Acquisition, Husbandry, And Veterinary Care Of Whale Sharks (*Rhincodon typus* Smith 1828*)* In An Aquarium Setting

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Abstract

Whale sharks, *Rhincodon typus* Smith 1828, are circumtropical planktivorous sharks and the largest fish in the ocean, yet remarkably little is known about this species. Long term management of a population of whale sharks in a large public aquarium in Atlanta, Georgia, USA, has provided new opportunities to gather biological data and develop appropriate species-specific husbandry techniques. For example: we have shown that they can be transported safely and effectively across intercontinental distances; they are amenable to operant conditioning; they can adapt to a range of diet delivery methods; they display remarkably diverse behavioral repertoire in the aquarium; and they can be safely and routinely handled for veterinary procedures, provided that careful consideration is given to restraint techniques for staff and animal safety. Previous research on whale sharks has focused on estimates of population size, migratory behavior and stock structure, but the maintenance of whale sharks in aquariums has afforded a greater physiologic and biological understanding of this incredible species. Future efforts with aquarium-held whale sharks should aim to build conceptual models of the physiology and homeostatic mechanisms of whale sharks, as well as documenting their sensory biology, behavior and maturation/reproduction process. This greater understanding of whale sharks serves to make these animals important ambassadors for international efforts concerning elasmobranch conservation and research.

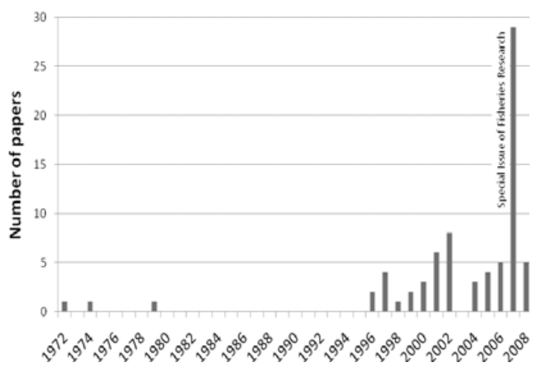
Key words: *Rhincodon*, whale shark, aquarium, husbandry, veterinary, transport

Introduction

Relative to bony fishes, remarkably little is known about the biology of whale sharks, despite their being the largest piscine species inhabiting the world's oceans. Colman (1997) was the first to review the biology of *R. typus*, citing just 34 peer-reviewed papers specifically about the species, which were evenly scattered over a 72 year period since 1925. A combined search of BioOne Abstracts, MEDLINE, and Aquatic Sciences and Fisheries Abstracts using the search term "*Rhincodon*" in the title field (as of June 15, 2009) returned 72 hits from peer-reviewed sources since Colman's review, which marks a 12-fold increase in publication rate (see Figure 1). Although these data are somewhat biased because 17 of these manuscripts appeared in the same 2007 special issue of Fisheries Research on the 2nd International Whale Shark Conference, there has nonetheless been a clear increase recently in scientific interest in this species. Two papers have reviewed changes in the state of whale shark science since Colman's paper, Stevens (2007) and Martin (2007), but at just 6 and 7 pages respectively, there remains relatively scant peer reviewed data in the literature.

The overwhelming majority of published research on whale sharks to date has been purely observational and has focused on isolated records and observations of animals at different locations, estimates of population sizes and attempts to track migratory behavior. These studies have shown that whale sharks may roam tremendous distances (Eckert and Stewart 2001), so it is no

Figure 1: Publication trends regarding research on whale shark biology, 1972-2009.



surprise that the two genetic studies that have explored stock structure in this species have shown that they either form a single panmictic population (Schmidt et al. 2009) or that gene flow is high within ocean basins (Castro et al. 2007). Other observational studies have examined diet composition based on stomach contents (Jarman and Wilson 2004), and growth and reproduction (Joung et al. 1996). One remarkable finding is that of a peculiar deep-diving behavior that whale sharks often display when swimming over open ocean water of abyssal depths (Graham et al. 2006, Brunnschweiler et al. 2009). These dives may exceed 1,500-meters in depth, or roughly 150 atmospheres of pressure, as well as spanning a temperature range of 25°C or more. The motivation for this behavior must be compelling because the physiological consequences are profound. Because specific physiological adaptations of this species are largely a mystery, these behaviors remain enigmatic and difficult to investigate. No studies to date have investigated the physiology or sensory biology of this species.

Acquisition and Transport

During the design phase for the Georgia Aquarium, whale sharks were identified as a key species for a meso-American open ocean exhibit conceived as the largest single fish aquarium yet built. Including the largest of all fish species was intended to provide an engaging guest experience and an unparalleled opportunity to educate the public about sharks and other pelagic species, as well as to gather new data about the biology of this species. Whale sharks had not previously been collected for display in any aquarium outside East Asia, nor had whale sharks ever been transported across intercontinental distances. Given the size of this species, the logistic challenges of such a transport were significant and are thus described here.

To assemble the Georgia Aquarium collection, two whale sharks were transported from Taiwan to Atlanta on each of three occasions between 2005 and 2007 (see Figure 2). The process began near Hualien, Taiwan, where animals were captured as part of a mixed-species pelagic trap net fishery that occurs in near-shore deep waters of Hualien Bay. After capture, whale sharks were held in deep-water net pens for 1 - 4 months of preliminary training and so that logistics could be arranged for the transport to Atlanta USA (Figure 2A). During this period, animals were desensitized to the presence of humans and trained to feed on krill dispensed manually from colored 1L plastic ladles attached to long poles, although some feeding on natural prey items that drifted through the holding pen was also observed. Animals of 4-4.5m in length were chosen for transport because they are at the smaller end of the size range captured in the Hualien Bay fishery, and more easily transported than larger animals, which commonly exceed 9m in length.

At the time of transport, the whale sharks were corralled into individual vinyl stretchers (Figure 2B) and lifted by deck crane into a temporary sea-water filled container on-

Figure 2: Long distance transport of whale sharks. **A:** Whale shark in sea-pen prior to transport; **B:** Loading the animal into the stretcher to be lifted into a temporary transit box for transport to harbour; **C:** Loading the animal into the transport container; **D:** Two transport containers in the cargo hold of the 747 aircraft; **E:** Animal and water are lifted from the transport container into the new aquarium; **F:** Divers guide the release of the whale shark into its new exhibit.

board a boat that transported the animal to Hualien harbor.

This was the only time when it was necessary for the animals to be lifted totally out of the water; all subsequent lifts were made with a "bladder" stretcher (see Figure 2C), wherein the animal remained immersed in water to provide good support to the body. When the transport boat reached harbor, the animals were transferred by crane and bladder stretcher into shipping boxes in the back of a semi-trailer truck (Figure 2C). These shipping boxes were custom-made fiberglass rectangular tanks 6.1m long, 2.1m deep and 2.3m wide, with integrated life-support systems built into the walls and lids, featuring paper cartridge filters, activated carbon to absorb organic wastes, and compressed oxygen supplementation. The truck was driven to Hualien Airport, for a short flight to Taipei, followed by transfer to a Boeing 747 for the longer leg from Taipei to Atlanta via Anchorage Alaska, a distance of approximately 13,000km.

After clearing customs and quarantine inspection in Atlanta, the shipping containers were loaded onto a semi-trailer truck and driven 14-km to the Aquarium. The boxes were then unloaded from the truck and the acclimation process began. After several gradual water changes and chemical additions so that transport water more closely matched exhibit water in temperature, pH and ammonia concentration (NH_3-N), a bladder stretcher was carefully slid under the animal and the whale shark, along with about 4,000L of water, was lifted from the shipping container (Figure 2E) and re-leased into the exhibit (Figure 2F).

Throughout the transport process the temperature, pH and dissolved oxygen (DO) concentrations in the transport boxes were monitored continually (see example in Figure 3) using Hach HQ40d handheld multi-probe meters (Hach Co., Loveland CO). NH_3-N ammonia concentrations were tested periodically using the salicylate-cyanurate method on a Hach DR890 portable colorimeter. Corrections to pH were made using bolus additions of sodium carbonate, whereas excess NH_3-N was ameli-orated using Amquel®Plus (Kordon LLP, Hayward CA) which binds ammonia into stable non-toxic amino-methanesulfonate. From early experiences it was learned that aggressive pH management early in trans-port provided the least amount of pH variation and minimized the time needed for acclimation at the Aquarium.

Figure 3: Example of a pH track in a transport box during international transport. The pH declined at the start of the transport and was rectified by 11 periodic additions of sodium carbonate, the effects of which were manifest as sharp increases. Over time, the rate of decline between additions dropped, partly as a result of increased alkalinity from carbonate additions, and partly as a result of decreased metabolism on the part of the animal, which calmed significantly after an initial excitement phase at being first placed in the transport box.

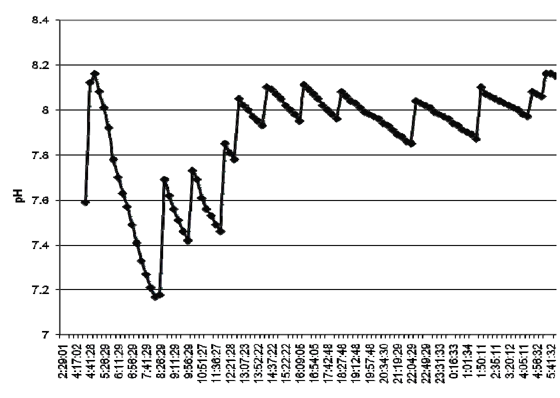


Exhibit design

The Ocean Voyager exhibit at Georgia Aquarium is presently the largest indoor fish aquarium in the world at approximately 24 million liters (6.3 million US gallons). The system is designed for long term exhibition of a large and diverse collection of teleosts and elasmobranchs, including large pelagic elasmobranchs such as whale sharks, great hammerhead sharks and manta rays. The tank is dumbbell-shaped in plain view, with a maximum depth of 10m deep and a minimum depth of 7-meters. The dumbbell shape of the exhibit is important for appropriate swimming behavior in elasmobranchs; it enables animals to turn both left and right as they circle the exhibit, which ensures healthy use of epaxial and hypaxial musculature on both sides of the body. The tank substrate is a mix of formed concrete rockwork, calcareous sand in some areas and siliceous beach sand in others. The walls are vertical epoxy-coated concrete. Public viewing occurs through nine acrylic windows, ranging from overhead domes to a tunnel that traverses the narrowest part of the tank, and a single large 23-meter wide by 10-meter high viewing window that looks down the long axis of the tank from the deep end. Lighting is provided through a combination of natural light from an overhead skylight and high intensety metal halide light fixtures in a range of color temperatures. There is a 300,000-L holding pool that is separated from the main exhibit by a gate at the shallow end, maintained on the same life support system.

The whole system, including the life support components, holds approximately 24 million liters of synthetic seawater and operates as a closed recirculation system. Primary water treatment occurs first by protein skimming and high-rate sand filtration driven by 70 fiberglass composite-bladed centrifugal Fybroc® pumps (36 @ 25hp, 34 @ 50hp; Met-Pro Corp., Telford PA), followed by ozone disinfection (up to 30% side stream) and sulfur-based autotrophic denitrification (<1% side stream, Zhang and Zeng 2006). Appropriate gas balance is main-tained by a large nitrification/de-aeration tower (1.5 million liters water capacity) containing a 2.5-meter thick layer of high surface area AccuPac® plastic media (Brentwood Industries, Reading PA) with a total surface area of 107,000m^2; from there, water is then gravity-fed back into the exhibit. Total system flow is 484,000 liters per minute, which results in a system turnover of around 1hr. A recovery system captures backwash water from sand filters, treats it by a combination of 2nd stage sand filters, protein skimming and ozone and then returns it to the system, reducing the need for addition of new water. A motorized catwalk that spans the width of the tank, suspended from ceiling rails, can be moved along the full length of the exhibit, allowing staff access to any area of the surface. In addition, two motorized gantry cranes facilitate the suspension of heavy items (including whale sharks) in the exhibit; these also provide lifting capacity for animals and equipment through a hoistway from the loading dock 2 stories below.

Whale shark husbandry

Despite those complications associated with size, whale sharks have proven very amenable to long term care in the aquarium setting. Animals held under conditions described above have shown growth of 0.3 - 0.5 m in length per year. They are relatively docile and not apparently prone to panic and “wall strike” (high speed flight or fright swimming, impacting a wall or other object) like other pelagic species in captivity such as scombrids, and their behavior can be modified using operant conditioning methods. Both traits help make the whale shark more manageable than many other pelagic species of teleosts and elasmobranchs kept in aquariums.

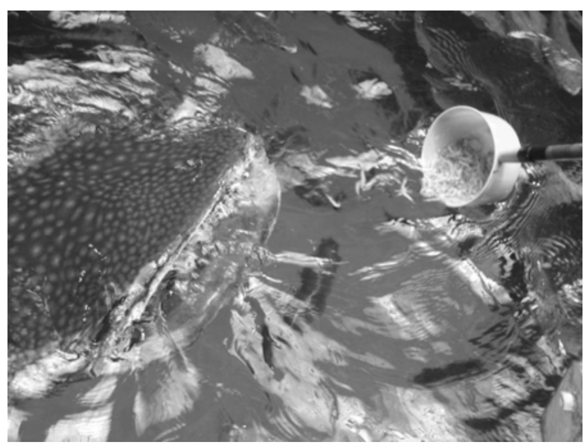
Successful feeding of whale sharks has been achieved using several approaches. The most common is by a static station, wherein whale sharks circle or swim in figure-8 patterns at a fixed point, at which a biologist can trail food back and forth, just ahead of the mouth, using a plastic ladle on a long pole (Figure 4). For larger animals, however, this approach is impractical, and a different method was developed wherein a biologist in a small inflatable raft maneuvers along a tagline, trailing food for the animal, which swims alongside the raft. It is difficult to estimate the ration as a percentage of body weight due to the challenge of accurately weighing the specimens, but even using conservative weight estimates, a figure of around 0.3-0.5% of body mass per day is likely (a 7-meter whale shark eats approximately 8 kg per day). This is modest for a growth ration in such a large species in warm water, which suggests that they are very efficient at feed conversion, and/or efficient swimmers. Diet in the aquarium setting consists primarily of commercially-harvested *Eu-phausia superba* and *E. pacifica* krill, sup-plemented with lance fish (*Ammodytes americanus*), juvenile silversides (*Menidia menidia*) and vitamin supplemented Mazuri® aquatic omnivore gel diet (Purina Mills LLC, St Louis MO).

Reproductive behaviors have not been observed in the whale sharks at Georgia Aquarium because, despite their size, all specimens are immature at the time of this writing. Reproductive maturation is expected to occur at around a 9-meter length in females and perhaps slightly less in males. Early signs of differentiation of the claspers were seen during necropsy in the first two males (see below) at body lengths of around 6.5 - 7.0m; this secondary sex characteristic heralds the onset of sexual maturation in other elasmobranch species.

Veterinary care.

The first two animals acquired in 2005, both males, stopped eating regularly in late 2006 and shortly thereafter stopped eating altogether; these individuals subsequently died in January and June of 2007. While a definitive cause of death could not be determined, their inappetence coincided with repeated exposures to tri-chlorofon (Dylox® Bayer AG, Germany), an organophosphate parasiticide that was applied at aquaculture industry standard doses to control a leech parasite infecting demersal elasmobranchs in the exhibit. Two female whale sharks that received fewer trichlorofon exposures, and two male sharks acquired later that received no treatments, have not displayed similar signs. These facts when taken together suggest that this species may be exquisitely sensitive to organophosphate compounds.

Figure 4: Whale shark feeding. Krill are dispensed using a ladle and the whale shark actively sucks in the food with the surface layer of water.



The decline and eventual death of the first two male specimens prompted the development of techniques to facilitate veterinary examination, diagnostics, and treatment of living whale sharks. These techniques span the husbandry and veterinary gamut, including restraint, anesthesia, phlebotomy, diagnostic imaging (ultrasound, endoscopy), injections, gavage-feeding, and biopsy. Anesthesia was used initially, employing approximately 75ppm MS-222 (Tricaine methanesulfonate, Finquel® Argent Laboratories, Redmond WA) in solution, but more recently, sedation using hyperoxia (120-150% saturation) has proven sufficient for most veterinary needs.

Research opportunities

Assembling a collection around a flagship species like the whale shark has provided an opportunity to bring significant research attention to an understudied species. This endeavor has been in two major spheres: field research in Mexico and in-house clinical research focusing on these animals.

In 2004, it was scientifically documented that whale sharks gather annually in the summertime, close to the coast of the Mexican state of Quintana Roo at the northeastern tip of the Yucatan peninsula. A burgeoning ecotourism industry in this area now takes advantage of this annual gathering, which occurs in response to high local plankton densities, in turn a result of tropical upwelling adjacent to the Yucatan coast (Merino, 1997). Project Domino (Proyecto Domino) is a multi-institutional research effort led by the Mexican government that aims to better understand the nature of whale shark aggregations in this area. In partnership with the Shark Research Group at Mote Marine Laboratory and the University of Southern Florida, Georgia Aquarium has contributed to this effort through support and participation in annual tagging activities, using both visual and satellite (PSAT and SPOT) tags (Wildlife Computers, WA), aerial surveys, and research on diet composition and functional feeding

Restraint is labor intensive and difficult and requires the use of divers, snorkelers, and "land-based" staff to corral the animal in a vinyl stretcher suspended in the exhibit. anatomy. These studies contributed to the establishment in May 2009 of the 146,000 hectare *Reserva de la Biosfera Tiburón Ballena*, or Whale Shark Biosphere Reserve, extending an existing marine and terrestrial reserve called Yum Balam towards the north and east to encompass the area in which the whale sharks aggregate (Mexican Government, CONANP department: http://www.conanp.gob.mx/reservas_biosfera.html).

In-house research efforts with whale sharks have focused on information gathered during veterinary examinations, as well as studies that seek to develop an ethogram of whale shark behaviors and shed light on behavioral aspects of their ecology. To establish normal baseline parameters for blood cell composition and serum chemistry analysis in captive *R. typus*, clinical studies have used standard veterinary hematology and clinical pathology (Dove et al. 2010), as well as more advanced methods such as proton NMR spectroscopy and mass spectrometry.

Future directions

The maintenance of whale sharks in an aquarium setting will continue to present opportunities to gather new knowledge about this species and about elasmobranchs in general. Specifically, research should explore the sensory biology of *R. typus* and continue to unravel the details of feeding behaviors and anatomical adaptations, so that we may better understand how whale sharks find and exploit concentrated patches of plankton (and each other) in the vastness of the open ocean. Studies should also aim to build a conceptual model of the physiology of this species. Field efforts should seek to understand the extraordinary crepuscular deep-diving behavior that has been observed in whale sharks, which clearly pushes physiological limits for vertebrate animals. Future studies centered on the Georgia Aquarium collection will involve continued in-house and field research efforts, but also a new direction that integrates both approaches. The aquarium setting has and should be used as a test site to develop new methods that will facilitate study of this species in the field, as has occurred in the past with other species such as dolphins (*Tursiops truncatus*). This is especially important because of the tremendous logistic challenges and safety risks associated with studying such a large animal; only when methods have been optimized in the aquarium setting can researchers go to the field with confidence that data and samples can be gathered from wild whale sharks effectively, safely and ethically. For example, hematology samples taken before and after a deep dive may uncover physiological changes that result from this behavior and may shed light on the specific adaptations required for deep diving by *R. typus.* We can build on collection-based experiences through this research and begin to better understand the biology of this extraordinary species.

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