as necessary. Gasterointestinal challenges (n = 2) were concluded to result from impaction with squid beaks and shrimp exoskeleton in young animals (i.e., < 2 years old).

Mortality attributed to parasites included: nematodes in the gills and brain (n = 3), systemic ciliated protozoan infections (n = 2; Stidworthy et al., 2013) and protozoal meningitis (n = 2). Large digenean trematodes were frequently found within the visceral cavity (n = 4) of *S. fasciatum*. In one case, the trematodes were identified as *Petalodistomum largus*. However, no adverse effects were ascribed to the presence of the trematodes, a finding in agreement with Benz and Bullard (2004) who describe digeneans to be unproblematic parasites for chondrichthyans. The copepod *Lepeophtheirus acutus* was described as a health concern for *S. fasciatum* (n = 3), but not a cause of death (Kik et al., 2011). Other previously documented *S. fasciatum* health challenges resulting from metazoans, protozoans and fungi have been summarized in Table 4.

Kunze and Simmons (2004), and Christopher and Thomas (2009), noted that some neonates would frequently swim in spirals for the first few days after hatching. Occasionally, this behavior persisted for 3 - 6 months and led to injury. This erratic swimming behavior was also noted at four other AZA institutions that have otherwise successfully reared *S. fasciatum* (Irvin, personal communication; Drinnen, personal communication; Celt, personal communication). Although the cause of this behavior is unknown, varying etiologies have been proposed, including: cerebellum development challenges, viral exposure or nutritional deficiency (Poll, personal communication).

Information about *S. fasciatum* health has been documented in the AZA and EAZA studbooks, including health screening protocols and guidelines for prophylactic treatments. Carers of *S. fasciatum* are encouraged to reference these

Table 4. Metazoan, protozoan and fungal health challenges encountered by zebra sharks, *Stegostoma fasciatum* (Hermann, 1783), in aquaria. The environmental source of the infection is indicated as either A = aquarium or W = wild.

Taxon	Species name	Site of infection	Isolated	Reference
Metazoans				
Digenea	*Petalodistomum largus*	Visceral cavity	W	
Cestoda	*Pedibothrium kerkhami*	Intestine	W	Caira, 1992
	Pedibothrium longispine	Intestine	W	Caira, 1992
	Pedibothrium veravalensis	Intestine	W	Caira, 1992
	Echinococcus granulosus	Hydatid cyst in spleen, liver and intestine	C	Assawawongkasem et al., 2000
Nematode	Capillariidae	Digestive tract	W	Moravec and Justin, 2010
Copepoda	*Lepeophtheirus acutus*	Eye	C	Kik et al., 2011
Protozoans				
Amoeba	Unknown	Brain	C	Goertz, 2004
Ciliates	*Philasterides dicentrarchi*	Systemic scuticociliatosis	C	Stidworthy et al., 2013
Fungi				
	Exophiala pisciphila	Liver, kidney, spleen	C	Marancik et al., 2011
	Mucor circinelloides	Liver, kidney, spleen	C	Marancik et al., 2011

documents and share the material with other stakeholders.

FUTURE DIRECTIONS

The collective goal for carers should be to maintain a genetically diverse, self-sustaining *S. fasciatum* population within aquaria, reducing pressures on wild stocks and providing a valuable living resource to learn more about this species. A variety of husbandry and management challenges must be addressed to realize this goal. Within EAZA studbook-managed institutions, additional breeding pairs need to be identified. Recommendations have been made to institutions within the AZA studbook-managed region, related to matching or transferring key breeding individuals, but many recommendations are yet to be accommodated. Within the AZA studbook region there are sufficient *S. fasciatum* founders to maintain a robust genetic pool, but only a few of these individuals are reproductively active. Population management through the AZA and EAZA studbooks would be further strengthened by the development of studbooks by other regional zoo and aquarium associations. Communication between studbook keepers and individual aquaria is integral to the successful implementation of regional species management programs. The use of integrated animal registration systems, such as Species 360 (www1) and/or TRACKS (www2), will help facilitate information sharing in a format that is efficient and useful for partner institutions.

Reproductive pairings based on the genetic composition of the population will help to maintain diversity and fitness for future *S. fasciatum* generations. Recommendations have already been made to discourage the breeding of genetically over-represented individuals within the AZA studbook region. To ensure a complete and robust breeding plan, genotyping of all potential founders must be completed, confirming the sire and dam of any offspring, as well as offspring resulting from parthenogenesis.

More research on the reproductive biology and husbandry of *S. fasciatum* is necessary to better understand triggers for breeding and to help induce reproductively inactive pairs to become successful breeders. Recent developments in the field of AI technology hold promise as another valuable tool to facilitate population management, allowing for the transport of *S. fasciatum* semen, rather than the more risky process of transporting live adult animals. AI also represents a mechanism to manage the

risk of chronic wounds and secondary infection resulting from repeated copulation attempts in aquarium conditions.

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INTERNET RESOURCES

www1 http://www.species360.org
www2 http://trackssoftware.com

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Chapter 42

Reproduction of spotted eagle rays, *Aetobatus narinari*, in aquaria

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Abstract: Spotted eagle rays, *Aetobatus narinari* (Euphrasen, 1790), are maintained in many aquaria worldwide. Despite their prevalence, few facilities have successfully bred *A. narinari*. To determine what factors affect *A. narinari* breeding success in aquaria, a survey was sent to facilities maintaining the species and the resulting data was compiled and analyzed. Six institutions reported a history of gravid *A. narinari* since 2000 with a total of 60 pregnancies, resulting in 82 live pups. Pregnancy was typically indicated by changes to the behavior, physical appearance and appetite of the gravid female. Of all successful pregnancies, 67% occurred where male *A. narinari* were outnumbered by females. Mean DW was 133.6 ± 9.3 cm and 152.2 ± 9.7 cm for sires and dams, respectively. Mean body mass (BM) for breeding *A. narinari* was 40 ± 5 kg and 60 ± 14 kg for sires and dams, respectively. Newborn pups had a mean size of 44.7 ± 5.1 cm DW (range: 35.5 - 58.0 cm DW) and a mean weight of 0.97 - 3.8 kg BM (range: 1.9 ± 0.7 kg BM).

INTRODUCTION

The spotted eagle ray, *Aetobatus narinari* (Euphrasen, 1790), is a large myliobatid ray found in temperate and tropical coastal waters and reef environments (Schleussel et al., 2010). Until recently *A. narinari* was historically considered to be a single circumtropical species. However, multiple species are now described across various global regions (Kyne et al., 2006; Richards et al., 2009; Schluessel et al., 2010; White et al., 2010; Naylor et al., 2012). *Aetobatus* spp. are classified by the IUCN Red List as "near threatened" globally, and "vulnerable" in Southeast Asia with a decreasing population trend (Kyne et al., 2006). Although protected in certain regions of Australia, the Maldives and the State of Florida (USA), this species is often taken by targeted fisheries elsewhere (Bianchi, 1985; Schluessel et al., 2010; Cuevas-Zimbron et al., 2011) and sometimes as bycatch (Kyne et al., 2006).

Since the opening of the first public aquarium in the mid-19th century, elasmobranchs have been popular exhibit animals (Koob, 2004). Over the past few decades, *A. narinari* has grown in popularity as a result of their visual appeal and swimming grace. *A. narinari* in public aquaria can act as ambassadors for their wild counterparts, by providing an opportunity to educate millions of guests. The conservation status of *A. narinari* (Kyne et al., 2006), coupled with the sustainable animal acquisition practices of zoos and aquariums, recommend the species for a well-planned breeding program. This chapter outlines some basic guidelines for the development of an *A. narinari* breeding program, supporting a better understanding of their reproductive biology and providing a source of healthy specimens for education display, while at the same time reducing pressure on wild stocks, and supporting aquatic animal medicine and *in situ* conservation efforts.

MATERIALS and METHODS

To quantify the frequency of, and predisposing conditions leading to, reproduction of *A. narinari* in aquaria, a questionnaire (hereafter "survey") was developed and distributed to facilities that had successful pregnancies. Exhibit characteristics, husbandry practices and animal status were assessed to determine what parameters correlated with successful reproduction. In addition, exhibit dimensions, residence time in the aquarium, sex ratio, reproductive status, pregnancy characteristics and neonate characteristics were examined to suggest minimum criteria necessary to establish a breeding program. Where quantifiable, results are reported as means ± standard deviations, as well as measured ranges.

RESULTS and DISCUSSION

Six aquaria displaying *A. narinari* reported pregnancies. The number of pregnancies at each institution ranged from 1 - 18 since 2000, with a total of 60 reported overall. Of these pregnancies, 52 resulted in 82 viable pups and the remaining eight pregnancies, deemed unsuccessful, resulted in aborted or stillborn pups.

Exhibit characteristics

In general, exhibit size is an important consideration when establishing a breeding program for elasmobranchs, as the spatial needs for active mating and copulation must be accommodated. Mean exhibit length, width, depth and volume for facilities (n = 6) with successful *A. narinari* pregnancies was 45.0 m, 28.6 m, 5.9 m, and 8,774.7 m^3, respectively. The smallest exhibit was 20 m x 9 m x 4 m with a volume of 322 m^3. Although *A. narinari* copulation has been observed mid-water (Uchida et al., 1990), successful breeding was reported in exhibits

with depths as shallow as 2.4 m. The survey revealed that exhibit shape was variable with no form common to facilities with successful pregnancies. Exhibit shapes were described as circular, oval, rectangular, figure eight, L-shaped and irregular.

Exhibit residence time

The mean length of time that breeding male and female *A. narinari* were maintained in a single aquarium, prior to parturition, was for 8.3 ± 2.6 years. A minimum of two years was reported for both genders before they successfully reproduced.

Husbandry

It is likely that food ration, behavioral conditioning, and animal health status influence the success of *A. narinari* reproduction in aquaria.

Feeding ration for breeding *A. narinari* was managed in a variety of ways: (1) a base ration was established for individual rays; (2) the entire population was fed a set ration; and (3) the entire population was fed to satiation during an allotted period of time. Vitamin supplementation was, in general, considered to be important, due to the loss of nutrients during freezing and thawing of food. According to the survey, weekly food ration for pregnant females was in the range of 5 - 20 % body mass (BM) with an mean of 9.7 ± 4.2 %BM per week. In some cases, female *A. narinari* food ration was increased by 1 - 2 %BM when pregnancy was confirmed. In other cases, food was offered to satiation. At some institutions, there was no change in food ration offered to pregnant female *A. narinari*.

Repeated capture and restraint for medical evaluations during pregnancy can increase the risk of premature delivery or fetal abortion. Behavioral conditioning can reduce the need for, and reduce stress associated with, animal handling. To reduce risks to pregnant females, *A. narinari* have been conditioned to station over a target for voluntary ultrasound readings (Corwin, 2012). This procedure requires patience and the application of advanced training techniques. In addition, the ultrasonographer is presented with some challenges (i.e., limited time for an ultrasound reading and movement of the ray during the procedure). However, this protocol imposes less manual handling and stress on *A. narinari* and therefore increases the likelihood of a pregnant female carrying healthy pups to full term (Reardon, personal communication; Kamerman, unpublished results).

Health challenges (e.g., parasite infestations, bacterial infections) that a healthy, non-pregnant female could overcome, may be sufficient to overwhelm a gravid *A. narinari,* in particular an animal nearing full term. Wherever possible, medical challenges should be addressed before a gravid female becomes symptomatic and is compromised.

Sex ratio

The mean number of *A. narinari* maintained at facilities where successful pregnancies were observed was 5.8 ± 1.8 per facility or one ray per 200 m^3 of water. Of the 60 documented pregnancies, 67% occurred when the total number of males was less than the total number of females, averaging 2.3 ± 1.0 males to 3.5 ± 1.4 females. A further 18% of pregnancies occurred in facilities where the sex ratio was one to one and the balance (13.3%) in facilities where male *A. narinari* outnumbered females. Population statistics were not recorded for one of the reported pregnancies.

To estimate the ratio of sexually mature males to females, a previously described (Tagliafico et al., 2012) correlate used for assessing wild *A. narinari*, disc width (DW), was employed. *A. narinari* with a DW >126 cm were deemed sexually mature, regardless of gender. The ratio of sexually mature males to sexually mature females, ranged from 1:1 - 3:3 with a mean ratio of one male to 2.9 females. A greater proportion (66.7%) of successful pregnancies occurred when the sexually mature male population was less than the sexually mature female population. Nearly one third (30.0%) of successful pregnancies occurred where there were equal numbers of sexually mature males and females. Only 1.7% of pregnancies occurred where sexually mature males outnumbered sexually mature females.

Sexual maturity and reproductive systems

Like other myliobatids, *A. narinari* is a matrotrophic viviparous species with trophonemata, which secretes histotroph to nourish the developing young (Schluessel et al., 2010). This reproductive strategy is also referred to as aplacental viviparity, whereby a placental connection between the mother and offspring is not formed (Carrier et al., 2004a). The reproductive tract of *A. narinari* (refer Figures 1 and 2) consists of a cervix, ostia, shell or nidamental gland, paired oviducts, ovaries and uteri (Hamlett and Koob, 1999) with only the left oviduct and uterus being functional (Henningsen et al., 2004; Schleussel et al., 2010; Stedman,

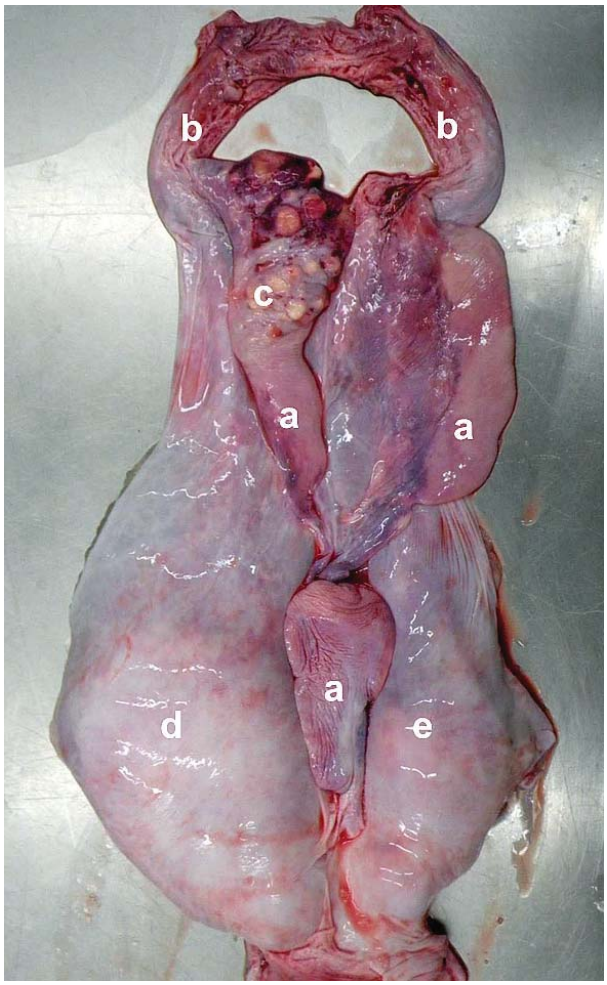

Figure 1. The reproductive tract of a recently gravid female spotted eagle ray, *Aetobatus narinari* (Euphrasen, 1790), showing: (a) epigonal organ lobes, (b) oviduct, (c) left ovary, (d) left uterus and (e) right uterus. Note the paired oviducts (b), the larger ovary on the left side (c), with various stages of oocyte development, and the larger left uterus (d). The right ovary is not observed. Photo: courtesy of Dr. N. Stedman.

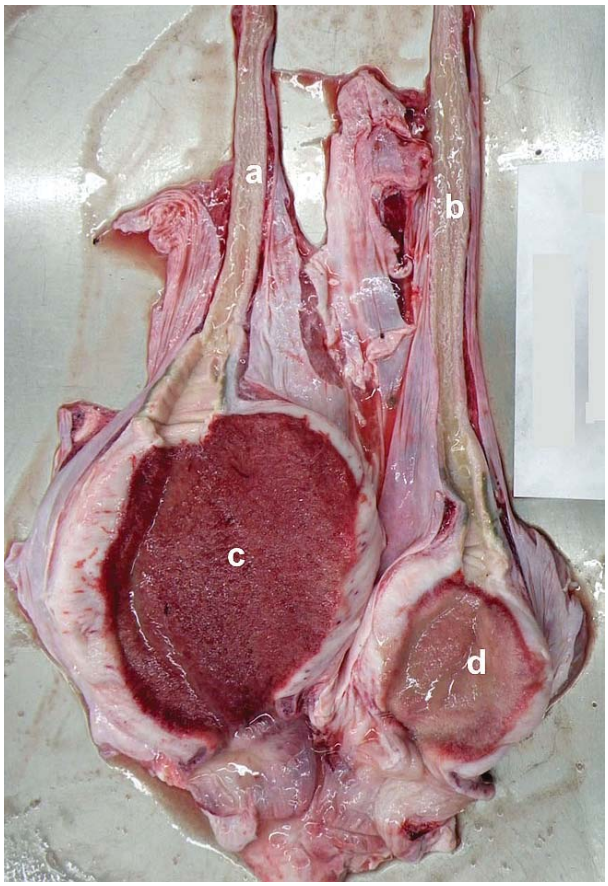

Figure 2. The reproductive tract of a recently gravid female spotted eagle ray, *Aetobatus narinari* (Euphrasen, 1790), showing: (a) left oviduct, (b) right oviduct, (c) left uterus with trophonemata and (d) right uterus. Note the left functional uterus with trophonemata (c) and the right, non-functional uterus (d). Photo: courtesy of Dr. N. Stedman.

personal communication). Functional and rudimentary reproductive organs, on the left side and right side, respectively, have been documented in other elasmobranch species (Schleussel et al., 2010), including the cownose ray, *Rhinoptera bonasus* (Mitchill, 1815).

The reproductive tract of male *A. narinari* is comparable with other elasmobranch species, consisting of testes and two functional claspers that lengthen and calcify with maturity (Schluessel et al., 2010). Other components of the elasmobranch male reproductive system are the epididymis, Leydig's gland, vas deferens, seminal vesicle, siphon sac (in some sharks) and clasper gland or alkaline gland (in some batoids) (Henningsen et al., 2004). The specific design of the claspers varies between elasmobranch genera (Carrier et al., 2004a), but both testes are active in all species (Henningsen et al., 2004).

Anatomical indicators, such as the presence of oocytes, ova and/or embryos, uterine size, and clasper length and calcification, are used to determine sexual maturity in elasmobranchs. Males are considered to be sexually mature when the claspers are large and rigid (fully calcified) (Schleussel et al., 2010). Researchers have used these indicators, along with DW, to establish size at sexual maturity in wild elasmobranch populations. Average size at maturity in *A. narinari* has been estimated to be 127.0 - 129.2 cm DW for males (Tagliafico et al., 2012; Bassos-Hull et al., 2014) and 134.9 - 150+ cm DW for females (Schleussel et al., 2010; Tagliafico et al., 2012). Survey data for *A. narinari* in aquaria indicated that the smallest sire and dam had a DW of 110 cm and 140 cm, respectively. Mean sire size was

133.6 ± 9.3 cm DW and mean dam size was 152.2 ± 9.7 cm DW. Breeding male *A. narinari* were, on average, 17 cm smaller than breeding females, consistent with sexual dimorphism observed in wild populations (Schluessel et al., 2010).

Estimated body mass (BM) of wild *A. narinari* is reported as 25 - 30 kg for mature males and 40 - 50 kg for mature females (Schluessel et al., 2010). Survey results corroborated these *in situ* observations. The smallest sire had a BM of 22 kg and smallest dam had a BM of 40 kg, with a mean BM of 40 ± 5 kg and 60 ± 14 kg for sires and dams, respectively.

Little data exists on the age of *A. narinari* at sexual maturity, although it has been estimated to occur at four to six years (Last and Stevens, 1994). Some of the sires and dams reported in the survey were born in aquaria, so their age-at-maturity is known. The youngest reported sire was five years old and the two youngest pregnant females were six years old. Based on estimated gestation times, the sire and dams were sexually mature at four and five years old, respectively. The average age for sires and dams was 12.8 ± 3.8 years and 10.7 ± 2.7 years, respectively. The smallest sire had a DW of 110 cm and was nine years old.

Mating behavior

Mating behavior by *A. narinari* has been documented in both wild and aquarium populations. Pre-copulatory behavior observed in wild males include following, biting, gouging the dorsal surface of the female with the lower dental plate and chasing females (Tricas, 1980; Uchida et al., 1990). Tricas (1980) observed a single male *A. narinari* attempting to mount an uncooperative female. After many unsuccessful attempts the female surfaced and the male began to "bob" and "sway", following her until she submerged again. In aquaria, *A. narinari* pre-copulatory behavior mimicked observed activity of breeding by wild conspecifics. In addition, "breaching" and "pelvic thrusting" was occasionally observed in male *A. narinari*, in aquaria, prior to copulation (Zimmerman, unpublished results). Although *A. narinari* copulation is rarely observed, post-mating evidence, such as bite marks on the pelvic fins or the caudal ridge of the pectoral fins, and/or abrasions or lacerations on the pectoral fins of females, are often observed in aquaria (Henningsen et al., 2004).

Copulation generally occurs abdomen-to-abdomen, mid-water, with the male inserting a single clasper into the cloaca of the female (Uchida et al., 1990). On one occasion, a male *A. narinari* was observed pushing a female through the water during copulation, with no resistance offered by the female (Zimmerman, unpublished results). Female *A. narinari* have been observed mating with up to four males in rapid succession, in both the wild and aquarium populations (Uchida et al., 1990). Polyandry, as well as multiple paternities within a litter, has been documented in many shark and ray species (Carrier et al., 2004b; Daly-Engel et al., 2006; Boomer et al., 2013), including *A. narinari* (Janse et al*.,* 2013) and the thornback ray, *Raja clavata* (Linnaeus, 1758) (Chevolot et al., 2007).

Pregnancy

A conclusive diagnosis of pregnancy in *A. narinari*, based on external and behavioral observations alone, is difficult. Once mating behavior has been observed, or if pregnancy is suspected, the female should be monitored closely for any physical or behavioral changes, such as increased food intake, abdominal swelling or changes in swimming behavior. Depending on the size of the pregnant female, the size of individual fetuses, and litter as a whole, the abdomen may or may not become visibly enlarged. The first observable sign of pregnancy may be swelling over the anterior section of the visceral cavity on the ventrum, growing to encompass the entire visceral cavity as pregnancy progresses. Approximately one month before parturition, pregnancy may be visible on the dorsal surface of the animal. A week before pupping, swelling on the ventrum of a gravid female may migrate caudally indicating that parturition is near (Janse, unpublished results). When multiple fetuses are present, the abdomen may periodically show distinct, separate bulges (Janse et al., 2010).

In some cases, obvious signs of pregnancy are absent. Facilities that maintain breeding *A. narinari* should consider regular examinations using ultrasound to monitor individual reproductive cycles. An enlarged, fluid-filled uterus, "swaying" trophonemata, and fetal movement and heartbeat, can all be indicators of pregnancy (DiRocco, personal communication) (Figures 3 and 4). More detailed information on elasmobranch ultrasonography can be found in Stetter (2004).

Gestation

The gestation period of *A. narinari* is difficult to determine, as the dates of successful copulation and fertilization are uncertain. In this study,

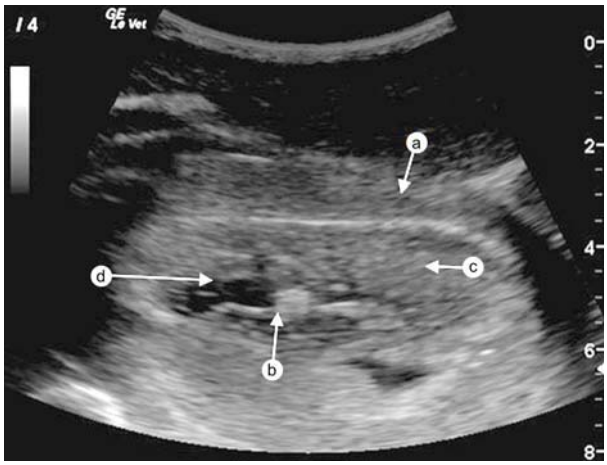

Figure 3. Ultrasound image of a spotted eagle ray, *Aetobatus narinari* (Euphrasen, 1790), fetus *in utero*. Notable structures include: (a) uterine wall, (b) fetus, (c) trophonemata, and (d) histotroph. Photo: courtesy of SeaWorld Orlando veterinary department.

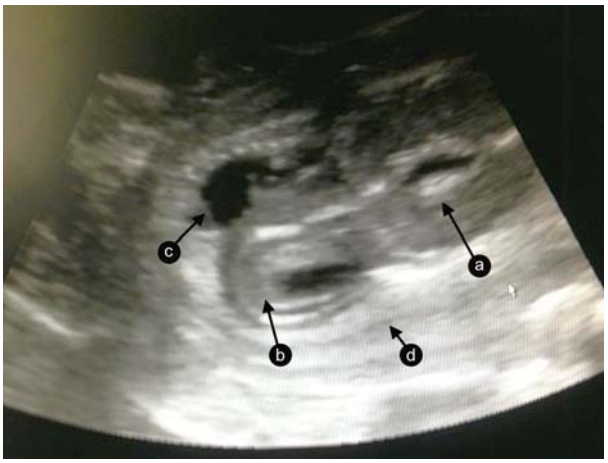

Figure 4. Ultrasound image of a late-term spotted eagle ray, *Aetobatus narinari* (Euphrasen, 1790), fetus *in utero*. Notable structures include: (a) fetal oral cavity, (b) fetal pectoral fin, (c) histotroph, and (d) trophonemata. Photo: courtesy of SeaWorld Orlando veterinary department.

breeding interval was used as an index of "gestation" as, in many cases, copulation was observed immediately following parturition. This phenomenon is not uncommon in aquaria, whereas in the wild there may be more of a gap between parturition and breeding (Henningsen et al., 2004). Various observations indicate that the reproductive cycle of *A. narinari* is continuous. These observations include: regular observations of mating behavior following parturition (Uchida et al., 1990), vitellogenesis proceeding in parallel with gestation, and mature oocytes being found in gravid and recently gravid females (Schluessel et al., 2010). In addition, the co-existence of mature ova and developing young can be found in other species of batoids, including the ocellated eagle ray, *Aetobatus ocellatus* (Kuhl, 1823), indicating there is no rest period between reproductive cycles in these species (Devadoss, 1998; Tagliafico et al., 2012).

Based on observations in aquaria, intraspecific gestation periods can be wide-ranging, possibly due to differential exhibit temperatures (Mahon et al., unpublished results; Schluessel et al., 2010), sperm storage and "over-gestation" (Henningsen et al., 2004).

Table 1. Eagle ray, *Aetobatus narinari* (Euphrasen, 1790), gestation times and exhibit water temperatures, for aquaria where successful pregnancies have been recorded. Data sorted by shortest to longest gestation time.

Aquarium name	Gestation period (days)	Water temperature (°C)
Underwater World Singapore	180 - 188	28.1 - 30.1
SeaWorld San Antonio	157 - 336	24 - 26
Discovery Cove	225 - 375+	23.5 - 26
Georgia Aquarium	~300	24.5
Burger's Zoo	239 - 435	25
Disney's The Seas	280 - 421	24 - 25
Okinawa Expo Aquarium	331 - 377	19.8 - 29.4
Ripley's Aquarium of the Smokies	328 - 399	23.5 - 24.5

Aquarium water temperature ranges and gestation times where *A. narinari* pregnancies were recorded have been summarized in Table 1. The shortest gestation range (180 - 188 days) was recorded in the aquarium (Underwater World Singapore) with the highest exhibit temperature range (28.1 - 30.1°C). Whereas, the longest gestation range (328 - 399 days) was recorded at the aquarium (Ripley's Aquarium of the Smokies) with the lowest exhibit temperature range (23.5 - 24.5°C).

Sperm storage of up to two years has been documented in some elasmobranch species (Henningson et al., 2004). A female *A. narinari* with a history of multiple successful parturitions, previously exhibiting a gestation period as short as 217 days, gave birth 375 days after the only male had been removed from the exhibit (Swider, unpublished results). This case is suggestive of long-term sperm storage. However, parthenogenesis must also be considered, as this mode of reproduction is more widespread in elasmobranchs than previously thought and has been observed in *A. narinari* (Chapman et al., 2007; Chapman et al., 2008; Robinson et al., 2011; Fields et al., 2015; Harmon et al., 2016). Mean *A. narinari* gestation time (or breeding interval) for surveyed aquaria was 285 ± 61 days (range: 157 - 435 days).

Parturition

A. narinari parturition has rarely been observed in aquaria. However, a number of near-term indications have been reported in the weeks leading up to parturition. These signs have included thickened or swollen pelvic fins, an enlarged abdominal area that has shifted towards the cloaca and dilation of the cloaca itself (Zimmerman, unpublished results). Other signs of impending birth included erratic swimming behavior, slower swimming, body twitches, decreased food intake (Zimmerman, unpublished results), increased attention from males (Uchida et al., 1990; Kamerman, unpublished results) and observable movement of young inside the female (Janse, unpublished results).

Janse et al. (2010) observed contractions, followed by accelerated swimming and rapid ninety-degree turns by a female *A. narinari* during parturition, presumably to aid the birthing process. During the birthing event, it took a single pup 14 minutes to emerge (Janse et al., 2010). During another reported parturition, Uchida et al. (1990) observed two pups born one minute apart. One of the pups was born head first and the other tail first. After parturition, the abdomen of female *A. narinari* has been observed to appear concave (Uchida et al., 1990; Zimmerman, unpublished results).

It has been suggested that parturition occurs seasonally in wild *A. narinari* (Tagliafico et al., 2012). The reproductive cycle of elasmobranchs in aquaria may differ from that of wild conspecifics for a variety of reasons, including the constant supply of food, water temperature ranges, the presence or absence of environmental cues, and other abiotic factors (Henningsen et al., 2004; Janse and Schrama, 2010). Recorded breeding and parturition of *A. narinari* in aquaria occurred during all months of the year.

Pups

A. narinari pups were first described in the literature in 1914 (Gudger, 1914). *In situ* studies report full-term *A. narinari* pups to have a DW of 17 - 57 cm (Bigelow and Schroeder, 1953; Schluessel et al., 2010). The wide range of pup DW reported could be due to experimental techniques causing premature delivery or fetal abortion, resulting in the inclusion of undersized specimens in the data set (Gudger, 1914; Schluessel et al., 2010). Mean size of *A. narinari* pups born in aquaria was 44.7 ± 5.1 cm DW (range: 35.5 - 58.0 cm DW), with a BM of 0.97 - 3.8 kg (range: 1.9 ± 0.7 kg BM). The weight of wild *A. narinari* pups is not well documented.

Litter size in wild *A. narinari* typically ranges from one to four pups, but a litter of ten pups has been documented (Bigelow and Schroeder, 1953; Schluessel et al., 2010; Tagliafico et al*.,* 2012). Tagliafico et al. (2012) studied wild caught *A. narinari* in Venezuela and reported that the DW of the mother did not correlate with litter size. Litter size in surveyed aquaria ranged from one to four pups, with a mean of 1.8 ± 0.6 pups per litter. No relationship was observed between female size and litter size, or the size of individual pups. Littermates were typically homogeneous in size, although two facilities did report a clear difference in pup size from the same female. In general, a solitary pup was larger than pups from multiple births.

LOOKING AHEAD

The future of *A. narinari* breeding programs will rely heavily on the careful documentation of factors that contribute to successful pregnancies.

The survey provided an invaluable opportunity to gather information on exhibit size, sex ratios, sexual maturity, gestation times, pup sizes and the behavior of near-term gravid females, which can serve as a baseline for establishing future breeding programs, as well as spark potential future investigations. However, more information is needed to further identify and fine-tune factors conducive to *A. narinari* reproduction in aquaria.

An important strand of future investigation should include the collection of data from aquaria with sexually mature, mixed-gender populations of *A. narinari* that have not had successful pregnancies. This information can then be compared to data from aquaria with successfully breeding *A. narinari* populations.

Extraction and assessment of hormone levels will aid an understanding of the *A. narinari* reproductive cycle. Previous hormone studies with other elasmobranchs (Manire et al., 1995) may provide a structural framework for these investigations. Hormone studies could provide better early pregnancy determination, clarify gestation durations and provide a predictor of parturition date.

Future research in the area of assisted reproduction (e.g., artificial insemination; AI) should also be explored. Although AI techniques have been successfully employed in many taxa in zoological parks, it is relatively new to elasmobranchs.

Behavioral husbandry training should be further developed and used to reduce excessive handling during physical examinations and medical procedures. If copulation is observed, or pregnancy is suspected, monitoring the gravid female using ultrasonography will be a valuable clinical tool, allowing assessment of fetus health and development.

With increased pressure on wild elasmobranch stocks, and rapidly changing legislation and permitting requirements for many elasmobranch species (Choromanski, 2004), there is a pressing need for coherent aquarium breeding programs. The aesthetic beauty and popularity of *A. narinari* make it a great candidate species to represent the plight of wild elasmobranchs, as well as a prime candidate for a structured multi-institution breeding and research program. A collaborative *A. narinari* breeding program is already in place in Europe and the Middle East, and is in its early stages in North America (Kinsler, personal communication). Regional and interregional cooperation is necessary to increase the success of a breeding program for this species. Collaborative efforts should include genetic management across the entire multi-institutional meta-population, as well as research on AI, nutrition and husbandry best practice.

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Chapter 43

Blacktip reef shark reproduction and neonate survivorship in public aquaria

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Abstract: *Ex situ* captive breeding programs play an important role in endangered species conservation by providing insurance against extinction in the wild, providing a source of individuals for reintroduction, and providing insight into the behavior and reproductive biology of a species, which can aid *in situ* conservation work (Primack, 2006). Between 2007 and 2014, breeding of blacktip reef sharks, *Carcharhinus melanopterus* (Quoy & Gaimard, 1824), was confirmed at 17 aquaria, predominantly European, resulting in 36 litters and 105 pups. Mean gestation time for *C. melanopterus* was 12.2 months, in the range of 7 - 15 months. A high proportion of *C. melanopterus* pups were stillborn (43.8%) and an additional 19.1% died within a week of parturition, leaving a survivorship of 37.1%. Many of the pups (44.8%) were born on exhibit, with a 42.6% survivorship. The balance of the litters were born in a separate quarantine tank (14.3%), with a 33.3% survivorship, in a floating cage within the main exhibit (25.7%), with a 74.1% survivorship, and in a pool connected to the main exhibit (15.2%), with a 87.5% survivorship.

INTRODUCTION

Conservation efforts aimed at maximizing long-term species persistence are commonly directed towards *in situ* (i.e., wild) populations. Increasingly, *ex situ* studies and aquarium breeding programs are informing and assisting *in situ* conservation efforts as the resulting life history data aids management decisions by conservation biologists in the wild (Simpfendorfer et al., 2011). Research on aquarium populations can provide insight into the behavior and reproductive biology of a species that, in-turn, can result in the development of new, or increasingly efficient, *in situ* conservation strategies (Primack, 2006). In extremis, *ex situ* breeding programs can play a role in endangered species conservation by providing insurance against extinction in the wild and a possible source of individuals for population augmentation or reintroduction programs.

There has been a worldwide decline in many elasmobranch populations (Cheung et al., 2005),

largely related to overfishing, by-catch and habitat destruction. Elasmobranchs are of particular conservation concern, due to their slow growth rates, late age at maturity, low fecundity and low reproductive output (Holden, 1974; Cailliet and Goldman, 2004). Despite the collective decline of elasmobranchs, public aquaria have historically lagged behind zoos in coordinating managed breeding programs. Although this discrepancy is beginning to be addressed, regional and international studbooks, and breeding programs for elasmobranchs, have only recently been established. Studbooks have been developed for nine elasmobranch species in Europe, through the European Association of Zoos and Aquaria (EAZA) (Janse, personal communication), and ten elasmobranch species in North America, through the American Association of Zoos and Aquariums (AZA; www1). Thus, public aquaria are increasingly in a position to provide information in support of *in situ* conservation efforts.

Blacktip reef sharks, *Carcharhinus melanopterus* (Quoy & Gaimard, 1824), are displayed in public aquaria worldwide, due to their small size, ready availability, familiarity with shallow waters, ability to navigate reef-like structures and adaptability to artificial environments (Compagno et al., 2005). *C. melanopterus* are found throughout the Pacific and Indian Oceans, where they inhabit shallow waters surrounding islands and atolls, and are an abundant meso-predator in these environments (Compagno et al., 2005). Population declines, primarily due to overfishing, have been observed across the natural range of *C. melanopterus* (Stevens, 1984; Chin et al., 2012), prompting the IUCN to classify the species as “near threatened” (Heupel, 2009).

In this chapter we examine conditions surrounding the reproduction of *C. melanopterus* in European public aquaria, with a particular emphasis on offspring survivorship.

METHODS

The European studbook for *C. melanopterus* is an extended monitoring program coordinated by EAZA. The first and second editions of the studbook (available from the authors on request) covered a period from 2007 to 2014. Throughout this time, annual questionnaires were circulated to collect data on reproducing *C. melanopterus*. Videos and photographs, documenting mating behavior and parturition, were also requested, to supplement quantitative data sets. Although this effort was predominantly directed toward European aquaria, it included participants from other continents (i.e., North America and Australia).

Parturition strategies

Two main strategies were employed by institutions for managing pregnant *C. melanopterus* in the lead-up to parturition:

***1. ‘Exhibit’ parturition*:** whereby no intervention was used to isolate pregnant *C. melanopterus* and therefore animals were not handled prior to pupping. Parturition took place within the main exhibit tank (Figure 1), surrounded by tank mates, including potential predators;

***2. ‘Isolation’ parturition*:** whereby pregnant *C. melanopterus* were isolated from the main exhibit to avoid intra- and interspecific predation or aggression. ‘Isolation’ parturition was achieved in

Figure 1. A blacktip reef shark, *Carcharhinus melanopterus* (Quoy & Gaimard, 1824), giving birth within the main exhibit at Timmendorfer SEA LIFE Centre, Germany.

one of three ways: (i) 'quarantine' parturition, whereby the gravid female was moved from the main exhibit into a separate quarantine tank elsewhere in the aquarium; (ii) 'cage' parturition, whereby the shark was isolated in a cage (usually floating) within the main exhibit, minimizing handling stress; or (iii) 'connected pool' parturition, whereby the shark was isolated in a tank connected directly to the main exhibit, usually with a movable barrier or gate, eliminating handling stress.

Using separate generalized linear mixed models (GLMM), with a binomial error distribution and a logit link function, we analyzed the relationship between pup survivorship and parturition strategy. To assess pup survival, we coded live pups as 'success' and stillborn pups as 'failure'. We then analyzed the data using two alternative approaches. First, we examined ('Analysis 1') pup survival between females giving birth in the main 'exhibit' to those giving birth in 'isolation'. Second, we split up the 'isolation' parturition category and examined ('Analysis 2') pup survival between females giving birth in the main 'exhibit', in 'quarantine', in a 'cage', or in a 'connecting pool'. The site where births took place (i.e., the facility) was included as a random effect in the statistical model, accounting for potential differences in pup survivorship due to different husbandry practices or varying conditions between different locations.

GLMMs were performed using the *glmer* function in the *lme4* package (Bates et al., 2014) in RStudio v3.1.2 (www2). Type II Wald chi-squared tests were used to report significant values from *glmer* models. Differences between two parturition strategies were assessed using a Tukey test, through the *glht* function in the package *multcomp* (Hothorn et al., 2008). We used the *overdisp_fun* function to assess over-dispersion in the models. The model for Analysis 1 was over-dispersed and could not be corrected, so the results should be interpreted with caution. The model developed for Analysis 2 was not over-dispersed and was more robust.

RESULTS AND DISCUSSION

Demographics and breeding

According to the EAZA studbook, at the time of writing, European aquaria have maintained a total of 223 *C. melanopterus*, consisting of 94 males and 129 females. All animals originated from the

Table 1. Blacktip reef sharks, *Carcharhinus melanopterus* (Quoy & Gaimard, 1824), born in European aquaria between 2007 and 2014, showing pup survivorship. Non-European aquaria have been included at the bottom of the table. (+) indicates that surviving pups had not yet reached one year of age at the time of writing.

Aquarium	No. of Litters	No. of pups	Pups born live	Pups surviving > 1 week	Pups surviving > 1 year
Aquazoo Löbbecke Museum	3	5	5		
Dierenpark Emmen	3	3	3	3	3
Kattegatcentret	1	1	1		
L'Aquarium de Paris	3	6	3	3	(+)
Legoland Windsor Resort	1	2			
Sea Life Centre, Billund	2	7	1		
Sea Life Centre, Günzberg	2	2	1	1	
Sea Life Centre, Konstanz	1	4	4		
Sea Life Centre, Oberhausen	5	19	12	10	3 (+)
Sea Life Centre, Scheveningen	2	6	5	2	2
Sea Life Centre, Speyer	1	6			
Sea Life Centre, Timmendorfer	3	8	2	2	(+)
Sea Life Centre, Weymouth	1	2			
Zoologischer Garten Berlin	1	2			
Las Vegas Aquarium	1	4	4	2	2
Underwater World Sea Life Aquarium	1	4	4	3	(+)
Shedd Chicago	5	24	14	13	12
Total	**36**	**105**	**59**	**39**	**22**
Percentage of total		**100.0%**	**56.2%**	**37.1%**	**21.0%**

Figure 2. Blacktip reef sharks, *Carcharhinus melanopterus* (Quoy & Gaimard, 1824), mating at the Birmingham National SEA LIFE Centre, UK.

wild, with the exception of six aquarium-bred pups. The majority of *C. melanopterus* were 8 - 11 years of age. The oldest *C. melanopterus* on record was a female at the Leipzig Zoo (Germany), estimated to be 26 years of age. A total of 62 responses were received to the annual EAZA studbook questionnaire, including responses from aquaria outside Europe. Breeding was confirmed at 17 sites, resulting in 36 litters comprised of 105 pups (Table 1).

Copulation

Observed copulation, or evidence of copulation, is useful to predict future date of parturition. Respondents to the EAZA studbook questionnaire reported many observations of *C. melanopterus* copulation events in aquaria. Staff frequently observed and captured images and film (Österberg, personal communication; Robson, personal communication) of *C. melanopterus* copulation events (Figure 2). When copulation was not directly witnessed, wounds around the pectoral fins, dorsal fins and abdomen evidenced recent copulation attempts (Figure 3).

Figure 3. Visual indications of blacktip reef shark, *Carcharhinus melanopterus* (Quoy & Gaimard, 1824), copulation at the Oberhausen SEA LIFE Centre, Germany. Note the lacerations around the abdomen, the pectoral fins and anal fins.

Gestation

Mean gestation time for *C. melanopterus*, as reported by questionnaire respondents, was 12.2 months, in the range of 7 - 15 months. In many cases, mating was not directly observed, but fresh lacerations on a female *C. melanopterus* were used as an estimate of copulation date and the beginning of gestation. Temperature is likely to be an important determining factor for *C. melanopterus* gestation time in aquaria. Comparative *in situ* evidence suggests a gestation period of ~10 months (Stevens, 1984; Porcher, 2005). Early signs of pregnancy are subtle in wild conspecifics, abdominal distension only becoming apparent after 3 - 4 months gestation (Figure 4a). Latter stages of pregnancy are more apparent (Figure 4b), females appearing more obviously obese (Porcher, 2005). In the weeks shortly before parturition, changes may be observed in the shape of the abdomen, just in front of the pelvic fins, which become squared-off and rectangular (Figure 4c).

Observations of near-term *C. melanopterus* in aquaria indicate that the pelvic fins may become slightly flared, especially in the days immediately prepartum (James, personal communication). In addition, a near-term female *C. melanopterus* may show the following signs:

1. Swimming with the head slightly elevated above the position of the caudal fin (Wille, personal communication; Watson, personal communication);
2. Dilation of the cloaca (Porcher, 2005; Stewart, personal communication);
3. Swimming slowly, with mouth agape (Wille, personal communication); and, occasionally
4. Fasting (Watson, personal communication; Stewart, personal communication).

Parturition

Female *C. melanopterus* in the wild are known to migrate to inshore waters to pup, with the offspring remaining in shallow water to avoid predation (Taylor, 1993). Papastamatiou et al. (2009) found evidence of *C. melanopterus* sex segregation at the Palmyra Atoll (Pacific Ocean), with a significantly higher number of female sharks inhabiting shallow sand flats than male conspecifics. It is rare for public aquaria to simulate shallow-water pupping grounds and, in

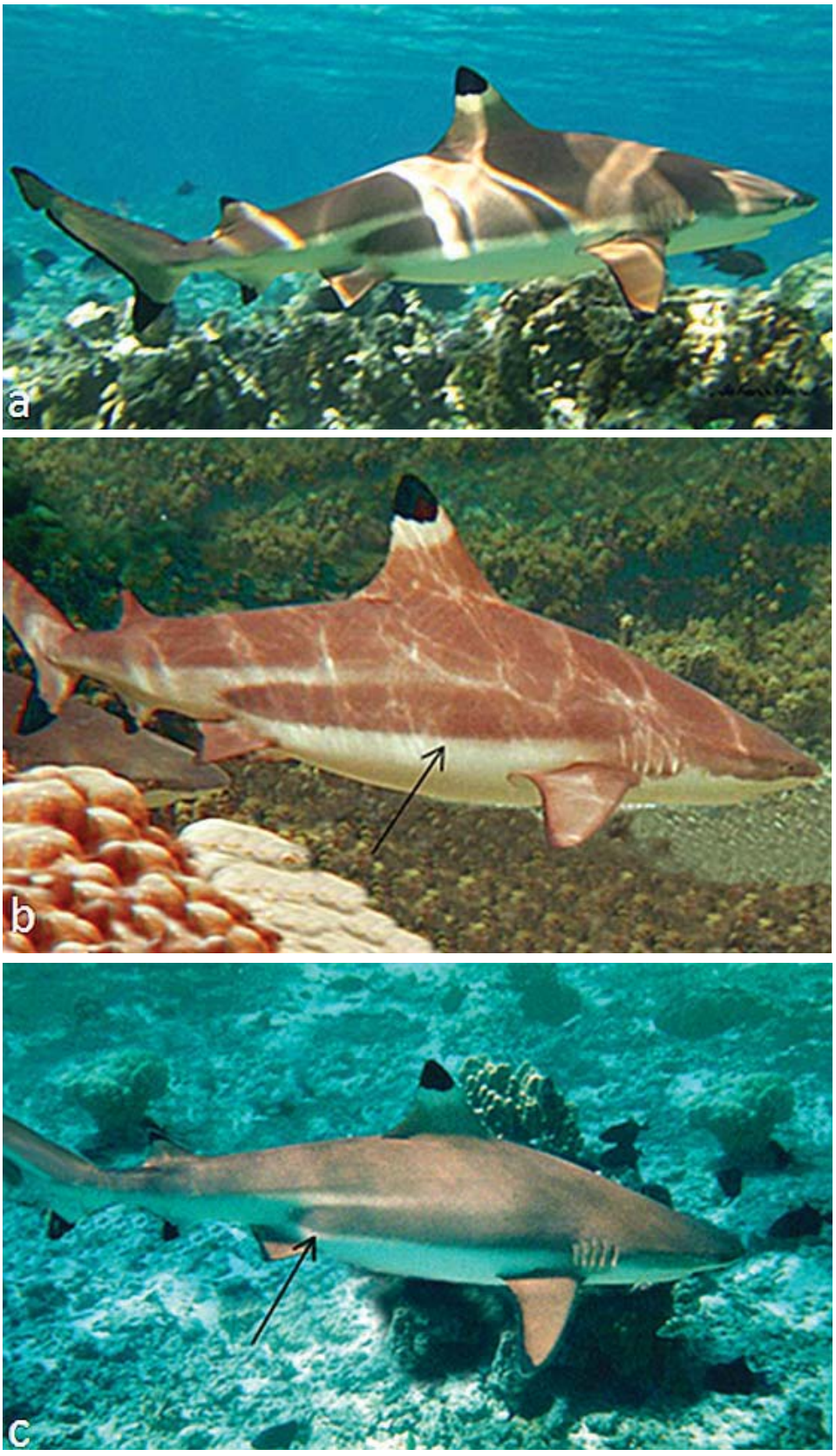

Figure 4. Various stages of pregnancy in wild blacktip reef sharks, *Carcharhinus melanopterus* (Quoy & Gaimard, 1824), in the waters off French Polynesia, showing (a) a female early in pregnancy; (b) obesity during mid-term pregnancy; and (c) bulging at the body wall, anterior to the pelvic fins, a few days prepartum. Figures after Porcher, 2005.

addition, gravid females are frequently surrounded by an abundance of potential predators or aggressors. This atypical pupping situation may account for the observed high proportion (i.e., 43.8%) of 'stillborn' pups (Table 1). However, it should also be noted that the incidence of stillborn *C. melanopterus* in the wild is unknown, so a direct *in situ* to *ex situ* comparison was not possible. An additional 19.1% of pups had died within a week of parturition, leaving a survivorship of 37.1%. After one year, only 21.0% of pups (n = 19) remained. It should be noted that predation by tank mates was excluded from this analysis. Of the 36 recorded parturition events, six of the gravid females died during, or shortly after, pupping.

Parturition strategy and pup survivorship

Parturition strategy appeared to influence *C. melanopterus* pup survivorship (Figure 5). Many of the pups (44.8%) were born using the 'exhibit' parturition strategy with a 42.6% survivorship. This parturition strategy was common, as many aquaria wished to avoid handling gravid females or did not have isolation facilities. The balance of the litters were born using the 'isolation' parturition strategy, broken down as follows: 14.3% in 'quarantine', with a 33.3% survivorship; 25.7% in a 'cage', with a 74.1% survivorship; and 15.2% in a 'connected pool', with a 87.5% survivorship (Figure 5).

When 'exhibit' parturition was compared to 'isolation' parturition (Analysis 1) the result was near, but not quite, significant ($X^2 = 2.79$, d.f. = 1, $p = 0.09$). When pup survivorship was compared between 'exhibit', 'quarantine', 'cage' and 'connected pool' parturition (Analysis 2), a significant difference was found ($X^2 = 13.36$, d.f. = 3, $p < 0.01$). Post hoc comparison revealed that pup survivorship was significantly higher when parturition took place in a 'connected pool' rather than parturition in a separate 'quarantine' tank ($z = -3.18$, $p < 0.01$). No significant differences were found between any of the other parturition strategies. Care should be exercised when interpreting these results as small data sets, with an over-representation from larger institutions (e.g., 93% of pups born using the 'connected pool' strategy were born at the Shedd Aquarium, Chicago, USA), coupled with site-specific husbandry techniques and environmental parameters, may have skewed the results. Husbandry staff capabilities and experience, the diet of gravid females, water quality and water temperature, and tank sizes, varied considerably between different aquaria and may have further impacted pup survivorship.

In addition to stillbirth and neonate death events, nine litters were recorded to be lost to predation by tank mates when the 'exhibit' parturition strategy was employed. The total number of pups preyed upon is unknown, as entire parturition events were not always witnessed. As gravid females are not easily identified, it is suspected that even more pups may have been lost to predation but were not recorded. Clearly, if 'exhibit' parturition is the chosen pupping strategy, pups should be removed from the exhibit quickly to avoid predation by tank mates.

CONCLUSIONS

Our investigation concluded that there is a substantial risk to leaving gravid females in an exhibit with other predatory fishes. Isolating pregnant females negates this risk, although, in some rare cases, the mother may prey on her own pups. Taking into account the high risk of predation of live pups, we recommend 'isolation' parturition to increase the survivorship of live-born pups and, given the caveats listed above, the 'connected pool' parturition strategy appears to be the best option. In the last two years, 64% of *C. melanopterus* parturition events were conducted using the 'isolation' strategy, as aquaria have striven to minimize pup losses due to predation. 'Connected pools' and 'cages' provided the highest rates of pup survival at birth, at 87.5% and 74.1%, respectively. A deeper investigation of a larger data set should be undertaken to determine various site-specific differences between aquaria, which are likely to be important in determining *C. melanopterus* pup survivorship.

We anticipate more reproduction of *C. melanopterus* in aquaria in coming years. Accurate record keeping and sharing of information is key to the future success of planned breeding programs, and to achieving the ultimate goal of a sustainable *C. melanopterus* population within the zoo and public aquarium community.

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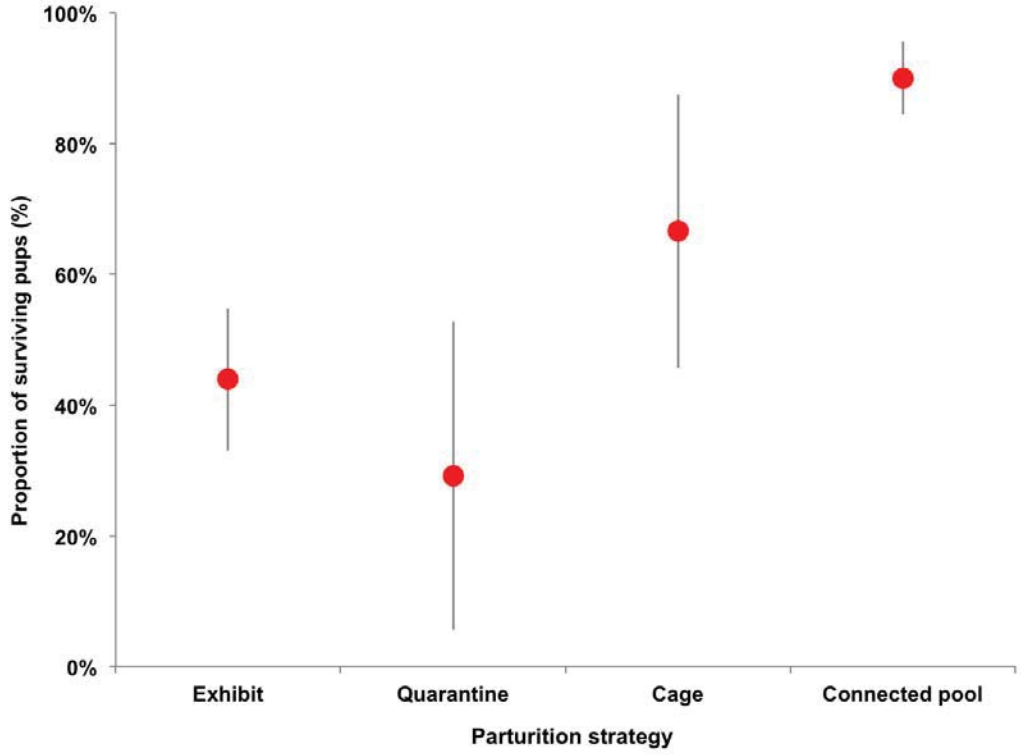

Figure 5. A comparison of parturition strategies for gravid blacktip reef sharks, *Carcharhinus melanopterus* (Quoy & Gaimard, 1824), in public aquaria. Grey bars represent standard deviations.

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The Elasmobranch Husbandry Manual II: Recent Advances in the Care of Sharks, Rays and their Relatives, pages 451-458.

Chapter 44

Fecundity, egg capsule size and neonate morphometrics of big skate, *Beringraja binoculata* (Girard, 1855)

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Abstract: Oviparous elasmobranchs tend to be prolific producers, especially when well-managed within controlled environments like public aquaria. Egg capsules were collected from three female big skates, *Beringraja binoculata* (Girard, 1855), maintained in the "Beyond the Golden Gate" exhibit at the Aquarium of the Bay (San Francisco, California, USA), during 2000 - 2002. Egg capsules were deposited by female *B. binoculata* as small as 91 cm total length (TL), substantially smaller than predicted size-at-maturity referenced in the literature (Hitz, 1964; Martin and Zorzi, 1993; Zeiner and Wolfe, 1993; Ebert et al., 2008). Mean (± SE) oviposition rate for the three skates was 6.2 ± 0.3 capsule pairs per month. Egg capsules ranged from 15.5 - 19.5 cm capsule total length (CTL), a much smaller size than previously reported by DeLacey and Chapman (1935) and Eschmeyer et al. (1983). Mean neonate length at hatching was 14.9 ± 0.9 cm TL, shorter than previously reported by DeLacy and Chapman (1935) and Ebert et al. (2008). It is possible that the stable and clement conditions within the aquarium exhibit, including, excellent water quality, abundant food and regular encounters with potential mates, may have led to early maturity and reproductive success, as well as smaller egg capsules and neonates, when compared to wild conspecifics.

INTRODUCTION

Big skates, *Beringraja binoculata* (Girard 1855), range from Alaska to Baja California, inhabiting shallow inshore waters and deep benthic habitats (Eschmeyer et al., 1983; Ebert, 2003). *B. binoculata* are oviparous, laying capsules in pairs over soft substrate, at regular intervals, for several months. Oviparous elasmobranchs typically insert one ovum per capsule, but *B. binoculata* is unique, depositing multiple ova within a single capsule. Based on this unique reproductive characteristic, Ishihara et al. (2012) proposed a new genus for the species, *Beringraja* (formerly *Raja*).

While it is generally accepted that most chondrichthyans are relatively long-lived and mature late in life, it is also acknowledged that methods for age and growth modeling require more standardization (Cailliet et al., 2006). Information relating to age and/or size at first reproduction allows development of effective management strategies (Goldman et al., 2012). There is a growing body of literature estimating size and age-at-maturity for *B. binoculata* in several eastern Pacific coastal populations: central California (Martin and Zorzi, 1993), Monterey Bay (Zeiner and Wolf, 1993), Gulf of Alaska (Gburski et al., 2007; Ebert et al., 2008), and British Columbia (McFarlane and King, 2006; King and McFarlane, 2010). The controlled environments of public aquaria offer an opportunity to track the reproductive activity of individual *B. binoculata* and shed light on age-at-maturity, fecundity and egg production rates.

MATERIALS and METHODS

Reproductive data from three female *B. binoculata* were recorded at the Aquarium of the Bay (San

Francisco, USA), from August 2000 to March 2002.

Animal acquisition

The three female *B. binoculata* used in this study were captured using otter trawl, or hook and line (i.e., heavily weighted, barbless hooks with steel leaders), in San Francisco Bay (California, USA). The three female skates #1, #2 and #3 had a total length (TL) of 91 cm, 94 cm and 120 cm TL, respectively.

Specimens were transported to the Aquarium in a 1.3 m^3 live well aboard the 9.75 m marine vessel *F/V Debbie K*. On arrival, the *B. binoculata* were given a gross physical examination, including an assessment of any injuries related to collection and the manual removal of external parasites.

The exhibit

The skates were introduced into the "Beyond the Golden Gate" exhibit at the Aquarium of the Bay, a 1,300 m^3 community exhibit with a sandy bottom and rock outcroppings. The exhibit was supplied with filtered water from San Francisco Bay on a semi-open system.

The animal population comprised a variety of local elasmobranch species, including broadnose sevengill sharks, *Notorynchus cepedianus* (Péron, 1807), leopard sharks, *Triakis semi-fasciata* (Girard, 1855), piked dogfish, *Squalus acanthias* (Linnaeus, 1758), brown smooth-hounds, *Mustelus henlei* (Gill, 1863), Pacific angel sharks, *Squatina californica* (Ayres, 1859), bat eagle rays, *Myliobatis californicus* (Gill, 1865), Pacific electric rays, *Torpedo californica* (Ayres, 1855), and longnose skates, *Raja rhina* (Jordan & Gilbert, 1880). Several large teleost species were also exhibited, including Chinook salmon, *Oncorhynchus tshawytscha* (Walbaum, 1792), white sturgeon, *Acipenser transmontanus* (Richardson, 1836), striped bass, *Morone saxatilis* (Walbaum, 1792), and several species of rockfish (*Sebastes* spp.).

Food and feeding

The skates, along with most of the exhibit inhabitants, were fed three times per week by broadcast feeding, with opalescent inshore squid, *Doryteuthis opalescens* (Berry, 1911), South American pilchard, *Sardinops sagax* (Jenyns, 1842) and capelin, *Mallotus villosus* (Müller,

Figure 1. Egg capsules from three big skates, *Beringraja binoculata* (Girard, 1855), showing identification tag placement. Note that the physical characteristics of each egg capsule are unique to the parent. Capsules, from left to right: Female #3 (TL: 120.0 cm), Female #2 (TL: 94.0 cm) and Female #1 (TL: 91.0 cm).

1776). Approximately half of the total food ration was supplemented with vitamins (Sea-Tabs, Pacific Research Laboratories Inc., San Diego, USA).

Egg capsules

Between 2000 and 2002, husbandry personnel collected any deposited egg capsules on a daily basis. If *B. binoculata* egg capsules were encountered they were removed from the main exhibit and placed into a floating basket within an adjoining husbandry pool.

Each egg capsule was cataloged and assigned an identification number (ID#). The ID# was written on colored shrink tubing, slipped over a retail clothing tag and inserted into the right anterior horn keel of the egg capsule (as per Ebert and Davis, 2007). Tags were attached using a standard retail tagging gun (Avery Dennison, Rancho Cucamonga, California, USA) with the ID# remaining visible on the flat side of the capsule (Figure 1). Capsules were candled with a flashlight to determine the number of egg yolks. The capsule total length (CTL) was measured to the nearest 0.1 cm, from the outermost tip of the anterior horns to the outermost tip of the posterior horns.

All capsules were placed into stacked tank shelving systems that were plumbed into the main exhibit life support system (Figure 2). The shelving systems were 180 cm high x 61 cm wide x 76 cm long. Each system accommodated four sliding bins, 20 cm high x 40 cm wide x 60 cm long. Each bin had its own water supply and contained ~38 L of seawater. When space was limited capsules were oriented on their lateral keels with attachment fibers facing towards the bottom. As new egg capsules were added they were leaned alongside previously cataloged capsules, much like books on a shelf.

Figure 2. Big skate, *Beringraja binoculata* (Girard, 1855), egg capsule incubation and neonate holding system.

Incubation

Throughout egg capsule incubation, the status of the rack system was checked at least twice a day to ensure uninterrupted operation. Capsules were surveyed for condition and occasionally candled with a flashlight to determine development progress. The crowded conditions and the absence of a natural water scouring effect meant that occasionally egg capsules needed to be agitated gently with a toothbrush. This process served to facilitate natural degradation (softening) of the outer layers of the egg capsule and reduce buildup of detritus and naturally occurring bacterial assemblages, which were otherwise amplified by the crowded conditions.

If a decaying capsule was found, it was partially cut open to expose the embryos for closer examination. If viable embryo(s) were discovered and appeared reasonably well developed (i.e., at least “stage 4”, as described by Hitz, 1964), the expired material was removed, and the capsule was scrubbed clean and returned to the bin. If viable embryos had significant yolk reserves and appeared underdeveloped (i.e., “stage 3” or less, as described by Hitz, 1964), the cut flap was detached entirely, decaying material removed

(Figure 3), and a clear plastic sheet installed (Howard, 2002) to minimize disturbance for the remainder of the incubation period. In some cases viable embryos were removed from the capsule temporarily, to improve access and allow for thorough and complete cleaning. To accomplish this cleaning process, the capsule was transferred, while maintained in a horizontal position, to a tub containing clean seawater. While submerged, the viable embryos were transferred to a one-liter beaker using a ladle. The interior of the egg capsule was then thoroughly scrubbed to remove expired material and bacterial films. The cleaned egg capsule and beaker were transferred to a second tub filled with clean seawater and the embryos were returned to the capsule while remaining submerged. The cleaned egg capsule was then returned to the incubation bin, or placed on exhibit, for the remainder of incubation. If handled carefully, and the integrity of the yolk remained undisturbed, embryos receiving this treatment developed normally.

Eggs surplus to this study were shipped to other public aquaria or research labs, in accordance with accredited transfer policies. Data from neonates resulting from eggs transferred to other institutions, or from deceased embryos, were not included as part of this study.

Neonates

When a hatchling was found in one of the incubation bins, it was transferred to a neonate bin (empty of capsules) containing a thin layer of fine sand substrate. Neonates were measured and total length (TL) and disc width (DW) recorded. The capsules in the source bin were examined to determine from which egg the skate had hatched and the corresponding incubation time recorded.

Neonates were offered North pacific krill, *Euphausia pacifica* (Hansen, 1911) and finely diced Atlantic surf clam, *Spisula solidissima* (Dillwyn, 1817) tongue, soaked in liquid vitamins (Vita-fish™, Marine Enterprises International, LLC., Baltimore, Maryland, USA) three times a day. Substrate in the neonate tank was cleaned with a 'hydro-vac' each morning.

RESULTS and DISCUSSION

Female sexual maturity

Egg capsules were deposited by female *B. binoculata* as small as 91 cm TL. This TL is smaller than predicted size-at-maturity referenced in much of the literature. Martin and Zorzi (1993) estimated female *B. binoculata*, along the central California coast (including San Francisco Bay), to mature at sizes >130 cm TL. In a separate study, Ebert et al. (2008) reported the smallest sexually mature wild female *B. binoculata* to be 125.8 cm TL. However, McFarlane and King (2006) reported results corroborating the findings of the current study, estimating 50% of females to be sexually mature at 90 cm TL.

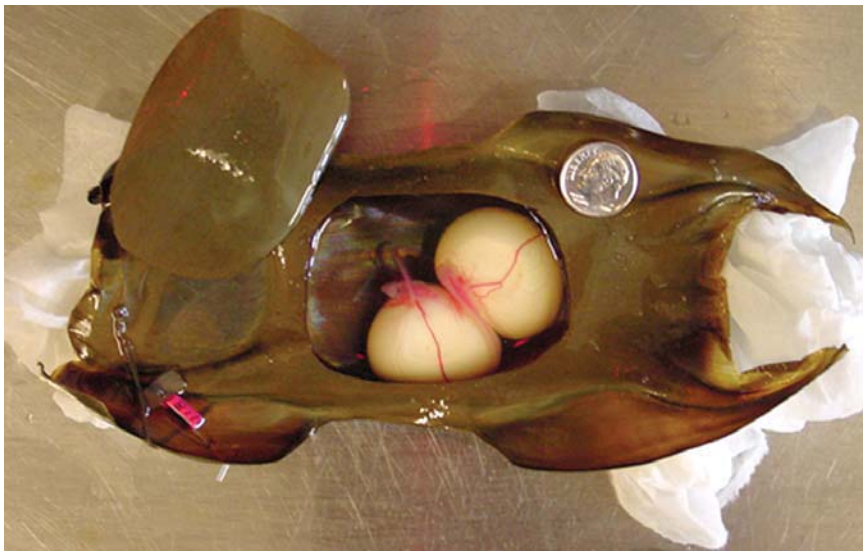

Figure 3. A big skate, *Beringraja binoculata* (Girard, 1855) egg capsule, cleaned and ready for window installation after the removal of an expired embryo.

The variable size-at-maturity for female *B. binoculata* in the literature emphasizes conclusions drawn by Cailliet et al. (2006), notably, that there needs to be consistency in the mechanisms used to produce estimates of age-at-maturity, size-at-maturity and longevity. Aside from methodology, there may be other factors (abiotic and biotic) that influence the observed size-at-maturity discrepancies. These could include, but are not limited to, challenging or clement environmental conditions, varying energy budgets, prey availability and nutrition, intra- and interspecific competition, the presence or absence of natural predators, and fishing pressures or other anthropogenic effects, all of which may affect reproductive biology and the onset of maturity *in situ*. It is possible that the stable and clement conditions within the aquarium exhibit, including excellent water quality, abundant food and regular encounters with potential mates, may have led to early maturity and reproductive success, compared to published data for wild conspecifics (Martin and Zorzi, 1993).

Egg-laying

The period of study (2000 - 2002) encompassed a complete egg-laying cycle for one of the skates (#2), lasting 308 days. During this time skate #2 laid 56 egg capsule pairs, or 5.6 pairs per month. Skate #2 then started a second egg-laying cycle, after a hiatus of approximately one month, from August 2001 until the end of the study period (March 2002). During the second cycle, skate #2 laid 43 egg capsule pairs, or 6.1 pairs per month. Skate #1 laid 43 egg capsule pairs from September 2001 to the end of the study period, at a rate of 7.2 egg capsule pairs per month, and skate #3 laid 53 egg capsule pairs from June 2001 to the end of the study period, at a rate of 5.9 egg capsule pairs per month. The mean (± SE) oviposition rate for the three skates was 6.2 ± 0.3 egg capsule pairs per month. As the exhibit was on a semi-open flow-through system, and subjected to seasonal temperature fluctuations, it is suggested that egg deposition in San Francisco Bay by wild *B. binoculata* may follow a similar annual pattern, starting in late summer and fall, a conclusion corroborated by Clemens and Wilby (1961).

Egg capsules

Egg capsule size appeared to be determined by female size (Figure 4). When multiple females were depositing egg capsules, it was relatively easy to attribute capsules to specific females through a variety of mechanisms.

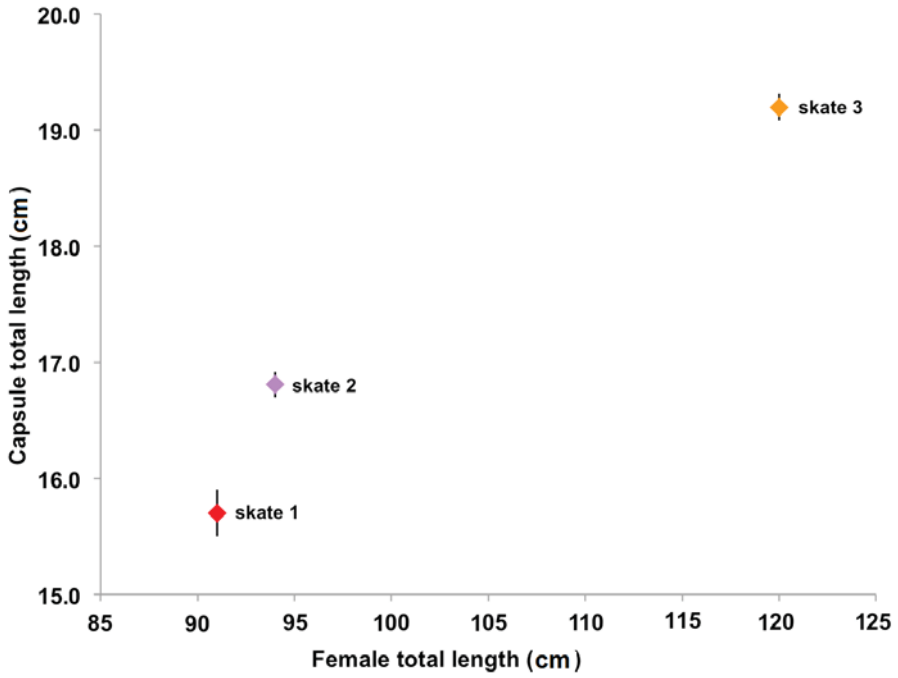

Figure 4. Big skate, *Beringraja binoculata* (Girard, 1855), mean egg capsule total length from three different females: #1 (TL: 91.0 cm), #2 (TL: 94.0 cm) and #3 (TL: 120.0 cm). Grey bars denote 95% confidence intervals. Skates #1, #2 and #3 laid 16, 99 and 44 egg capsules, respectively.

Capsules in the final stages of development, within the oviducal gland, could be observed as prominent bulges on the dorsal surface, anterior to the base of the tail, on each side of the midline of a gravid female (Luer and Gilbert, 1985). Furthermore, intervals between capsule depositions remained relatively constant for each female, allowing accurate prediction of oviposition. Finally, and most importantly, each female *B. binoculata* laid capsules with subtle but unique differences in shape, attributes that remained constant throughout the egg capsule-laying cycle (Figure 1).

Egg capsules laid by female *B. binoculata* in this study ranged from 15.5 - 19.5 cm CTL (Figure 4) much smaller than previously reported. DeLacey and Chapman (1935) sampled *B. binoculata* capsules from Puget Sound, which ranged in size from 26.5 - 30.5 cm TL (n = 20), and Hitz (1964) reported *B. binoculata* capsules ranging from 23.5 - 30.0 cm TL (n = 281). Eschmeyer et al. (1983) list *B. binoculata* capsules to be nearly 30.0 cm TL, and Ebert and Davis (2007) report capsules of 21.0 - 28.0 cm TL. It is important to note that the CTL measurement used in this study, the greatest TL of the capsule, is a different measurement to egg capsule length (ECL), measured from apron to apron, as originally described by Ishiyama (1958) and used by Ebert and Davis (2007). Had ECL been recorded in this study, instead of CTL, capsule TL measurements would have been even shorter than previously published.

Capsule size is directly related to female size in other skate species (Ishiyama and Ishihara, 1977; Templeman, 1982), so it is not surprising to find a similar pattern in *B. binoculata*. This finding further highlights the small TL and precociousness of the reproductive females in this study and may further reflect the Aquarium pre-conditions for the observed size-at-maturity described above. It should also be considered, however, that the absence of capsules < 20.0 cm TL reported in the literature may be an artifact of incomplete or biased sampling, whereby smaller mature wild *B. binoculata* have fewer opportunities to reproduce, or that smaller gravid females had reduced access to deposition grounds where *in situ* sampling occurred.

Incubation

Water temperature is likely to play a role in determining the rate of development and incubation times for *B. binoculata* embryos. Mean incubation time for egg capsules from skate #2 at 10°C and 13 - 15°C were 234.4 and 187 days, respectively. DeLacy and Chapman (1935) speculated that incubation duration for *B. binoculata* in Puget Sound (San Juan Islands, USA), where annual average sea

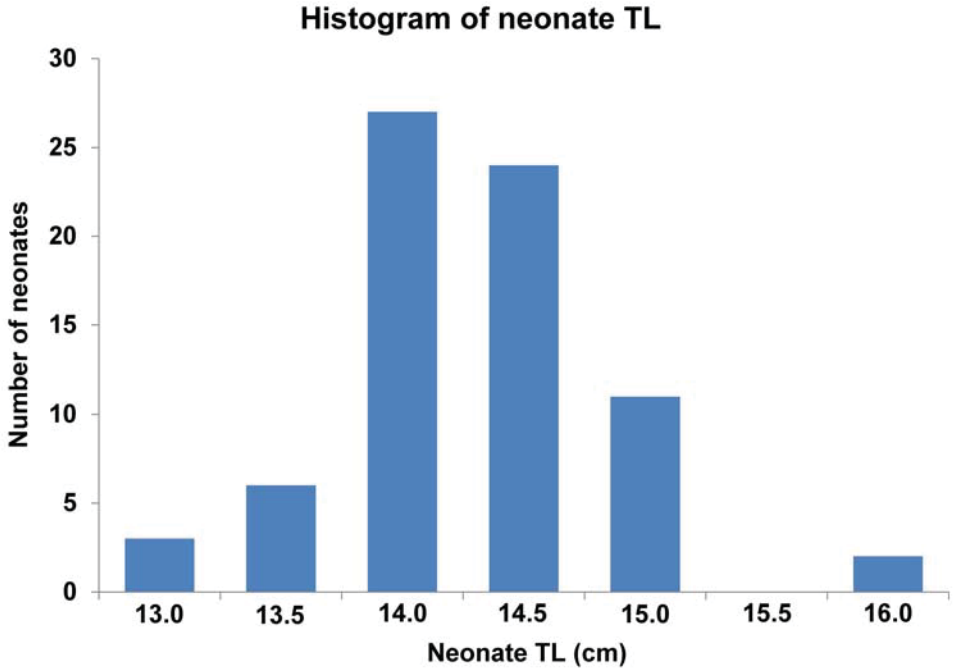

Figure 5. Frequency histogram of neonate (n = 73) total length (TL) at time of hatching, from three female big skates, *Beringraja binoculata* (Girard, 1855).

surface temperatures were ~10°C, might be as much as 365 days.

Nenoates

Mean neonate length at hatching was 14.9 ± 0.9 TL cm (n = 73) and demonstrated a normal distribution (Figure 5). Neonate TL was shorter than previously reported (DeLacy and Chapman, 1935; Ebert et al., 2008).

CONCLUSIONS

In their natural habitats, skates have been identified as one of the most vulnerable groups of marine fishes (Dulvy and Reynolds, 2002). Skates can serve as great ambassadors for oviparous elasmobranchs in public aquaria. Highlighting the body form and function, reproductive biology, and ecological roles of *B. binoculata*, are all great ways to narrate the story of skates, and their kin, in particular the critical challenges facing these vulnerable species.

Female size-at-maturity, egg capsule size and neonate size-at-birth, of *B. binoculata* observed in this study, were smaller than reported in the literature for *in situ* conspecifics. It is possible that the stable and clement conditions within the aquarium, including, excellent water quality, abundant food and regular encounters with potential mates, may have led to early maturity and reproductive success in the aquarium specimens.

Public aquaria play a key role in developing and implementing best management practices and husbandry techniques for culturing and rearing skate egg capsules and neonates. Well-managed breeding programs can provide a supply of specimens for public display and reduce pressure on wild stocks. Partnering with research facilities allows the strategic use of complimentary skill sets to advance the collective knowledge of elasmobranch biology. Breeding programs, in conjunction with conservation biology research initiatives, may provide opportunities for wild stock management strategies, and even *in situ* stock enhancement should other conditions dictate. At the very least, public aquaria can contribute resources and skills that can be applied to learning more about the reproductive biology of elasmobranchs.

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The Elasmobranch Husbandry Manual II: Recent Advances in the Care of Sharks, Rays and their Relatives, pages 459-464.

Chapter 45

Annually recurring parthenogenesis in a zebra shark, *Stegostoma fasciatum*

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Abstract: A zebra shark, *Stegostoma fasciatum,* held in captivity at the Burj Al Arab aquarium, produced embryos and pups in the absence of a male. A total of 15 pups were produced from eggs laid within the aquarium over a period of four consecutive years, commencing 2007. Parthenogenesis was confirmed through DNA analysis for three pups sampled during the first two consecutive egg cycles and is presumed to be the method of reproduction responsible thereafter.

INTRODUCTION

The zebra shark *Stegostoma fasciatum* (Hermann 1783) is a medium-sized, demersal shark that can be found inhabiting sea floors distributed within shallow, coastal, sub-tropical and tropical waters of the western Pacific and Indian Oceans (Compagno, 2002; Dudgeon et al., 2008). *S. fasciatum* has an average total length (L_T) of *c.* 300 mm at birth (Kunze and Simmons, 2004) and is considered to be a large adult when in excess of 1,800 mm L_T (Dudgeon et al., 2008). In April 2008, a neonate *S. fasciatum* was hatched from an egg produced by a female housed in the Burj Al Arab aquarium facility in Dubai. Parthenogenesis was the suspected method of reproduction as the female in question was imported into the aquarium in 2001 with an L_T of 1200 mm; the exact age at importation was unknown and the female was presumed to be sexually immature. This shark was the only member of its species

exhibited during the following six years before egg production began.

Two alternatives to parthenogenesis were considered as possible methods of reproduction: (1) hybridization with a male blacktip reef shark *Carcharhinus melanopterus* (Quoy and Gaimard 1824) that was housed with the *S. fasciatum*; and (2) sperm storage from a mating prior to captivity. As far as it is known, hybridization between *S. fasciatum* and *C. melanopterus* has never been recorded.

Parthenogenesis is the production of offspring without fertilization by a male; it is a form of asexual reproduction in which an embryo develops from an unfertilized female gamete. Automictic parthenogenesis (automixis) is a type of asexual reproduction characterized by the production of an egg through meiosis, subsequently followed by the fusion of the ovum with one of its sister polar bodies, producing a diploid zygote with elevated homozygosity compared to its mother (Schuett et al., 1998; Chapman et al., 2008). There are two forms of parthenogenesis through automixis: (1) central fusion, where the offspring exhibit heterozygosity and result from fusion of the egg nucleus to the first polar body and (2) terminal fusion, where the offspring exhibit elevated homozygosity and result from the fusion of the egg nucleus to the second polar body (Lampert, 2008).

Parthenogenesis is rare in vertebrate species, which usually reproduce after fusion of male and female gametes (Watts et al., 2006). Parthenogenesis has been reported in *c.* 70 vertebrate species (roughly 0·1%) (White, 1973; Avise et al., 1992) and has been identified conclusively using molecular markers in only a handful of cases, such as in reptiles (Schuett et al., 1998; Groot et al., 2003; Watts et al., 2006; Booth et al., 2010) and in sharks (Chapman et al., 2007, 2008; Feldheim et al., 2010).

Facultative parthenogenesis in vertebrates has only been found in captive animals, but might simply have been overlooked in natural populations (Lampert, 2008). Unfortunately, even though facultative parthenogenesis has been reported in almost all vertebrate groups, vertebrate species that are able to switch between sexual and clonal reproduction have never been reported from natural populations (Lampert, 2008). With recent advances in molecular methods, the cytological and molecular mechanisms of clonal reproduction can now be investigated much more easily, and scanning of natural populations for parthenogenesis becomes much more feasible (Lampert, 2008). As automixis is easily overlooked in wild vertebrate populations and there is only a rudimentary understanding of its breadth of evolutionary occurrence and frequency (Chapman et al., 2007; Lampert et al., 2007), it is of general biological interest to determine how widespread and common it is among sharks (Chapman et al., 2008).

The female *S. fasciatum* started producing eggs in 2006, however, all eggs in that year's cycle were discarded. In 2007, the same animal produced 30 eggs and the decision was made by the curatorial staff to retain them for the sole purpose of observing the possibility of parthenogenesis. Three of the eggs developed embryos, one survived past hatching but died after 80 days. All of the deceased embryos were female. All of the pups were assisted in hatching from their egg case. A summary of the number of eggs, embryos and neonates produced each year is given in Table 1.

Table 1. A summary of the egg cycle for each consecutive year from an isolated specimen of *Stegostoma fasciatum* (Hermann 1783).

Year	Number of eggs	Number of embryos	Number of pups	Number alive to date
2007	30	3	1	0
2008	40	15	9	3
2009	28	9	5	0
2010	28	3	1	1

In July 2010, the female entered her fifth egg cycle and completed it in late November. Development of three embryos was confirmed with the successful hatching of one neonate from the cycle. The gestation period of all eggs from all egg cycles was *c.* 160 days at 26ºC. To date, there are three pups surviving from the 2008 egg cycle and one from the 2010 egg cycle. The reasons for mortalities of the other pups varied.

Tissue samples were collected from two deceased embryos (one from each of 2008 and 2009) and also from the only pup to survive past hatching from the 2007 egg cycle. DNA was extracted using QIAamp DNA mini kit (Qiagen; www.qiagen.com) according to the manufacturer's instructions.

Eleven polymorphic microsatellite markers, previously developed for *S. fasciatum* (Dudgeon et al., 2006), were employed for parentage analysis. Three other loci for *S. fasciatum* were screened but either failed to amplify in the offspring or were proven uninformative.

The blacktip shark *Carcharhinus limbatus* (Müller and Henle 1839) markers employed in this study were found by Keeney and Heist (2003) to result in amplification for other carcharhinids with *C. melanopterus* amplifying for 13 of the 16 markers used in their study. Seven of the 16 *C. limbatus* markers characterized by Keeney and Heist (2003) amplified for the male *C. melanopterus* sample tested in this study and were used to investigate possible genetic contribution in the offspring.

All forward primers were end-labelled with fluorescent M13 primers. The markers were assigned in different cocktails based on their annealing temperature and products size as shown in Tables 2 and 3.

The PCR reactions were used in a final volume of 25 l containing *c.* 50 ng of genomic DNA, 0·025 pmol of forward primer, 0·25 pmol of reverse primer and M13-labelled primer, 1·5 mM of $MgCl_2$, 200 µM of each dNTPs and 0·5 U of Fast start *Taq* DNA polymerase (Roche; www.roche.com). The PCR amplification was performed in MJ research thermocycler (Biorad; www.bio-rad.com) with an initial denaturation temperature of 95°C for 10 min followed by 35 cycles of 15 s

Table 2. *Stegostoma fasciatum* (Hermann 1783) microsatellite loci.

Marker	Primer sequence (5 - 3)	Annealing temperature (°C)	Multiplex name	Allele range (bp)
Sfa205	F: TGGGCCAAAGTCCTTATTTA R: AAATAACATTCCAGTTATAGGAAATGA	52	A	355 - 391
Sfa335	F: GATGGGCATGAAACAAGATTT R: GTGGCCTGCCTTCTTGATT	57	B	355 - 411
Sfa387	F: CGCCCTCCCCTAAAATAGAC R: ACATCCTCGTTGCCTTTGAT	57	C	220 - 245
Sfa454	F: TGAAGGTGCAGCAAGAATTG R: ATGTGCATGCATGTTTTGGT	57	C	172 - 210
Sfa418	F: TGGAAGTTGCATTGCTGAAG R: GCACCATCAGTTTTCCAGGT	57	C	215 - 232
Sf2	F: GACTTCACTTCCTCCATCAG R: ACACCCCATACTTGCTACAG	60	D	170 - 193
Sf41	F: AGGGCATACTCTGCATTGCT R: ACTCCCACAAAGGACCACAG	60	D	190 - 237
Sfa236	F: AGACAGGCAGACAGATAGACAGA R: GAGGGAATAATGCTGCCTCA	60	E	235 - 279
Sfa221	F: AAACAGATTGCGATCATTAGCA R: AGGATCATCTCAGCACTGGAA	60	E	231 - 260
Sfa248	F: CATTCAGCTTTTTCTTTAAGTTGTCA R: GCAGAAATAGATGCATAGACAGCA	60	E	280 - 346
Sf72	F: GATAGCCTCAACCAGGATCA R: GCTTTCTGAACAAGATGGAA	60	F	200 - 286

Table 3. *Carcharhinus limbatus* (Müller and Henle 1839) microsatellite loci.

Marker	Primer sequence (5 - 3)	Annealing temperature (°C)	Multiplex name	Allele range (bp)
Cli-103	F: GCTTCATTCCATGAGAG R: TTTCTCTGTCCTGGTGTTTC	52	A	130 - 145
Cli-111	F: ACTTACGAACTGTTGCTAACTC R: GGGAGATAAACGACAAATGTG	52	A	105 - 180
Cli-102	F: GACTGGCTGACCTAACTAAGC R: ATCCTGTGGTCCTTCTATC	54	B	130 - 150
Cli-107	F: GGATTCACAACACAGGGAAC R: CTCATTCTTAGTTGCTCTCG	56	C	110 - 135
Cli-110	F: GAGGGAAGACTTAAACACAAGG R: TTTCCTTTGGCTGTCGCTG	58	D	145 - 175
Cli-2	F: CTTTGAGGAAGTTGGTACTGATG R: GCCACTCTTGTTCTGAATTTTTCCG	58	D	190 - 220
Cli-12	F: TCCCAGTCACATTTACACATGC R: GGAAGACCATTGAACCCAATC	58	E	195 - 220
Cli-103	F: GCTTCATTCCATGAGAG	52	A	130 - 145

denaturation at 95°C, 30 s annealing at 52 - 64°C, 30 s extension at 72°C and 45 min final extension at 72°C.

The PCR products were diluted 1:10 in deionized formamide (Applied Biosystem; www.appliedbiosystems.com) and denatured for 5 min at 95°C. The labelled products were electrophoresed and detected on an ABI Genetic Analyzer 3730xl (Applied Biosystem) and the results were analysed using GeneMapper v 4.0 software package (Applied Biosystem).

Elevated homozygosity was observed in the pups relative to the mother (Table 4). These results would be expected if reproduction by automictic terminal fusion parthenogenesis had taken place. Tissue sample SH06 and SH07 amplified for all of the markers, SH03 amplified for eight of the markers and sample SH02 from the mother amplified for all 11 *S. fasciatum* markers used. The only non-maternal allele was seen in sample SH03 for marker *Sfa248*, this sample was tested three times with the same result. This anomaly could possibly be explained by a non-stepwise mutation, but is not considered to be a significant result on its own to suggest any paternal contribution.

Apart from this single anomaly, the samples from the offspring were found to be homozygous at each marker for one maternal allele. Sample SH04 from the male *C. melanopterus* amplified for four of the *S. fasciatum* markers and possessed a common allele on all, except marker *Sfa335* where the two alleles carried by the male were different to that of all the pups, suggesting that it is unlikely that any paternal contribution took place. In addition, the fact that marker *Sfa335* amplified in *C. melanopterus* displays two different allele sizes that present for either the mother or embryos. This indicates that there is a difference in the genome and no paternal contribution for at least that region of the genome.

The *C. melanopterus* male amplified for seven *C. limbatus* markers. Neither samples from the mother nor offspring amplified for any of the carcharhinid markers (Table 5). *C. limbatus* markers only amplified the *C. melanopterus* genome and not those of the mother or embryos. Amplification for the carcharhinid markers would be expected in the offspring if hybridization had occurred. As with Chapman et al. (2007), the genetic results coupled with the captive history of the mother indicate that parthenogenesis is the most tenable explanation for the development of the offspring.

Several species of shark are known to store sperm for several months after copulation, raising the possibility that sperm storage could have been a possible method of reproduction. The *S. fasciatum* in question was imported into the Burj Al Arab aquarium in 2001 and the possibility of the female storing sperm for 6 years after a

Table 4. Composite genotypes of the adult female and three offspring *Stegostoma fasciatum* (Hermann 1783) and the male *Carcharhinus melanopterus* (Quoy and Gaimard 1824) at 11 *S. fasciatum* microsatellite loci.

Marker	Female *S. fasciatum* SH02 (M)	Pup, 2008 SH06 (F3)	Embryo, 2008 SH03 (F2)	Embryo, 2009 SH07 (F4)	Male *C. melanopterus* SH04 (BT)
Sfa205	382, 366	366, 366	366, 366	382, 382	
Sfa335	367, 379	367, 367	379, 379	367, 367	373, 385
Sfa387	228, 228	228, 228	228, 228	228, 228	226, 228
Sfa454	192, 204	204, 204	204, 204	204, 204	
Sfa418	218, 220	218, 218		220, 220	
Sf2	183, 185	185, 185	185, 185	183, 183	185, 187
Sf41	201, 199	199, 199	199, 199	199, 199	
Sfa236	265, 249	249, 249	249, 249	249, 249	
Sfa221	249, 249	249, 249	249, 249	249, 249	245, 249
Sfa248	301, 313	301, 301	313, 285	313, 313	
Sf72	219, 217	219, 219	217, 217	219, 219	

previous mating is considered highly unlikely. These facts coupled with the genetic results showing elevated homozygosity relative to the mother make sperm storage an unlikely explanation for the reproduction.

Other previous genetically confirmed cases of parthenogenesis in sharks have occurred in aquarium settings. In 2001, a bonnethead shark *Sphyrna tiburo* (Linneaus, 1758) gave birth to a pup that was produced *via* parthenogenesis (Chapman et al., 2007). In 2007, a female *C. limbatus* pup was found in the uterus of an adult female during a *post mortem* examination; parthenogenesis was confirmed in this pup by DNA analysis (Chapman et al., 2008). The third such example was presented by Feldheim et al. (2010) in a white-spotted bamboo shark *Chiloscyllium plagiosum* (Bennett 1830) where two parthenogens were produced.

After investigating all possibilities, it was concluded that parthenogenesis was the most scientifically plausible explanation for the production of offspring analysed in this case and for every egg cycle of this female shark to date.

Table 5. Composite genotypes of the male *Carcharhinus melanopterus* (Quoy and Gaimard 1824) at seven *Carcharhinus limbatus* (Müller and Henle 1839) microsatellite loci.

Marker	Male *C. melanopterus* SH04 (BT)
Cli-103	136,138
Cli-111	116,132
Cli-102	139,139
Cli-107	121,123
Cli-110	165,165
Cli-2	200,200
Cli-12	209,209

Although genetic samples were only taken in the first two egg cycles, the environment of the *S. fasciatum* in question had not changed and she had not been exposed since 2001 to any member of the same or similar species where fertilization could have occurred. This finding is thought to provide the first genetically confirmed successive virgin birth for chondrichthyans as well as the fourth verified case of parthenogenesis in sharks and the first for the family Stegostomatidae.

To date, there are only two documented records of successive parthenogenetic reproduction in a vertebrate species: in the Burmese python *Python molurus bivittatus* (Groot et al., 2003) and in the boa constrictor *Boa constrictor* (Booth et al., 2010), although only the latter was verified genetically. Chapman et al. (2007) stated that it remained unknown whether parthenogenesis can be a repeated facultative response to an absence of male sharks. This finding in chondrichthyans of annually recurring parthenogenesis is probably proof that it can be a repeated facultative response in *S. fasciatum* in the absence of a mate. It remains to be seen if any of the four surviving parthenogens will develop to maturity and be reproductively viable.

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General Index

Abramites, 104
abrasion, 44, 233
acanthias, 125, 128, 130–32, 142, 145, 198, 254, 286, 290, 299, 301, 322, 348, 452
Acanthochondria, 127
Acanthurus, 72
  olivaceus, 72
acclimation, 78, 114, 409
acclimatization, 24, 55, 57, 207, 231, 312
acid-base balance, 144, 165, 354
Acipenser, 452
  transmontanus, 452
acronotus, 5, 11, 122, 142, 283, 294, 304, 313
activated carbon, 45, 49, 184–85, 404
acutidens, 8, 259
acutus, 124, 131, 274, 299, 302, 427, 429, 431
adenovirus, 123, 128, 285
aeglefinus, 83
Aeromonas, 117, 128, 188, 286
Aetobatus, 121, 126, 129, 147–49, 151, 264–65, 268, 274, 294, 297, 301–2, 433–34, 436, 438, 440–42
  narinari, 126, 129, 142, 147–49, 151, 264–65, 274, 294, 297, 301–2, 329, 433–34, 436, 438, 440–42
  ocellatus, 126, 438, 442
Aetomylaeus, 334, 347
  bovinus, 334, 347
africanum, 223–27
agalactiae, 117, 128
age-at-maturity, 76–77, 412, 451
aggression, 26, 28, 54, 57, 72, 103, 109, 119, 123–26, 219, 332, 405, 423, 425, 444
aiereba, 100, 104, 106–7, 111
alalunga, 7
albacares, 5–6
albimarginatus, 53–57, 334
albisella, 350
alfredi, 59–61, 65, 256–58, 366
Alfred manta, 258, 366
alkalinity, 62, 90, 115, 184
alletteratus, 26–27, 426
altavela, 127, 130, 366
amblyrhynchos, 54, 212, 270, 274
americana, 125–26, 129–30, 252, 294, 299–300, 305, 307, 314, 316, 318–19, 329–30, 357, 359–62, 365, 372–73
American lobster, 72
American pilchard, 399, 413, 452
americanum, 123, 125, 128
americanus, 72
amikacin, 8
ammonia, 38–39, 49, 60, 62, 68, 82, 89–90, 101, 115, 119, 280, 326, 404, 408, 425
amphipod, 35
amphotericin B, 273
ampullae of Lorenzini, 122, 125, 170–71, 175–76
Amquel, 101
Amyloodinium, 118, 121, 124, 128
analgesic, 297, 335, 340, 343, 348, 354
Anarhichas, 35
ancylostoma, 67–74, 125, 142, 213, 220, 334, 346, 365
ancylostomae, 69
anemia, 40, 87, 126, 136, 139–40, 269–70
anesthetic, 56, 205–7, 257, 260–61, 296, 304, 331–33, 335–36, 339–48, 350–51, 354–56, 360, 365
angel shark, 412, 419
Anisotremus, 121
  virginicus, 121
annulatus, 334
Anoplopoma, 7
Anoplopoma fimbria, 7
anorectic, 26, 45, 49–50, 56, 78–79, 105, 107, 296
anorexia, 23, 48–49, 64, 139, 358
anostomid, 104
Anoxypristis, 76
Anthosoma, 121
  crissum, 121
antibiotic, 7–8, 48–50, 55, 270, 295, 350, 353, 355–56, 358–59
anticoagulant, 120, 137, 257
antifungal, 129, 273, 333
anti-inflammatory, 297
appetite, 8, 56, 59, 64, 83, 163, 226, 232, 349, 395, 434
appetite stimulant, 8
AQUI-S, 79
aquila, 334, 366
arabicum, 306
arafurense, 69
Argulus, 107, 118, 127
argus, 72
Argyrosomus, 51, 298
  japonicus, 298
Arripis, 394
Arripis trutta, 394
artificial insemination, 305, 363–64, 368, 370–73, 383, 385, 409, 423, 431–32, 440
asperrimus, 142, 213
assisted feeding, 64, 106, 116, 203, 297, 340, 348, 418, 426
assisted swimming, 205–6, 399
asterias, 278
atipamezole, 56, 336-337, 340-342, 344, 346
Atlantic blue crabs, 72
Atlantic bonito, 116, 141

Atlantic butterfish, 116
Atlantic guitarfish, 125, 128, 286
Atlantic herring, 45, 104, 116, 139, 141, 326, 426
Atlantic horse mackerel, 350
Atlantic krill, 91
Atlantic mackerel, 116, 139, 141
Atlantic rays, 179
Atlantic salmon, 45, 141, 166, 353
Atlantic sharpnose shark, 27, 122, 128, 132, 166, 213, 274
Atlantic silverside, 59, 63, 93
Atlantic stingray, 290, 373
Atlantic surf clam, 454
atlanticus, 25, 27, 30
Atlantic wolffish, 35
auratus, 105, 164, 167, 333, 337, 356
Australian blacktip shark, 7
Australian bonito, 154, 394
Australian bull ray, 369
Australian freshwater whip ray, 366
Australian salmon, 394
australis, 154, 368–71, 394, 399
avidus, 124
bacteria, 50, 125, 183–84, 188, 264, 285–87, 358, 407, 430
bamboo shark, 132, 252–54, 318, 373, 432, 463
banded houndshark, 123, 369
barndoor skate, 172
basking shark, 307
bat eagle ray, 127, 151, 452
batoid, 65, 84, 268, 441
becaplermin, 295
behavioral enrichment, 26, 28, 32, 105, 110, 112, 221
Benedenia, 78
Benedeniella, 117, 121, 126, 132, 263, 266–68, 275, 299, 302
  posterocolpa, 121, 126, 132, 263, 266–67, 275, 299, 302
Beringraja, 451–56
  binoculata, 451–56
bicarbonate, 49, 71, 89, 101, 298
big skate, 451, 453–55, 457–58
bigtooth river stingray, 104
binoculata, 125, 451–58
biochemistry, 11–12, 70–71, 120, 123–27, 129–30, 132, 145, 151, 156, 249, 252–54, 301–2, 351, 355–56, 373
biological filter, 184, 406–7
biopsy, 120, 245–46, 252, 254, 271, 306
biosecurity, 114, 405
BioSisT, 295
birostris, 59, 61, 142, 151, 220, 256, 267, 314, 319, 376
blackchin guitarfish, 346, 348, 365–66
blacknose shark, 5, 11, 294, 304, 313
blackspot sharks, 57
blacktail reef shark, 212, 270
blacktip reef shark, 212, 278, 283, 292, 334, 346, 443–47, 449, 460
blacktip shark, 7, 27, 212, 294, 309, 319, 366, 461, 464
black-tailed antenna ray, 106
blood, 38–40, 96–98, 116–18, 120, 130–32, 142–44, 165–67, 197–200, 217–21, 250–53, 255–62, 294, 299–302, 349–51, 354–56
blood chemistry, 39–40, 98, 120, 351
blood glucose, 40
blood sampling, 140, 165, 221, 251, 255–57, 259, 261, 294, 340
bluefin tuna, 5–6, 11–12
bluefish, 8, 14, 32, 394
blue runner, 83
blue shark, 43–47, 49, 51, 252, 305, 355
blue-spotted stingray, 283
bluestreak cleaner wrasse, 299
bluntnose sixgill shark, 412
BM, 1–3, 5–10, 16, 18–21, 27, 80, 105, 137–38, 150, 411, 413–16, 421–22, 424–27, 434–35, 437
BM/week, 49, 56, 82, 105, 138, 426–27
body mass, 1–3, 5, 12–13, 19–23, 34, 49, 116, 147–48, 340–42, 344, 350, 413–14, 416–17, 421–22, 434–35
bonasus, 120–21, 126–27, 131–32, 150–51, 172–73, 264–69, 274–75, 277–78, 280–81, 283–84, 299–302, 312–13, 317, 361–62, 365–66
bonito, 2, 7, 83, 116, 141, 154, 394
bonnethead, 123, 128–31, 139, 145, 151, 270–71, 274, 277–78, 283, 286, 291, 300, 305, 311, 365–66
Boulengerella, 104
bovinus, 51, 334, 347
bowmouth guitarfish, 67–69, 71, 73–74, 125, 213, 220, 346, 365
Brachyplatystoma, 104
brain, 34, 118, 122–24, 253–54, 280, 284–86, 290, 301, 306, 308, 317, 324–25, 327–28, 427, 429
Branchellion, 126–27, 130–31, 263, 268–70, 273–74, 299
  torpedinis, 126, 130–31, 263, 268–69, 274, 299
branchitis, 118, 130, 281, 284
Branchiura, 118
branchiuran, 127
Branchotenthes, 69, 73
Branchotenthes robinoverstreeti, 69, 73
breeding, 109, 123–24, 156, 363–65, 367–73, 375–78, 385–89, 391–95, 399–400, 403–9, 422–26, 430–31, 434–35, 437–41, 443–46
brevicaudata, 299, 368–70, 372
brevipinna, 334
brevirostris, 5, 9, 11, 14, 71, 121–22, 129–30, 132, 135, 151, 301–2, 305, 356, 364–65, 373
broadnose sevengill shark, 9, 27, 213, 321, 369, 412
brownbanded bambooshark, 260–61
brown smooth-hound, 305

bullnose eagle ray, 366
bull ray, 51, 334, 369
bull shark, 9, 13, 27, 212, 254, 258, 334
buoyancy, 9, 26, 202, 205, 207, 310, 358, 398–99
Burmese python, 464
butorphanol, 56, 301, 331, 334–37
butterfly ray, 127, 130
calcium, 39, 71, 84, 135–36, 138, 140, 145, 257, 298, 408
Californian anchovy, 413
California skate, 6, 458
californica, 127, 132, 151, 411–19, 452
californicus, 452
californiensis, 105–6, 110
Callinectes, 72
  sapidus, 72
calorie, 26, 427
campechanus, 27
canadum, 27
canicula, 123, 172–73, 213, 277–78, 281, 283, 290, 299, 301, 348
canis, 123, 128, 131–32, 142, 145, 171–72, 272, 285–86
capelin, 83, 104, 116, 139, 141, 214, 216, 225, 326, 413, 426, 452
capensis, 224
Capsalidae, 117, 132, 266, 275
capture, 1–3, 16–17, 38–40, 43–46, 55, 75–76, 78–79, 127–28, 130–31, 150–51, 248, 251–52, 337, 340–41, 356
Carangidae, 26
Caranx, 27, 83, 350
  crysos, 83
  latus, 350
Carassius, 105, 164, 167, 333, 337, 356
  auratus, 105, 164, 167, 333, 337, 356
carbohydrate, 139
carcharhini, 122, 129, 270, 274
carcharhinid, 7, 13, 24, 57, 122, 151, 210, 441, 462
Carcharhinidae, 44, 122, 129, 132, 197, 213, 274, 302, 387
Carcharhinus, 5–7, 11–13, 26–27, 53–55, 57, 122, 128–31, 212, 257–58, 274, 341–42, 348–51, 443–47, 449, 460–64
  acronotus, 5, 11, 122, 142, 283, 294, 304, 313
  albimarginatus, 53, 55, 57
  amblyrhynchos, 54, 212, 270, 274
  brevipinna, 334
  dussumieri, 7
  falciformis, 27, 131, 212
  galapagensis, 6, 212
  leucas, 9, 13, 27, 78, 130, 151, 196, 199, 212, 252, 254, 257–58, 365, 379, 409
  limbatus, 27, 128, 212, 220, 294, 309, 319, 441, 461–64
  melanopterus, 122, 128–29, 212, 221, 277–78, 280, 283, 286, 292, 346, 355, 443–47, 449, 460, 463
  obscurus, 151, 212
  plumbeus, 5, 9, 12, 44, 48, 128, 131, 274, 299, 302, 341–42, 344, 346, 348–51, 355
  sealei, 57
  tilstoni, 7
Carcharias, 144–45, 153, 155–57, 196–97, 252–54, 310–11, 333–35, 341–42, 356–58, 375–77, 379–85, 387–89, 391–93, 395–97, 399–401
  taurus, 144–45, 153, 155–57, 196–97, 252–54, 310–11, 333–35, 341–42, 356–58, 375–77, 379–85, 387–89, 391–93, 395–97, 399–401
Carcharodon, 1–5, 7, 9, 11–13, 18, 27, 31, 34, 212, 240, 306
  carcharias, 1–5, 7, 9, 11–13, 18, 27, 31, 34, 212, 240, 306
Caribbean spiny lobster, 72
carnpanulatum, 69
carpet sharks, 98,129, 400, 431
Carpobothrium, 69, 74
  rhinei, 69, 74
cartilage, 34, 40, 72, 137, 203, 232, 256, 271, 283, 325, 327–28
catfish, 104
catheter, 306, 318, 353, 368, 370, 423
catshark, 123, 223–25, 227, 278, 280, 312, 373
cefovecin, 296, 302
ceftazidime, 48–50, 359
ceftiofur, 361
cemiculus, 346, 348, 365
centroura, 125, 142
cepedianus, 9, 13, 27, 32, 129, 213, 305, 307, 321, 329, 363, 365, 367–70, 373, 386
Cephalobothrium, 69, 74
  neoaetobatidis, 69, 74
cephalopod, 115, 224
Cephaloscyllium, 123, 142, 305, 321, 372, 388
  ventriosum, 123, 142, 305, 321, 388
cephalus, 34–35, 154, 337, 394, 399
cestode, 73, 118-19, 285
Cetorhinus, 307
  maximus, 307
chain catshark, 123, 312
chaophraya, 212
chiller, 37, 406
Chiloscyllium, 124, 129, 132, 142, 179, 252–54, 260–61, 296, 302, 306, 366, 368–70, 373, 405–8, 423
  arabicum, 306
  plagiosum, 124, 142, 179, 252–53, 296, 302, 318, 369–70, 373, 423, 432, 463
  punctatum, 124, 129, 142, 167, 179, 252–53, 260–61, 347, 366, 368–69, 405–8
Chimaeridae, 127
Chimaeriformes, 198
Chinook salmon, 2, 452
chitin inhibitors 118, 121
Chlamydiales, 113, 118, 123, 126, 129, 132, 281

chloral hydrate, 291, 300
chloramine, 286, 404
chlorine, 115, 119, 404–5
chloroquin diphosphate, 78
chokka squid, 224, 227
chromium, 136
chub mackerel, 2, 413
Cichla, 104
cichlid, 104
ciliata, 399
ciliatosis, 285, 432
circinelloides, 118, 272, 427
cirratum, 26–28, 30, 32, 124, 129, 179, 196, 206, 212, 220, 305–7, 347, 350, 364–66, 372
CITES, 77, 87, 100, 240, 256
Citrobacter, 117
clam, 216, 426, 454
clasper, 153, 395, 422–23, 436–37
clavata, 76, 125, 213, 277–78, 280, 348, 366, 437
clearnose skate, 172, 366, 369, 373, 409, 423, 431, 458
clindamycin, 118, 127, 295
cloaca, 118–19, 122, 124, 129, 353–54, 359, 361, 368, 370, 395, 418, 422–23, 437, 439, 446
cloudy catshark, 123, 373
clove oil, 333, 337, 354, 356
Clupea, 45, 83, 104, 116, 139, 141, 165, 326, 413, 426
  harengus, 45, 104, 116, 139, 141, 165, 326, 426
  pallasii, 413
CO2, 4, 84, 260
cobalt, 136, 138
cobia, 27
coccidia, 113, 118, 120, 126–27, 274, 277, 283, 296
coccidiosis, 266, 283, 285–86
coelom, 63, 116, 118, 120, 122, 126, 130, 252, 265, 305–6, 316, 321, 349, 353, 355
collection, 33–41, 43–51, 53–55, 59–61, 68–70, 77, 113–14, 119–20, 177–89, 191–95, 199–200, 219–20, 237–40, 286, 368–69
colliei, 51, 127, 131, 212
Colossoma, 104
common eagle ray, 366
common guitarfish, 72, 125, 283
common stingray, 130, 346
competition, 6, 26, 57, 83, 110, 116, 150, 156, 186–87, 211, 219, 326, 455
conditioning, 28, 61–62, 64, 80, 83, 91–92, 110, 164, 193, 196, 206, 209–11, 217, 219–21, 435
conger, 350
Conger conger, 350
consumption, 1–4, 6, 9–13, 68, 94, 135, 140, 144, 153, 155–56, 224, 241, 411, 414, 417–18
contact lesion, 49, 87
copepod, 38, 41, 69, 73, 87, 121–22, 429
copper, 78, 103–4, 115, 119, 121–22, 126, 130, 132, 135–36, 138, 267, 275, 299, 302
copulation, 153–54, 329, 359, 376, 378, 383, 394–95, 398–400, 418, 422–23, 430, 434, 437–38, 440, 446
corticosteroid, 270, 297, 354
cortisol, 39, 166–67, 343
Coryphaena, 6, 51
  hippurus, 6, 51
cownose ray, 132, 151, 172, 266, 268, 274–75, 278, 280, 301–2, 306, 312–13, 315, 317, 366, 436
cranchii, 69
Crangon, 413
  franciscorum, 413
crayfish, 105
Crenicichla, 104
crissum, 121
crysos, 83
Cu, 135–36, 141, 143
cuvier, 2, 8, 23–32, 47, 51, 54, 71, 74, 121, 155, 172, 212–13, 268–69, 356, 366
cyanocobalamin, 139
daily ration, 1, 6–8, 11, 14, 32, 63
dalyensis, 366
damselae, 117
damsel fish, 350
Dascyllus, 350
  albisella, 350
dasyatid, 105, 118, 125–26, 325–26
Dasyatidae, 111, 125, 129, 185, 213, 302, 441
Dasyatis, 10, 111, 125, 129–30, 142, 179, 252, 268, 299, 318–19, 329–30, 334, 359–62, 365–66, 368–69
  americana, 125, 129–30, 212, 252, 294, 299, 305, 307, 314, 316, 318–19, 329–30, 357, 359–62, 365
  brevicaudata, 299, 368–69
  centroura, 125, 142
  kuhlii, 111, 132, 283, 441
  marmorata, 334
  pastinaca, 125, 130, 346
  sabina, 125, 172, 179, 290, 366
  thetidis, 334
  uarnak, 334
  violacea, 5, 10, 12, 330
dasypogon, 142
deaccession, 77, 237–43, 405
Decacotyle, 150
  floridana, 150
decompression, 38
dehydration, 49, 79
Dendromonocotyle, 117, 125–27, 129, 132
  californica, 127, 132
  octodiscus, 127
  pipinna, 125
denitrification, 61, 90
dentex, 83
denticle, 102, 198, 203, 293, 304
Dermophthirius, 78, 117, 122, 128–29, 301

melanopteri, 122
nigrellii, 122
penneri, 128
desensitization, 94, 96, 117, 193, 196, 202–3, 206, 212
deslorelin, 372
devil ray, 64, 315, 366
dexamethasone, 116, 351, 353
diagnostic, 109–10, 119–20, 126, 128, 246, 248–49, 251, 265–67, 279–80, 284–85, 303–5, 307–9, 311, 313, 355–57
diazepam, 116, 335
dicentrarchi, 124, 282, 287, 427, 432
diet, 6–9, 22–23, 26–28, 31–32, 63, 72, 74–75, 93–94, 104–5, 108, 135–37, 139–42, 144–45, 251–52, 299
diflubenzuron, 121, 299
dimidiatus, 299
dinoflagellate, 118, 121, 124
Diphyllidea, 119
Dipturus, 172
laevis, 172
discus ray, 100
disc width, 60, 102, 106, 120, 126, 150, 180–81, 183, 214–16, 360, 413, 435, 454
disease, 129–30, 135, 137, 140, 144–45, 187, 241, 246–47, 249, 264, 271–74, 277–87, 303, 372–73, 405
disinfection, 61, 90, 114, 120, 184, 249, 326, 353
dissolved oxygen, 38–39, 55–56, 60, 68, 75, 78–79, 82, 89, 114–15, 121, 124, 260–61, 404, 408, 413
diving, 5, 36, 80, 191, 193–95, 197, 199–201, 203, 205, 207, 226, 231, 401
djiddensis, 57, 72, 142, 334
dogfish shark, 132, 301
Dollfusiella, 69
michiae, 69
dolphinfish, 6, 51
dorsal saddle, 203, 206, 257, 292, 335–36
Doryteuthis, 83, 452
opalescens, 83, 452
dose, 8, 30, 40, 49, 55–56, 290–91, 296–99, 302, 332, 335, 340, 342–44, 348, 351, 354
Dracunculoidea, 118
dronabinol extract, 116
duorarum, 72
dusky shark, 151, 212, 335–36
dusky smooth-hound, 123, 171
dussumieri, 7
DW, 60, 102, 106, 120, 150, 181–82, 326, 360, 413, 434–37, 439, 454
dystocia, 250, 296, 319, 357, 361
eagle ray, 147, 265, 294, 317, 366, 377, 388, 434, 436, 438, 440–42
Echeneis, 30
naucrates, 30
ectoparasite, 24, 55, 302
egg, 172, 223–24, 267, 270–71, 299, 305, 357–59, 364, 371, 406, 408, 421–25, 429, 451–61, 463–64
eglanteria, 125, 172, 252, 365–66, 368–70, 373, 409, 423, 431, 458
Eimeria, 118, 126, 131–32, 263–66, 274, 283, 313
southwelli, 118, 126, 131–32, 263–65, 274, 283
electricity, 169, 171, 173–75, 204
electric ray, 198
electrolyte, 40, 116, 297, 348
electromagnetic field, 170
elongata, 38, 41
elongatus, 122, 128
embryo, 154, 172, 269, 371, 424, 453–54, 460, 463
embryogenesis, 329
encrasicolus, 224
endoscope, 120, 306
endotherm, 2
energy, 2, 6, 8–10, 14, 21, 23, 26, 50, 69, 94, 116, 135, 141, 148, 166
energy budget, 10, 148
Engraulis, 20, 160, 224, 413
encrasicolus, 224
japonicus, 20, 160
mordax, 413
enrichment, 26, 28, 32, 99, 105, 108–10, 112, 164, 221
enrofloxacin, 7–8, 30, 48, 50, 55, 70, 74, 293–94, 296, 300, 353, 355
Enterococcus, 358
Entobdella, 117, 125
envenomation, 99, 101–2, 182, 200, 205
eosinophil, 84, 351
epaulette shark, 129–32, 252, 287, 366, 369
eperlanus, 139, 326
Epinephelus, 57, 81, 128, 398
lanceolatus, 57, 128
epitheliocystis, 118, 126, 129, 132, 277, 281, 285
Ergosan, 50
erinacea, 125, 130–31, 252, 296, 373
Erpocotyle, 117, 123, 128–29, 322
tiburonis, 123, 129
erythrocyte, 429
estradiol, 365, 368, 373, 380–81, 383
estrogen, 372, 380
ethogram, 28–30, 160–61, 164, 198
etomidate, 339–40, 343–51, 354, 356
etorphine, 335
Eucrossorhinus, 142, 268
dasypogon, 142
Euphausia, 20, 59, 63, 91, 93, 160, 232, 454
pacifica, 20, 59, 63, 93, 232, 454
superba, 20, 59, 63, 91, 160
European anchovy, 224
European conger, 350
European smelt, 139, 326
European sprat, 45, 326

European squid, 350
euthanasia, 39–40, 181, 237–38, 242–43, 341, 343, 405
Euthynnus, 26–27, 426
alletteratus, 26–27, 426
Exocoetidae, 232
Exophiala, 118, 272–74, 427
pisciphila, 118, 272, 274, 427
eye, 11, 35, 41, 49–50, 139, 202, 245, 253, 266, 296, 325, 327–29, 350, 427
fai, 142
falciformis, 27, 131, 212
falkneri, 111, 372
fasciata, 123, 142, 452
fasciatum, 121, 124, 216–21, 272, 274, 277–78, 282, 284, 299–300, 315, 369–70, 372, 377, 421–32, 459–64
Fe, 135–36, 141, 143
fecundity, 109, 376, 391, 412, 444, 451, 453, 455, 457
feeding, 6–13, 22–32, 49–50, 56–57, 63–65, 82–83, 91–98, 103–6, 110–17, 153–55, 159–65, 214–19, 223–26, 232, 425–27
fenbendazole, 121, 131, 286, 291
ferrugineus, 257–58
fertilization, 305, 370–71, 383, 418, 437, 460, 464
filter, 22, 45, 49, 59, 61, 63, 87, 90–91, 98, 103, 108, 171–72, 184–85, 298, 406–8
filtration, 4, 43, 45–46, 61, 80, 82, 89–90, 103, 114, 121, 160, 182–85, 404–7
fimbria, 7
flapnose ray, 283
flathead grey mullet, 154, 394
Flavobacterium, 117
floridana, 150
fluoride, 404
fluoroquinolone, 355
folic acid, 138–39
food, 6–11, 26–31, 63–64, 72, 82–83, 91–97, 103–11, 115–16, 153–56, 160–61, 224–26, 232, 393–95, 413–14, 424–27
food consumption, 6, 9–10, 13, 140, 153, 156, 411, 414, 417–18
food intake, 6, 10, 83, 103, 109, 115–16, 148, 153, 155–56, 393, 395, 411, 414, 437, 439
fork length, 38
formalin, 40, 120, 128, 278, 283
francisci, 124, 171, 305, 322, 348
franciscorum, 413
freminvillei, 127
freshwater ray, 110, 172
freshwater stingray, 99–100, 102, 106, 108, 110–11, 127
freshwater whipray, 212
fungal, 23, 78, 113, 118, 120, 129, 131, 174, 263, 271–74, 280–81, 283, 285, 330, 430–31
fusariosis, 285
Fusarium, 118, 123, 125, 128–29, 131–32, 271–74, 283
solani, 118, 123, 128–29, 131–32, 271, 274, 283
Gadus, 35, 145, 337
morhua, 145, 337
ogac, 35
gairdneri, 145
galapagensis, 6, 212
Galapagos shark, 212
Galeocerdo, 8, 23, 25, 27, 29–32, 47, 51, 54, 71, 74, 199, 212, 334, 356, 386
cuvier, 8, 23, 25, 27, 29–32, 47, 51, 54, 71, 74, 199, 212, 334, 356, 386
Galeorhinus, 6
galeus, 6
galeus, 6
gallbladder, 280, 283, 286, 305, 321–22
gastric, 10, 50, 87, 117, 119, 122, 132, 248, 254, 266, 283, 286, 310, 353, 355
gastrointestinal, 64, 82, 118, 120, 126–27, 265, 304–5, 312–13, 318, 358, 426
gastropod, 35, 104, 224, 299, 426
gavage, 116, 121, 291–92, 297–98, 304
gel food, 59, 63, 93–94
genealogy, 241, 380–82, 397
genetic, 85, 109, 156, 241, 364, 376–77, 383–84, 387, 389, 412–13, 418, 422–23, 430–31, 440, 461–64
geophagines, 104
Geophagus, 104
georgianus, 155
gestation, 109, 129, 306–7, 325–27, 329–30, 363, 365, 371–72, 378, 381, 412, 437–41, 443, 446, 449
ghost shrimp, 105
giant grouper, 57
giant guitarfish, 57, 72
giant manta, 151, 220, 267, 314, 319
giant shovelnose, 72, 124
gibbesi, 72
gigas, 369
gill, 65, 120, 122–23, 126–27, 129–32, 148, 212–13, 221, 260–61, 280–86, 291, 295–96, 304–6, 322, 452
Ginglymostoma, 26, 32, 124, 129, 196, 206, 212, 220, 305, 307, 347, 350, 364, 366, 372
cirratum, 26, 32, 124, 129, 196, 206, 212, 220, 305, 307, 347, 350, 364, 366, 372
Ginglymostomatidae, 124
glass shrimp, 105, 110
glauca, 43–51, 128, 172, 252, 305, 355
Glaucostegus, 72, 124, 142
typus, 72, 124, 142
glucose, 39–40, 71, 84, 116, 136, 248, 298, 368, 371
Gnathanodon, 72
speciosus, 72
golden trevally, 72

goldfish, 105, 164, 166–67, 333, 337
gonadosomatic index, 368
gonadotropin, 381, 388
grabata, 366
Grapsus, 72
  grapsus, 72
grass shrimp, 413
great hammerhead shark, 272
great white shark, 212, 306
Greenland cod, 35
Greenland halibut, 35
Greenland shark, 33–35, 37, 39, 41–42
green sawfish, 76, 85
griseus, 412, 419
growth, 1–4, 8–14, 31–32, 81–82, 105–6, 110–12, 136–37, 139–40, 232–33, 362, 364–65, 400, 411–15, 417–19, 457–58
growth rate, 1, 9, 14, 16, 21, 232, 325, 413, 415, 418
GSI, 368
guitarfishes, 213, 292, 440
Gymnura, 127, 130, 268, 366
  altavela, 127, 130, 366
  japonica, 127
Gymnuridae, 127, 213
haddock, 83
hake, 45, 83, 224
halleri, 172–73, 330
Haller's round ray, 172, 366
haloperidol, 300
hammerhead, 5–6, 12, 51, 54, 57, 176, 252, 254, 271–72, 274, 285, 313, 346, 356, 464
handling, 39–41, 79–80, 100–102, 122–24, 197, 199, 201–5, 207, 214, 220–21, 248–53, 291–92, 297, 331–33, 335–36
Haploblepharus, 212
harkemai, 121–22, 132, 274, 302
heal, 30, 70, 102, 119, 173, 218, 232
Heliotrygon, 100, 106
hematocrit, 39–40, 71, 131, 253, 258–59, 269, 351, 356
hematology, 120, 122–25, 127–28, 165, 250, 252, 257–58, 262, 351, 355
Hemiodus, 104
Hemiscylliidae, 124, 213
Hemiscyllium, 124, 129–32, 142, 179, 252, 287, 347, 366, 369
  ocellatum, 124, 129–32, 142, 179, 252, 287, 347, 366, 369
hemoglobin, 39, 84, 351
henlei, 104, 109, 305, 340, 387, 452
heparin, 38, 120, 257
hepatitis, 272, 282, 351, 427
herpes, 123
herring, 45, 83, 104, 116, 139, 141, 165, 326, 413, 426
Heterocotyle, 117
Heterodontidae, 124
Heterodontus, 124, 130, 171, 252, 278, 281–82, 305, 322, 348, 364
  francisci, 124, 171, 305, 322, 348
  japonicus, 124, 282
  portusjacksoni, 124, 130, 252, 278, 281–82, 364
hexabothrid, 299
Hexabothriidae, 73, 117, 129
Hexanchus, 412, 419
  griseus, 412, 419
Himantura, 125–26, 129, 142, 212, 366
  chaophraya, 212
  dalyensis, 366
  fai, 142
  jenkinsii, 142
  uarnak, 125, 129, 142, 366
  undulata, 142
hippoglossoides, 35
hippurus, 6, 51
histology, 114, 246, 278, 281, 283, 285–86, 327, 418
histotroph, 306, 319, 359–60, 372, 435, 438
Homarus, 72
  americanus, 72
Honeycomb stingray, 125, 334, 366
hook, 24, 34, 37–38, 46, 54, 119–20, 122, 131, 248, 303, 308, 313, 319, 348–51, 353–55
hormone, 120, 194, 219, 364–65, 368, 373, 380–81, 383, 388, 440
horn shark, 171, 305, 322, 366
horse-eye jack, 350
hound shark, 212-13, 277, 283, 387
Huffmanela, 122, 263, 270–71, 273–74, 299, 302
  carcharhini, 122
  markgracei, 274
hydrodynamics, 48, 50, 254
Hydrolagus, 51, 127, 131, 212
  colliei, 51, 127, 131, 212
hyperactivity, 44, 69, 165, 205, 343–44
hyperechoic, 359
hyperemia, 119
hypnotic, 332, 354
hypophagia, 16
hypostoma, 64, 142, 299–300, 315, 366
hypoxia, 119, 132, 252, 254, 348
ICe, 292, 294, 296–97
IM, 48–50, 55–56, 70, 140, 206, 257, 259–60, 292–94, 296, 299–300, 331, 335–36, 341–43, 346, 353
imaging, 245–46, 251, 303–9, 311, 313, 315, 317, 319, 321, 323, 330, 349, 355, 381, 389
immature, 34, 40, 74, 155, 383, 459
immersion, 115, 117, 121–22, 124–27, 191–95, 257, 291, 295–99, 331, 333–34, 337, 339–40, 347, 349, 354–55
immobilization, 256–57, 260–62, 289, 302, 331, 333, 335, 337, 339, 348–49, 354–56
inappetent, 8, 64, 72, 116, 123, 126, 161, 203, 399, 418
incubation, 371, 421–22, 424–25, 453–54, 456, 458

infection, 78, 80, 82, 101–2, 106–8, 120–22, 127–32, 174, 264–67, 269–72, 274, 277–79, 281–86, 427, 429–31
injection, 8, 48–49, 55, 203, 206–7, 256–57, 259–60, 292–93, 296, 300–302, 326, 335, 341–46, 349–50, 354–55
inornata, 6
inositol, 138
insemination, 305, 363–64, 368, 370–73, 379, 383, 385, 398, 409, 423, 431–32, 440
International Union for Conservation of Nature, 241, 325, 363, 422
intestine, 71–72, 96, 117, 122, 129, 264, 409, 427, 429
intracoelomic, 131, 348–49, 351, 353, 355
intramuscular, 8, 49, 114, 206, 256–57, 259, 292, 301–2, 333–34, 337, 341, 346, 349
intrasinus, 294
intravenous, 70, 101, 256, 259, 300–301, 313, 342, 346, 352–53
iodine, 49, 135–36, 138, 140, 145, 249, 279, 299–300, 350, 360
iron, 135–36, 138, 270, 300
isoflurane, 333
Isurus, 5, 11, 13–14, 27, 32, 44, 235, 252, 254, 305
  oxyrinchus, 5, 11, 13–14, 27, 32, 44, 254, 305
IUCN, 74, 77, 85, 87, 98, 227, 241, 243, 389, 391, 400–401, 431–32, 434, 441, 449
IV, 101, 259–60, 291–92, 294, 297, 300, 313, 341–42, 346, 348, 400
iwamae, 106, 111
jamaicensis, 121, 127, 129, 131, 172–73, 176, 213, 268–69, 272, 274, 286, 312, 366
Japanese anchovy, 20, 160
Japanese bullhead shark, 124, 282
Japanese butterfly ray, 127
Japanese meagre, 298
Japanese wobbegong shark, 57, 124
japonica, 127
japonicus, 2, 6–8, 10, 20, 51, 57, 124, 160, 282, 298, 413
javanica, 283
jenkinsii, 142
juvenile, 1–2, 11–13, 25–26, 28, 67–68, 71–72, 78–79, 81–83, 121–24, 166, 230, 232–33, 408–9, 421, 429–30
Kcal, 8–10
kelp, 138, 350, 412
keratitis, 49, 124
ketamine, 335, 340–42, 344, 348
kilocalorie, 94
king snake eel, 27
kishinouyei, 160
krill, 20, 59, 63, 87, 91, 93, 104, 160, 230, 232, 299–300, 454
kuhlii, 105, 111, 125–26, 132, 283, 299, 334, 347, 441
kyphosis, 247, 251
Labroides, 266, 299
  dimidiatus, 299
Labrus, 268
lactate, 71, 120, 130, 151, 300, 354
laevis, 172
lanceolatus, 57, 128
largespot river stingrays, 372
largetooth sawfish, 76, 85, 171, 292, 346
largus, 427, 429
Latropiscis, 72
  purpurissatus, 72
latus, 350
Lecanicephalidea, 73–74, 119
leech, 69, 116, 126, 130–31, 263–64, 268–69, 274, 299
lemon shark, 5, 8–9, 11, 14, 135, 167, 259, 356, 373
lentiginosus, 125, 128, 286
leopard shark, 132, 309, 322, 366
leopoldi, 104, 108–9, 127
Lepeophtheirus, 124, 131, 274, 299, 302, 427, 429, 431
  acutus, 124, 131, 274, 299, 302, 427, 429, 431
Leporinus, 104
lesion, 50, 122, 269–70, 272, 284, 349
lesser devil ray, 64, 315, 366
lesser sandshark, 334
lesser-spotted dogfish, 281, 290
lethargic, 35, 106, 230
leucas, 9–10, 13, 27, 32, 78, 130, 151, 196, 199, 212, 252, 254, 257–58, 334, 365
Leucoraja, 125, 131, 252, 290
  erinacea, 125, 131, 252
  ocellata, 290
levamisole, 122, 131, 271, 274, 299, 302
lewini, 5–6, 8–9, 11–13, 51, 57, 124, 171, 271–72, 274, 313, 346, 356
lice, 107
life support system, 26, 37, 61, 77, 90, 103, 114, 174, 179, 182–86, 189, 226, 231, 393, 406–7
light, 30–31, 34, 81–82, 184–85, 191, 265, 267, 341, 343, 368, 370, 393–94, 413, 421, 424
lilacinus, 118, 272
limbatus, 27, 57, 128, 212, 220, 294, 309, 319, 441, 461–64
Litopenaeus, 45, 72, 326
  setiferus, 72
  vannamei, 72
little skate, 131, 252, 296, 302
little tunny, 26–27, 426
liver, 116, 118–20, 122–24, 139–40, 156, 272, 279–80, 305–7, 321–22, 324, 351, 353, 355, 427, 429
Loligo, 45, 48, 93, 224, 326, 350, 426
  vulgaris, 224, 350
longevity, 11–12, 23–25, 31, 41, 46, 237, 245, 357–58, 394, 405, 455
longnose skate, 457–58
long-tailed river stingray, 106

LSS, 37, 211, 226, 404, 406–7
lucens, 20
Lumbriculus, 105
  variegatus, 105
Lumbricus, 105
  terrestris, 105
Lutjanus, 27
  campechanus, 27
lymma, 125–26, 132, 211, 214, 216, 299, 305, 325–29, 347, 365–66
lymphocyte, 84, 265, 351
maccoyii, 5–6
mackerel, 2, 11, 83, 98, 116, 139, 141, 153, 350, 399–400, 413, 426, 431
macrothela, 69, 73, 132
maculatus, 124, 142, 305, 316–18, 346
Madecassol, 48, 50
magnesium, 39, 71, 74, 136, 138, 298
magnetic field, 170, 175
magnetic resonance imaging, 251, 303, 306–8
Mallotus, 83, 104, 116, 139, 141, 326, 413, 426, 452
  villosus, 83, 104, 116, 139, 141, 326, 413, 426, 452
malnutrition, 64
Manta, 51, 59–65, 142, 151, 220, 256, 258–60, 267–68, 314, 319, 366, 376
  alfredi, 59–60, 65, 256, 258, 366
  birostris, 59, 142, 151, 220, 256, 267, 314, 319, 376
manta ray, 65, 256, 314, 319, 376
marbled stingray, 334
marbofloxacin, 350–51, 353, 355–56
marcescens, 117, 129, 286
markgracei, 122, 132, 270, 274
marmorata, 334
MatriStem, 295
maturity, 16, 76–77, 326, 329–30, 376, 378, 380, 383, 389, 391, 412, 422, 435–37, 451, 454–58
maximus, 307
Mazuri, 7, 27, 63, 72, 82, 93–94, 104–5, 116, 138, 146, 160, 413
mechanical filtration, 45, 80, 89, 184
medetomidine, 56, 331, 334–37, 339–40, 342, 344, 346, 348
medical, 49–50, 62–63, 67–70, 96–97, 102, 113–15, 131–32, 190, 198, 200, 203–4, 219–20, 241–42, 259–60, 295–96
medication, 19, 104, 108, 121, 206, 219, 266, 292, 295–97, 300
Megalops, 27, 30
  atlanticus, 27, 30
megalopterus, 366
megestrol acetate, 116
Melanogrammus, 83
  aeglefinus, 83
melanopteri, 122
melanopterus, 13, 57, 122, 128–29, 142, 212, 221, 277–78, 280–81, 283–86, 292, 346, 355, 443–49, 460–63
melleni, 266
Melogonimus, 69, 73
  rhodanometra, 69, 73
meloxicam, 361
Menidia, 59, 63, 93, 413, 426
  menidia, 59, 63, 93, 413, 426
meningoencephalitis, 118, 124, 129, 282, 284
Merlangius, 83, 172
  merlangus, 83, 172
merlangus, 83, 172
Merluccius, 45, 83, 224
  capensis, 224
metabolic, 2, 5–6, 8, 10–13, 166, 251–52, 254, 291, 297, 337, 360, 426
metabolomic, 249–52, 262
metazoan, 113, 128–29, 264, 266, 273–74, 430–31
methanesulfonate, 40, 333, 347, 355–56, 360–61
methylprednisolone, 48–49
methylquinoline, 333
metomidate, 343
metritis, 118, 128, 357–59
meyeni, 125, 129, 132, 142, 308, 319, 334
Miamiensis, 124
  avidus, 124
michiae, 69
Microbothriidae, 117, 128, 132, 274, 302
microcephalus, 33–42
microchip, 17, 107
microdon, 76–78, 80, 82–85, 142, 171, 292, 346
Microsporidia, 119
midazolam, 17, 256, 259–62, 291, 335
mineral, 82, 105–6, 135–41, 143–45, 270
Mitosyl, 48
Mobula, 51, 64, 142, 299, 315, 334, 366
  hypostoma, 64, 142, 299, 315, 366
  kuhlii, 334
Mobulidae, 59, 65
mobulid, 59, 65, 90, 151, 268
mokarran, 54, 142, 272
Monocotyle, 69, 125
  ancylostomae, 69
Monocotylidae, 117, 132
monocyte, 84, 351
monogenea, 73, 128–29, 132, 275, 297, 418
Monopisthocotylea, 266
morbidity, 114, 117–19, 123–24, 126–28, 269
mordax, 104, 413
morhua, 145, 337
Morone, 452
  saxatilis, 452
morphometric, 16, 18–19, 40, 51, 107, 150, 214, 254, 418, 422
mortality, 44, 47, 114, 117–19, 123–24, 126–30, 132, 140, 230, 272, 274, 379, 423, 425, 429
motoro, 100, 104, 109–10, 127, 213, 366, 368–69

MRI, 306–7, 323–24
MS-222, 40, 107
mucometra, 357, 359
Mucor, 118, 272–73, 427
  circinelloides, 118, 272, 427
Mugil, 154, 394
  cephalus, 154, 394
Mugilidae, 83
mullet, 83, 154, 394
multi-meter, 173–75
multivitamin, 27, 48, 427
Mustelus, 123, 128, 131–32, 142, 145, 171, 272, 278, 280, 285–86, 305, 334, 340, 387, 440
  asterias, 278
  canis, 123, 128, 131–32, 142, 145, 171, 272, 285–86
  henlei, 305, 340, 387, 452
  mustelus, 280, 334
mycobacteriosis, 118, 128–29, 285–86
Mycobacterium, 102, 129, 131, 190, 281, 286–87
mykiss, 104, 167
myliobatid, 105, 118, 126, 196, 263, 265, 434, 442
Myliobatidae, 126, 129, 185, 213, 262, 274
Myliobatiformes, 198, 305
Myliobatis, 127, 132, 151, 366, 368–69, 452
  aquila, 366
  australis, 368–69
  californicus, 452
  freminvillei, 127
myoinositol, 139
Myoxocephalus, 35, 130
  scorpius, 35
myxozoa, 118–19
naltrexone, 336–37
nana, 106, 111
Narcine, 268
Narcinidae, 213
narinari, 56, 126, 129, 142, 147–51, 264–68, 274, 283, 294, 297–99, 301–2, 329, 334, 347–48, 433–42
Narkidae, 213
natural seawater, 24, 36, 82, 137, 230–32, 382, 392–93
naucrates, 30
Nebrius, 257–58
  ferrugineus, 257–58
necropsy, 34, 40, 50, 69, 71, 114, 120, 129, 137, 242, 272, 286, 429
Negaprion, 5, 8–9, 11, 14, 71, 121, 129–30, 132, 135, 151, 301–2, 305, 356, 364, 373
  acutidens, 8, 259
  brevirostris, 5, 9, 11, 14, 71, 121, 129–30, 132, 135, 151, 301–2, 305, 356, 364, 373
nematode, 121–22, 128, 263–64, 281, 427
neoaetobatidis, 69, 74
Neobenedenia, 266, 299
  melleni, 266
Neodermophthirius, 117, 121–22, 132, 274, 302
  harkemai, 121–22, 132, 274, 302
Neoentobdella, 117, 125
neonate, 83, 406–7, 413, 424–25, 429–30, 434, 443, 448, 451, 453–57, 459, 461
Neotrygon, 105, 125, 299, 347
  kuhlii, 105, 125, 299, 347
Neotrypaea, 105
  californiensis, 105
Nesippus, 69, 73
  vespa, 69
neutrophil, 84, 351
NH3, 184, 425
nightcrawler, 110
nigrellii, 122
nile tilapia, 27, 293
niloticus, 27, 293
nimble spray crabs, 72
nitrate, 62, 82, 90, 103, 106, 137, 225, 253, 279, 302, 326, 350, 408, 425
nitrite, 39, 62, 82, 90, 326, 408, 425
nitrous oxide, 304, 337
noise, 48, 159–61, 163–67, 169, 171, 175–76, 226, 252
northern pink shrimp, 72
North Pacific krill, 93
Notorynchus, 9, 13, 27, 32, 129, 213, 305, 307, 321, 329, 363, 367, 369, 373, 412
  cepedianus, 9, 13, 27, 32, 129, 213, 305, 307, 321, 329, 363, 367, 369, 373, 412
novaezelandiae, 399
nursehound, 123, 277–78, 283, 348
nurse shark, 151, 157, 196, 206–7, 212, 254, 258, 348, 350, 356, 387, 389, 391, 400–401, 409
nutrition, 70, 72, 74, 98, 100, 104–5, 111–12, 135–38, 140, 144–46, 160, 251–52, 427, 431, 440–41
Nybelinia, 69, 74
  southwelli, 69
obesity, 83, 106, 151, 249, 447
obesus, 57, 121–24, 128, 130, 196, 213, 293, 306–8, 314, 323–24, 365–66, 369, 373
obligate ram ventilator, 44, 68, 354
obscurus, 151, 212, 334–36
ocellata, 290, 301
ocellated eagle ray, 438
ocellate river stingray, 213
ocellatum, 124, 129–32, 142, 179, 252, 287, 347, 366, 369
ocellatus, 126, 438, 442
octodiscus, 127, 129
octopus, 27, 224
Odontaspididae, 121, 388, 400
Odontaspis, 145, 156, 307, 348, 388, 400
  taurus, 145, 156, 307, 348, 388, 400
ogac, 35
olivaceus, 72
Ommatokoita, 38, 41
  elongata, 38, 41

Oncorhynchus, 2, 83, 104, 167, 452
mykiss, 104, 167
tshawytscha, 2, 452
oocysts, 264–66, 283
oomycota, 107, 118
oophagy, 13, 153, 305, 376, 392
oophoritis, 118, 128
opalescens, 83, 452
opalescent squid, 83
operant conditioning, 28, 59, 61–62, 64, 83, 108, 164, 196, 209–10, 355
opioids, 332
orangespot surgeonfish, 72
orbignyi, 104, 111
Orconectes, 105
orectolobid, 124
Orectolobidae, 124, 213, 254, 400
Orectolobus, 57, 124, 132, 142, 253, 268, 305, 316–18, 346, 371, 383
japonicus, 57, 124
maculatus, 124, 142, 305, 316–18, 346
ornatus, 124, 132, 253, 371, 383
Oreochromis, 27, 166, 293
niloticus, 27, 293
organophosphate, 69, 126, 270
orientalis, 5–7, 73
ormetoprim, 266
ornate wobbegong, 57, 124, 132, 253, 366, 371
ornatus, 57, 124, 132, 253, 371, 383
ORP, 184
Osmerus, 104, 139, 326
eperlanus, 139, 326
mordax, 104
osmoregulation, 169, 246, 254, 356
Osteoglossum, 104
ovariectomy, 357, 359–62, 372
overdose, 79, 259, 299, 341
oviduct, 223, 326, 360, 370, 423, 435–36
oviparous, 223, 305, 308, 330, 364, 371–72, 380, 389, 421, 451, 457
oviposition, 423–24, 451, 455
ovoviviparous, 391, 412
oxygen, 1–4, 37–39, 45, 55–56, 60, 78–82, 89, 101, 114–15, 121, 184, 260–61, 344–45, 347, 359
oxygenation, 37, 45, 54, 63, 79–80, 129, 323
oxyrinchus, 5, 8–9, 11, 13–14, 27, 32, 44, 254, 305
oxytetracycline, 70, 126
ozone, 61, 82, 90, 114–15, 131, 184, 253, 299, 326, 393, 405
pacifica, 20, 59, 63–64, 93, 232, 454
Pacific angelshark, 411, 413–15, 417, 419
Pacific bluefin tuna, 5–6, 11
Pacific electric ray, 452
Pacific herring, 413
pacu, 104, 356
Paecilomyces, 118, 272–73
lilacinus, 118, 272
Palaemonetes, 105, 110
pallasii, 413
palpation, 165, 306, 340
pancreas, 280, 284, 305
pancreatitis, 118, 125
Pancreatonema, 123, 125, 128
americanum, 123, 125, 128
Pandarus, 69, 122
cranchii, 69
smithii, 69
Panulirus, 72
argus, 72
papillomatosis, 285
Paralebion, 122, 128
elongatus, 122, 128
parasite, 69, 114, 117–18, 121, 123, 125–27, 130, 132, 264–66, 268, 274–75, 285, 297, 299, 302
Paratrygon, 100, 104, 106, 111
aiereba, 100, 104, 111
parthenogenesis, 384, 387–89, 423, 430, 432, 441, 459–64
parturition, 326–27, 329, 359, 361, 365, 378–79, 381, 392, 394–96, 398–99, 411–12, 435, 437–41, 443–46, 448–49
pastinaca, 125, 130, 346
paternity, 154, 383–84, 387, 389, 423, 440–41
pathogenesis, 245, 247–48, 251, 269, 272
paucimaculatus, 369–71
pectinata, 75–78, 80, 82–85, 376
pelagic, 4–8, 10, 12–13, 44, 48, 50, 69–70, 72, 119, 121, 125–26, 148, 150–51, 220, 392–93
pelagic stingray, 5, 13, 302, 330
Penaeus, 45, 72, 326
duorarum, 72
penneri, 122, 128
Peprilus, 116
triacanthus, 116
Percnon, 72
gibbesi, 72
pericardium, 119, 122
peritoneum, 264, 353, 355, 360–61
perotteti, 76, 85
Petalodistomum, 427, 429
largus, 427, 429
pH, 4, 38–39, 60, 62, 82, 89–90, 101, 103, 106, 114–15, 124, 126, 247, 257–58, 408
pharmacodynamics, 289, 291
pharmacokinetics, 289–91, 300–302, 356
pharmacology, 120, 246, 254, 262, 289, 291, 293, 295, 297, 299, 301–2, 337, 348, 356
phenoxyethanol, 56–57, 331, 333–34, 337, 347
Philasterides, 124, 282, 287, 427, 432
dicentrarchi, 124, 282, 287, 427, 432
phosphorus, 84, 136, 258
Photobacterium, 117
damselae, 117
photoperiod, 39, 75, 81–82, 84, 109, 185, 329, 378–

79, 382–83, 393, 408
physical exam, 107
Piaractis, 104
piked dogfish, 125, 198, 290, 322, 348, 452
pipinna, 125
Piscicolidae, 118, 132
pisciphila, 118, 272, 274, 427
piscivorous, 105–6, 111, 156
PIT, 114, 225, 414
plagiosum, 124, 142, 179, 252–53, 296, 302, 318, 369–71, 373, 423, 432, 463
plankton, 87
plasma, 40, 120, 129–30, 132, 137, 144–45, 166, 252–54, 257–58, 290, 365, 370, 373, 380, 388–89
platyhelminths, 266, 269, 273
Platyrhinoidis triseriata, 366
Pleocyemata, 83
Plesiotrygon, 100, 106, 111
iwamae, 106, 111
nana, 106, 111
plumbeus, 9, 12, 26–28, 30, 32, 122, 128–29, 131, 270–71, 274, 299, 341–42, 344, 346–51, 354–55
Poecilostomatoida, 118
pole feeding, 6, 29, 83, 226
polyandry, 154, 376, 384, 419, 437
polychaete worm, 224
Polyonchoinea, 266
polyopisthocotylean, 117
Pomatomus, 8, 14, 32, 394
saltatrix, 8, 14, 32, 394
ponazuril, 118, 127, 266
Pontobdella, 69, 73, 125
macrothela, 69
porcupine ray, 213
porkfish, 121
Poroderma, 223, 227
africanum, 223, 227
Port Jackson shark, 130, 252, 278, 281–82
portusjacksoni, 124, 130, 252, 278, 281–82, 284, 364, 368
positive reinforcement, 32, 117, 164, 196, 210–11, 220–21
posterocolpa, 121, 126, 132, 263, 266–67, 275, 299, 302
post-partum, 69, 327
post-release, 2, 8, 23, 166, 230, 233, 241–42, 356
Potamotrygon, 100, 102–4, 110–11, 121, 127, 179, 213, 366, 368–69, 372
falkneri, 111, 372
henlei, 104
leopoldi, 104, 127
motoro, 100, 110, 127, 213, 366, 368–69
orbignyi, 104, 111
schroederi, 100, 111
tigrina, 104, 111
Potamotrygonid, 102, 109, 111, 185
Potamotrygonidae, 99–100, 110–12, 127, 213
prawn, 225
praziquantel, 55, 69, 78, 117, 121, 126, 129–30, 132, 150, 267–68, 274–75, 291, 296–99, 301–2, 418
pre-copulatory, 156, 388, 392, 422, 437
pregnancy, 109, 150, 305, 326–27, 329, 359–60, 364–65, 368, 370, 395, 434–35, 437, 440, 446–47
Prionace, 43–47, 49, 51, 128, 172, 252, 305, 355
glauca, 43–47, 49, 51, 128, 172, 252, 305, 355
Pristid, 75, 84
Pristidae, 75–76, 84–85, 213
Pristiformes, 85, 171, 198
Pristis, 76, 81–83, 85, 142, 171, 268, 292, 299, 346, 376
microdon, 76, 85, 142, 171, 292, 346
pectinata, 76, 85, 376
perotteti, 76, 85
zijsron, 76, 85, 142
Procambarus, 105
productus, 125
progesterone, 365, 368, 373, 380–81
progestin, 380
prolapse, 122, 132, 254
prophylaxis, 102, 301
propofol, 256, 260–62
protein skimmer, 49, 184, 326, 406–7
Protovit, 48
protozoa, 277, 282–83, 285
Pseudocaranx, 83, 155
dentex, 83
georgianus, 155
Pseudomonas, 102, 117, 358
Pseudoplatystoma, 104
Pteroplatytrygon, 125, 302, 366
violacea, 125, 302, 366
punctatum, 124, 129, 142, 167, 179, 252–53, 260–61, 347, 366, 368–69, 371, 405–8
purpurissatus, 72
pyridoxine, 139
quarantine, 45, 47–50, 55–56, 67–70, 72–73, 77–80, 107, 113–31, 133, 246–47, 266–68, 327, 406–7, 445, 448
quinine sulfate, 78
Rachycentron, 27
canadum, 27
rainbow smelt, 104
rainbow trout, 104, 145, 167
Raja, 125, 130, 277–78, 280, 290, 296, 301, 365–66, 369, 373, 423, 431, 437, 451–52, 457–58
binoculata, 125, 457–58
clavata, 125, 213, 277–78, 280, 348, 366, 437
eglanteria, 125, 172, 252, 365–66, 369, 373, 409, 423, 431, 458
erinacea, 130, 296, 373
inornata, 6
rhina, 125, 290, 452, 458
undulata, 278, 280

Rajidae, 125, 213
ranitidine, 117
ration, 6–11, 14, 16, 27, 72, 93–94, 104–6, 108, 110, 193, 210–11, 224–26, 232, 425–27, 435
red snapper, 27
refugia, 90, 115, 124
reinforcement, 32, 103, 117, 164, 196, 210–11, 215–16, 218, 220–21
reinforcer, 210, 216–18, 225
Reinhardtius, 35
  hippoglossoides, 35
remora, 30
reproduction, 73–75, 84, 106–9, 153–57, 364, 370–73, 375–79, 385–89, 391–95, 397–401, 421–23, 433–35, 439–45, 459–60, 462–64
reproductive biology, 2, 12, 154, 326, 330, 376, 387–89, 391, 400, 430, 434, 441, 443, 455, 457
requiem shark, 53, 387, 441, 464
research, 8–11, 13, 21–22, 32–34, 73–74, 135–36, 165–66, 251–55, 356, 370–73, 384–89, 403–5, 407–9, 440–43, 457–59
restraint, 63–65, 79, 84, 96, 126–27, 165, 198, 202–5, 219–21, 292, 332–33, 335–36, 350, 352, 354–56
retifer, 123, 301, 312
Rhina, 67, 69, 71, 73–74, 121, 125, 142, 213, 220, 268, 334, 346, 365
  ancylostoma, 67, 69, 71, 73–74, 125, 142, 213, 220, 334, 346, 365
Rhincodon, 15–22, 87–89, 91–98, 159–63, 166, 213, 220–21, 229–31, 233–35, 251, 256–60, 262, 268, 306, 308
  typus, 15–22, 87–89, 91–98, 159–63, 166, 213, 220–21, 229–31, 233–35, 251, 256–60, 262, 268, 306, 308
rhinei, 69, 74
Rhinidae, 73, 125, 213
Rhinobatidae, 68, 73, 125, 213
Rhinobatos, 72, 121, 125, 128–29, 283, 286, 334, 346, 348, 365
  annulatus, 334
  cemiculus, 346, 348, 365
  lentiginosus, 125, 128, 286
  productus, 125
  rhinobatos, 72, 125, 283
Rhinoptera, 131–32, 150–51, 264, 266, 268, 274–75, 277–78, 280, 283, 301–2, 312–13, 315, 317, 361–62, 365–66
  bonasus, 131–32, 150–51, 264, 266, 268, 274–75, 277–78, 280, 301–2, 306, 312–13, 315, 317, 361–62, 365–66
  javanica, 283
Rhizoprionodon, 27, 122, 128, 130, 132, 142, 145, 166, 213, 270, 274, 380, 388
  terraenovae, 27, 122, 128, 130, 132, 142, 145, 166, 213, 270, 274, 380, 388
rhodanometra, 69, 73
Rhynchobatus, 57, 72, 142, 268, 334
  djiddensis, 57, 72, 142, 334
  laevis, 366
ribbontail stingray, 211, 325, 328, 366
riboflavin, 139
Ringer's solution, 353
robinoverstreeti, 69, 73
rockfish, 51, 452
rosette river stingray, 100
roughtail stingray, 125
round ribbontail ray, 132, 308, 319, 366
round stingray, 366
sabina, 125, 172, 179, 290, 302, 366
sablefish, 7
sagax, 399, 413, 452
sakura shrimp, 20, 160
salar, 45, 141, 166, 353
salinity, 75, 78–79, 82, 84, 115, 117, 120–21, 124, 297, 300, 326, 393, 408, 423, 425
Sally Lightfoot crabs, 72
Salmo, 45, 83, 141, 145, 166, 353
  gairdneri, 145
  salar, 45, 141, 166, 353
salmon, 2, 12, 45, 83, 141, 166–67, 353, 394, 452
saltatrix, 8, 14, 32, 394, 399
sandbar shark, 5, 9, 81, 128–29, 131, 212, 252, 274, 299, 302, 346, 348–51, 353, 355, 366
sand filtration, 61, 82, 90
sand sillago, 399
sand tiger shark, 156, 201, 203, 205, 207, 253–54, 310–11, 314, 366, 376–78, 387–89, 391–93, 395, 397, 399–401
sapidus, 72
saprolegniales, 107
sarda, 2, 7, 51, 83, 116, 141, 154, 394
Sarda, 2, 7, 51, 83, 116, 141, 154, 394
  australis, 154, 394
  orientalis, 7
  sarda, 51, 116, 141
Sardinops, 165, 399, 413, 452
  sagax, 399, 413, 452
satiation, 72, 82–83, 105, 435
sawfishes, 75–77, 79, 81–83, 85, 198, 213, 268, 292, 440
saxatilis, 452
scalloped hammerhead shark, 5, 51, 356
schroederi, 100, 111
scoliosis, 122, 247–48, 251, 304, 307, 398
Scomber, 2, 51, 116, 139, 141, 413, 426
  japonicus, 2, 51, 413
  scombrus, 116, 139, 141
Scombridae, 83
scombrus, 116, 139–41
Scopthalmus, 268
scorpius, 35
Scuticociliate, 282
Scuticociliatida, 113, 118, 123–25, 282

Scyliorhinidae, 123, 213, 223, 372
Scyliorhinus, 123, 151, 172, 213, 277–78, 280–81, 283, 290, 301, 312, 348, 369–70
canicula, 123, 172, 213, 277–78, 281, 290, 301, 348
retifer, 123, 301, 312
stellaris, 123, 151, 277–78, 283, 348
torazame, 123, 369–70
scyllium, 123, 368–69
seal, 12
sealei, 57
sea louse, 131, 274, 299, 302, 431
sea urchin, 35
Sebastes, 51, 452
sedation, 79–81, 181, 205, 207, 255–57, 259–61, 332–33, 335–37, 339–43, 345–46, 348, 365
sedative, 17, 256, 332–33, 335–37, 339–40, 345, 356
semen, 364, 368–71, 385, 423, 430
semifasciata, 123, 132, 142, 309, 322, 340–41, 348, 366
seminal vesicle, 267, 423, 436
sepsis, 117, 279, 281, 429
Sergeant baker, 72
Sergia, 20, 160
kishinouyei, 160
lucens, 20
Serratia, 117, 129, 286
marcescens, 117, 129, 286
serum, 132, 137, 139, 143–44, 165, 246, 248–51, 253, 299–300, 307, 349, 351, 373, 380, 388–89
setiferus, 72
sex ratio, 379, 382, 389, 399–400, 405, 434–35
sexually mature, 72, 106, 376, 391, 435–37, 440, 454
shallowwater cape hake, 224
shark wand, 201, 206
sharptooth houndshark, 366
shortfin devil ray, 334
shortfin mako shark, 5, 11, 27
shorthorn sculpin, 35
short-tail stingray, 369
shovelnose guitarfish, 125
shrimp, 20, 45, 49, 72, 104–5, 110, 139, 141, 145, 160, 214, 216, 224, 426, 429
shy sharks, 212
sicklefin lemon shark, 259
silky shark, 27, 131, 212
Sillago, 399
ciliata, 399
silver trevally, 155
silvertip shark, 53, 55, 57, 334
Siphonostomatoida, 73, 118, 131, 274, 302, 431
siphon sac, 196, 436
size-at-maturity, 451, 454–57
skate, 172, 176, 252, 290, 296, 301–2, 366, 369, 373, 384, 389, 423, 431, 451, 453–58
small-spotted catshark, 277
smalltooth sawfish, 76–77, 85, 376
smithii, 69
smooth back river stingray, 104
smooth dogfish, 123, 128, 131–32, 145, 272, 286
smooth-hound, 123, 171, 278, 305
smooth nose wedgefish, 366
sodium bicarbonate, 49, 89, 101, 298
sodium carbonate, 49, 89
solani, 118, 123, 128–29, 131–32, 271–72, 274, 283
solidissima, 454
Somniosus, 33–35, 37, 39, 41–42
microcephalus, 33–35, 37, 39, 41
sorubim, 104
sound, 54–55, 126, 159, 163–67, 211, 214, 224–26, 241, 307, 317, 319, 365, 368, 373, 456–57
South American freshwater stingray, 100, 127
South American pilchard, 399, 413, 452
southern bluefin tuna, 5–6, 12
southern stingray, 27, 130, 196, 212, 294, 314, 316, 318, 359–62, 366
southwelli, 69, 118, 126–27, 131–32, 263–65, 274, 283–86
sparsely-spotted stingaree, 369
speciosus, 72
sperm, 156, 329, 363–64, 368, 370–73, 383, 395, 398, 418, 423, 438–39, 460, 462–63
spermatogenesis, 364, 368, 373
Sphyrna, 5–6, 11–13, 51, 123–24, 128–31, 142, 270–72, 274, 277–78, 283, 285–86, 311, 313, 346, 365–66
lewini, 5–6, 11–13, 51, 57, 124, 171, 271, 274, 313, 346, 356
mokarran, 54, 142, 272
tiburo, 123, 128–31, 139, 142, 145, 151, 270–71, 274, 277–78, 283, 286, 291, 300, 305, 365–66
Sphyrnidae, 123, 213
spine, 101–2, 120, 122, 127, 198–200, 205, 379, 398
spinner shark, 334
spinning, 139
spiny butterfly ray, 130
spiracle, 104, 305
spiral valve, 119, 126, 264, 305–6, 320
Spirurida, 118
Spisula, 454
solidissima, 454
spleen, 264, 272, 280, 284, 286, 305, 321, 427, 429
sporocysts, 265
sporozoans, 78
spotted eagle ray, 147, 265, 294, 317, 366, 377, 388, 434, 436, 438, 440–42
spotted ratfish, 127, 212
spotted stingaree, 369, 373
spotted wobbegong, 124, 305, 316–18, 346
Sprattus, 45, 326
sprattus, 45, 326
Squalidae, 41, 125, 213
Squalus, 125, 128, 130–32, 142, 145, 198, 223, 254, 286, 290, 301, 322, 330, 348, 452

acanthias, 125, 128, 130–32, 142, 145, 198, 254, 286, 290, 301, 322, 348, 452
Squatina, 268, 411, 414–17, 419, 452
Squatina californica, 411, 414–17, 419, 452
squid, 45, 48–49, 56, 82–83, 104, 110, 137, 141, 216, 224–25, 227, 320, 326, 426, 429
starry smooth-hound, 278
station, 26, 28, 56, 64, 80, 83, 93, 209, 212, 214–16, 218, 277, 327, 427, 435
Stegostoma, 213, 216–18, 221, 268, 272, 274, 277–78, 315, 366, 369–70, 421, 425, 427–32, 459–61, 463–64
fasciatum, 213, 216–18, 221, 272, 274, 277–78, 315, 320, 366, 369–70, 421, 425, 427–32, 459–61, 463–64
Stegostomatidae, 124, 464
stellaris, 123, 151, 277–78, 283, 285, 348
Stenopus, 266
stereotypical, 95–96, 198
steroid, 253, 373, 380–81, 383, 388–89, 441
stingaree, 369, 373
Stoibocephalum, 69, 73
arafurense, 69
stomach, 34–35, 40, 105–6, 122, 205–6, 283, 297, 305–6, 319–20, 322, 324, 353, 355, 358, 426
Streptococcus, 117, 128
agalactiae, 117, 128
stress, 2–4, 30–31, 70–71, 79–80, 113–14, 117–18, 124–26, 151, 160–61, 164–67, 181, 193–94, 202, 217, 219–20
stretcher, 37–39, 49, 60, 63–65, 79, 88–89, 96–97, 204, 215, 219–20, 230, 233, 327, 335–36, 352–53
striped bass, 452
striped bonito, 7
striped catshark, 223, 225, 227
studbook, 76–77, 84–85, 109, 326, 377, 400, 422, 425–26, 430–31, 444–46
subconjuctival, 296
substrate, 29, 62, 78, 90, 108, 114, 119, 124–27, 185, 231–32, 267–69, 413–14, 421–22, 425, 454
sucralfate, 117, 296
sulfadimethoxine, 118, 266
sulfamethoxazole, 296
superba, 20, 59, 63–64, 91, 93, 160, 232
superba krill, 59, 63
supplement, 63, 80, 82, 140, 177, 277–78, 350, 418, 426, 444
survivorship, 2, 68, 110, 166, 241, 356, 379, 443–45, 447–49
suture, 107, 296, 353, 360–61
swellshark, 305, 321, 388
swim-and-glide, 186
swimming behavior, 6, 8, 23–24, 28–29, 39–40, 42, 47–50, 54–56, 93, 95–96, 122–23, 125, 159–65, 423, 437
swimming speed, 11, 35, 38, 95, 164, 195, 198, 394
swordfish, 44
synthetic seawater, 61, 90
Taeniura, 125, 129, 132, 211, 214, 216, 299, 305, 325, 328, 347, 365–66
grabata, 366
lymma, 125, 132, 211, 214, 216, 299, 305, 325, 328, 347, 365–66
Taeniurops, 125, 142, 308, 319, 334
meyeni, 125, 142, 308, 319, 334
tag, 2, 8, 14, 23, 25, 30, 36, 107, 114, 230, 233, 419, 452–53, 458
tapeworm, 69
target, 45, 64, 72, 80, 89, 92, 108, 114–15, 210–12, 214–19, 221, 224–26, 291–92, 330, 426
tarpon, 27, 30
taurine, 138
taurus, 57, 120–22, 137, 144–45, 153–57, 205–7, 247–49, 251–54, 306–7, 341–44, 356–59, 362–63, 369–71, 375–89, 391–401
tawny nurse shark, 258
teeth, 35, 68, 72, 75–77, 79–80, 107, 137, 178, 197–98, 206, 305
temperature, 4–6, 11–12, 35–39, 80–82, 114–15, 347–48, 371–72, 378–79, 393–95, 399–401, 408, 412–13, 424–25, 438–39, 461–62
terraenovae, 27, 122, 128, 130, 132, 142, 145, 166, 213, 270, 274, 380, 388
terrestris, 105, 110
territoriality, 28, 54
testosterone, 302, 365, 368, 373, 380, 387
Tetraphyllidea, 74, 118
therapeutic, 8, 95, 111, 246–47, 251, 289, 292, 294–96, 355
thermography, 305
thetidis, 334
thornback guitarfish, 366
thornback ray, 213, 278, 437
thornback skate, 125
thorntail stingray, 334
Thunnus, 5–7, 12, 45
alalunga, 7
albacares, 5–6
maccoyii, 5–6
orientalis, 5–6
thyroid, 140, 246, 252, 279, 305, 307, 316, 381
tiburo, 123–24, 128–31, 139, 142, 145, 151, 270–71, 274, 277–78, 283–86, 291, 296, 300, 305, 365–66
tiburonis, 123, 129
tiger shark, 23, 25, 27, 29, 31–32, 156, 201, 203, 212, 253–54, 310–11, 376–78, 387–89, 391–93, 399–401
tiger stingray, 104, 106, 111
tigrina, 104, 106, 108–11
tilstoni, 7
TL, 1–3, 16–21, 26–28, 34–38, 45–46, 53–56, 68–69, 75–77, 79–80, 223–25, 398–99, 411–15, 421–22, 451–52, 454–57

tobramycin, 49
toltrazuril, 118, 127, 266
tomography, 303, 306–7
tonic immobility, 70, 130, 203–4, 207, 218, 346, 350, 354–56, 359, 365, 373
tope sharks, 6
torazame, 123, 369–70
Torpediniformes, 198
torpedinis, 126, 130–31, 263, 268–69, 274, 299
Torpedo, 268, 452
  californica, 452
torpedo ray, 213
total gas pressure, 115
total length, 1–3, 16–21, 23–25, 34, 45, 53, 181, 183, 223, 230, 411–12, 415, 421, 451–56, 459
touch pool, 131, 173, 177–89, 253
toxicity, 101, 103, 121, 130–31, 135, 140, 145, 247, 286, 290, 348, 356
toxicoses, 280
toxicosis, 277–78, 280
trachurus, 350, 399
Trachurus novaezelandiae, 399
  trachurus, 350
train, 61, 64, 117, 210, 217–18, 220, 224, 226
tranquilizer, 332
transmontanus, 452
transport, 1–6, 24, 35–41, 43–51, 53–57, 59–61, 67–69, 73–75, 77–81, 88–89, 100–101, 112–14, 203–5, 239–40, 251–54
treatment, 49–50, 78, 117–19, 121–24, 126–29, 200, 260–61, 264, 266–67, 269–74, 290–92, 295–301, 355, 357–59, 404–7
trematode, 69, 282
triacanthus, 116
Triaenodon, 57, 121, 128, 130, 196, 213, 293, 306–8, 314, 323–24, 365–66, 369, 373
  obesus, 57, 121, 128, 130, 196, 213, 293, 306–8, 314, 323–24, 365–66, 369, 373
Triakidae, 123, 213
Triakis, 123, 132, 142, 309, 322, 340, 366, 368–69, 373, 452
  megalopterus, 366
  scyllium, 123, 368–69
  semifasciata, 123, 132, 142, 309, 322, 340, 366
tricaine methanesulfonate, 40, 333, 347, 355–56, 360–61
trichlorfon, 69, 78, 121–22, 124, 132, 267, 270, 275, 297, 299, 302
trimethoprim, 125, 296
triseriata, 366
trophonemata, 296, 305–6, 314, 319, 326, 359, 435–38
trovafloxacin, 296, 302
trutta, 394
Trygonorrhina, 142
  fasciata, 142
Trypanorhyncha, 73–74, 119
tshawytscha, 2, 7–8, 452
turnover time, 183–84, 231, 407
Tylocephalum, 69
  carnpanulatum, 69
typus, 15–22, 65, 72, 87–98, 124, 129, 142, 159–66, 220–21, 229–35, 251, 256–62, 268, 306, 308
uarnak, 125–26, 129, 142, 334, 366
ultrasound, 109, 116–17, 120, 129–30, 304–5, 307–8, 314–17, 325–30, 353, 356, 358–62, 364–68, 372–73, 435, 437–38
ultraviolet sterilization, 103
undulata, 142, 278, 280
undulate ray, 278, 280
urea, 39–40, 71, 116, 120, 298–99, 351, 354, 368, 370–71
uric acid, 84
Urobatis, 121, 129, 131, 172, 176, 213, 268–69, 274, 286, 312, 330, 366
  halleri, 330
  jamaicensis, 121, 129, 131, 172, 176, 213, 268–69, 274, 286, 312, 366
urogenital papilla, 368, 423
Urogymnus, 142, 213, 268
  asperrimus, 142, 213
Urolophidae, 213
Urolophus, 172, 369–70
  gigas, 369
  halleri, 172
  paucimaculatus, 369–70
Uronema, 50, 118
Urophycis, 83
Urotrygonidae, 127
uterine lavage, 357–59
uterus, 34, 40–41, 153, 267, 306, 308, 318–19, 327, 358–60, 362, 370–73, 383, 389, 392, 435–37
vannamei, 72
variegatus, 105–6
vasculitis, 118, 124, 280
VBGF, 413
venipuncture, 120, 127, 131, 196, 253, 317, 356
venom, 102, 111, 127, 132, 198, 200
ventilation, 4, 29, 63, 68, 78, 97, 172, 198, 204, 206, 306, 341, 345, 348, 398
ventriosum, 123, 142, 305, 321, 388
vespa, 69
vetrap, 101
vibration, 159–61, 163, 165, 167
Vibrio, 117, 130, 188, 286–87
villosus, 83, 104, 116, 139, 141, 326, 413, 426, 452
violacea, 5, 10, 12–13, 125–26, 302, 330, 366
virginicus, 121
Vitafish, 105
vitamin, 59, 63, 72, 74, 82, 94, 99, 105, 116, 119–20, 124–25, 135–45, 270–71, 426–28, 435
vitamin A, 135, 140, 144
vitamin B, 8, 116, 270
vitamin B2, 138

vitamin B6, 138
vitamin B12, 138
vitamin C, 30, 94, 116, 119, 135, 137, 140, 144, 248, 270–71
vitamin D3, 145
vitamin E, 74, 116, 135, 137, 140, 144–45, 270
Vita-Zu, 7, 27, 72, 94, 138, 160, 413, 427
vitellogenesis, 364, 380, 438
viviparous, 326, 329, 364, 371–72, 380, 435
voltage, 169–75, 280
von Bertalanffy growth function, 2, 9, 413
voriconazole, 118, 123, 273
vulgaris, 224, 350
water mold, 107, 127
water movement, 62, 90, 174, 183, 226
water quality, 48, 56–57, 61–62, 82, 89–90, 102–4, 114–15, 182–85, 189–90, 326, 393, 404, 408, 424–26, 430–31
wedgefish, 366
whale shark, 16, 19, 21–22, 88–89, 91, 94–98, 162–63, 220–21, 229–31, 233–35, 251–52, 256–60, 262, 306, 308
whip ray, 212, 366
whiptail stingray, 213
white blood cell, 120
white-blotched river stingray, 104, 127
whitecheek shark, 7
whiteleg shrimp, 72
white shark, 1–5, 7, 9, 11–14, 22, 27, 31–32, 212, 240, 306
white shrimp, 72
whitespotted bamboo shark, 318
white sturgeon, 452
whitetip reef shark, 196, 213, 293, 306, 308, 314, 323–24, 366, 369
white trevally, 83
whiting, 83, 172
winter skate, 290
wobbegong, 57, 124, 132, 213, 253–54, 305, 316–18, 346, 366, 371
wound, 28, 30, 70, 84, 102, 198, 200–201, 206, 295–97, 301, 313, 317, 352, 354, 356
xylaxine, 340
yellowfin tuna, 5–6, 11
yellow ray, 268–69
yellow stingray, 129, 172, 176, 213, 286, 312, 366
yellowtail horse mackerel, 399
yolk, 110, 267, 305–6, 325, 327–28, 371, 392, 412, 423–25, 453–54
young-of-the-year, 2, 12, 31, 68–69
zebra shark, 198, 272, 278, 315, 320, 366, 369, 377, 421, 425, 431–32, 441, 459, 461, 463–64
zijsron, 76–78, 80, 82–85, 142
zinc, 48, 50, 135–36, 138, 248, 280, 299
zoonosis, 184, 187–88