DRUM *and* CROAKER

A Highly Irregular Journal for the Public Aquarist

Volume 39 *Jan. 2008*

TABLE OF CONTENTS
Volume 39, 2008

A Letter from the Editor: 50 Years of Drum and Croaker

Fellow Aquariophiles,

This milestone almost escaped me. My editorship has been a fun way to recharge my aquatic batteries for 15 years. It has become a comfortable habit. While the history of Drum and Croaker is briefly covered on the main page of this website, it is appropriate to reflect on where we have been, and perhaps where we are going.

The periodical began in 1958 as a mimeographed newsletter that was circulated among the small number of big-city aquarium facilities then in existence. A *what* newsletter? See http://en.wikipedia.org/wiki/Mimeograph for more information on this lost process. It was pioneered by Thomas Edison…'nuff said. A number of years ago I began recovering the old issues that are now posted on the web site hosted by the Columbus Zoo and Aquarium (only 1-2 copies remained of some). The original muddy mimeos had often been recopied on early Xerox machines which produced even muddier reproductions. Early copier paper came on a roll and had all the feel and permanence of cheap holiday wrapping paper. The resulting document was smudged, spotted, and streaked. Much of the earlier material was therefore beyond the help of OCR software and was retyped by hand by the office staff at SeaWorld Ohio. Fortunately, print shops were used for production starting in the mid 1960s, and I've been able to convert many of these to pdfs with less effort. I still plan to eventually have all old issues available online.

I first ran across a pile of old issues of Drum and Croaker in 1977 after starting graduate school under Eugenie Clark, "the shark lady". She was well acquainted with Craig Phillips, then the director of the National Aquarium in downtown Washington D.C. Craig was a D&C editor for a number of years and his unique and amazing geometrical line drawings of aquatic life can be found throughout the early issues. I contributed my first article in 1984, as an aquarist with less than three years experience. It was a design for building an underwater collecting container out of a trash can. Seemed like an important idea at the time! Shortly thereafter, the journal dropped out of site for nearly 5 years. It was initially resurrected as a commercially produced newsletter, and then finally restored to industry editorship through the moral support of the attendees of the June 1992 Regional Aquatics Workshop (all 21 of us), and the graciousness of then-editor John Kuhns, the inventor of AmQuel®.

When I started editing D&C in 1993, it was still printed on 11 x 17" paper and circulated by snail mail to a hundred or so facilities. It was collated, folded and stapled by hand with the help of my then-little kids. Not every institution had email access and the idea of paperless journals was unpopular. That soon changed. By 2000 the document was being distributed by email as a pdf file, but a few folks still needed to receive paper copies. The Columbus Zoo offered some space on its web site in 2001 and the 2002 issue was posted solely on the web, as is the practice today.

Drum and Croaker is the only periodical that effectively reflects milestones in the field that have occurred over the past half century. While archiving many of the early issues, I discovered that many of the "advances" I thought were new in the 1980s had been previously written about in the 1960s. As our field expanded many of the experienced pioneers retired or moved on to upper management and were replaced by fresh faces from other institutions. The result was a loss of "institutional memory". Basically we all began "reinventing the wheel" because communication in the early 1980s was nowhere as good as it is today. We spent a lot more time on the phone

then, and the only time we networked as a group was at the AAZPA (now AZA) national meetings, which at the time featured many husbandry-oriented paper sessions. Most of our productive meetings came at casual after-hours gatherings during these conferences, and these were the catalyst that resulted in the first RAW meeting in 1989. The AquaticInfo listserve, moderated by Brian Nelson (New England Aquarium), has since turned networking into a daily activity.

Each Drum and Croaker since 1994 has started with "D&C ## Years Ago" by Rick Segedi. By reprinting bits from the earliest issues, Rick has been able to point out how much we have changed as an industry, how much we have stayed the same, and how much we still rely on the ingenuity of individual husbandrists to care for our delicate charges. Rick was present at the birth of D&C as a young aquarist and helped pioneer the development of artificial seawater formulas. In this issue, Rick reaches back to 1958, as he did in 1994, and also updates you on the "current events" of 1978.

What is the future of Drum and Croaker? I had begun to wonder if the blogosphere was going to have an impact, but after a relatively slow year for papers in 2007, I've been flooded with contributions for 2008, and this issue is over 110 pages! Abstracts of the annual Regional Aquatics Workshops are also captured here and there is synergy between the two entities. I don't think that anything can replace the permanence and usefulness of a well written technical paper…OK, a mind-meld, maybe. Coming years will certainly bring more changes in the way we use and access data. Linking to D&C with a hand-held device was certainly not something anticipated in 1958, when vacuum tubes were still in common use, and the "transistor radio" was the iPod® of its day.

Look forward to seeing you all soon.
Pete Mohan, Editor
Manager, Living Collections
Akron Zoological Park

DRUM & CROAKER 50 YEARS AGO
and
30 YEARS AGO

Richard M. Segedi

D & C - June 1958

NEWS FROM THE AQUARIUM WORLD

Vancouver Aquarium

This new Aquarium, located in Stanley Park, has already become one of the city's leading attractions. At 8:30 P.M. on Friday, March 21, 1958, they experienced something that all of us shudder about -- the one-inch thick, non-tempered glass in the largest tank (12,000 gallons) broke and spilled water and fish into the corridor. No one was hurt and most of the fish were saved. Depth of water in the tank has been altered to provide a greater safety factor for the future.

Ocean Park (California)

The only thing left intact on Ocean Park pier is Lawrence Welk's bubble bath ballroom. Everything else is being ripped out and rebuilt with funds from the Columbia Broadcasting Company and the Los Angeles Turf Club. The amount being spent is thought to be somewhere between 7 and 9 million dollars. The "Sea Circus" section is under the guidance of Ross McBride and will contain several porpoise and marine mammal tanks as well as one large tank devoted to dual Martine-designed diving bells. "Neptune's Kingdom", another section, is a fabulous dry land aquarium in which giant models of underwater organisms move about in a natural manner with the aid of hidden motors.

D & C - Spring 1978

Japanese Aquarium Tour
Charles Farwell, Scripps Aquarium

The large number of aquaria [in Japan] presents the problem of how to see as much as possible in a small period of time. This tour would include 14 aquaria in 18 days. In addition, some free time would be available throughout the tour. [!!!]

The price for this tour will be approximately $2000 which includes airfare, ground transportation, lodging and a guide-interpreter. Two nights lodging will be at Japanese Inns, and meals will be provided for these two days. [I wonder what this would all cost today!]

Her Underwater Yard Has Plenty of Room for Marine Research
Barry Schatz, Miami Herald, December 18, 1977

[Mary Lou] Klay recently opened up the natural education resources around her and calls it the Grassy Key Marine Study Center. Her two-month old idea takes its roots from 10 years of involvement in marine projects with her former husband Jerry Klay . . . shark capturing expert.

Two large above - ground cement aquariums . . . [and] Several offshore wire holding pens temporarily house larger sharks. A nearby building houses a basic laboratory setup where baby sharks, including one nurse shark born in captivity, are more closely monitored.

Since the property was once used to hold sharks and prepare them for live shipment to universities, aquariums, and oceanariums the world over, Klay says the new study center is naturally well-suited to shark research.

D & C - Fall 1978

Planning
Marc Fleischmann, Exhibit Designer, New England Aquarium

A problem that faces many institutions and affects the smooth development of various projects is the scheduling and coordination of different departments and persons involved in the many stages from concept through construction and evaluation.

Whenever a project is initiated, a CONCEPT WORKSHEET and its associated project file [are] started by the Design department. The individual whose original idea it is completes the "concept description" block of this form. At this point, care must be taken to insure that physical characteristics of the concept are kept to a minimum so as not to hinder the design process by the introduction of preconceived ideas.

THE HUSBANDRY OF PORIFERANS IN PUBLIC AQUARIA

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Abstract

Sponges, phylum Porifera, are the simplest and oldest multicellular animals on the planet. Poriferans are also incredibly varied, compromising in excess of 8,000 species worldwide and holding title as the most chemically diverse phylum on Earth.

Despite the simplicity of these animals, their captive care and basic husbandry needs remain largely a mystery. This paper highlights the biology, collection, and husbandry for sponges in seawater systems. Restorative techniques, including fragmentation and reaggregation, for treating ailing sponges will also be explored.

Figure 1. A flower sponge, possibly of the family *Axinellidae*, demonstrates obvious excurrent canals terminating in a single osculum on each leaf of the sponge. When flow or position within the tank is changed these canals collapse and the sponge reconfigures its structure to match the changed conditions.

Introduction

Sponges are rather uncomplicated in terms of physical structure. They are made up of a series of tunnels that begin at the incurrent pores, ostia, and terminate at the excurrent pores, oscula. The outer layer of a sponge is composed of contractile cells, termed pinacocytes. These

cells allow the sponge to undergo minor shape changes and play a role in regulating water flow through the sponge by varying the diameter of incurrent openings. The pinacocytes allow the sponge to react to changing environmental conditions by modifying the shape of the sponge and orientation of ostia/oscula to conform to prevailing currents.

Oscula prove to be the most useful indicator of sponge health within captive systems. Sponges that have a combination of proper flow, suitable food, lighting, and sedimentation should soon open their oscula to begin feeding. Oscula formation should be relatively quick; changes can appear as quickly as a few hours. A sponge that does not open its oscula for more than a day or two should be evaluated, and changes in flow or location should be considered.

For the purposes of this paper, sponges can be categorized into two informal groups, amorphous and structured, based upon their oscular arrangement.

Amorphous sponges are those that do not have a highly organized oscular structure. These sponges are able to arrange their pinacocytes to adapt to various environmental conditions. Amorphous sponges can, to a degree, absorb and generate oscula from their body tissue in order to best feed within any given current. These sponges typically include the encrusting, boring, ball, rope, and rod varieties.

Amorphous sponges prove to be easier to rear than structured sponges. The pinacocytes are able to restructure the sponge to a much greater degree than those of the more highly structured and organized sponges. In the case of amorphous sponges, the outer layer of the sponge is able to absorb non-useful oscula when current or position is changed. In a similar response to change, these unstructured sponges are able to generate new oscula better suited to prevailing environmental conditions.

Structured sponges comprise those sponges that have a more organized, rigid oscular arrangement; including vase, tube, and barrel sponges. These sponges typically have one large osculum around which the body is the sponge is centered. While the pinacocytes can change the ostia on these sponges, the large osculum is in a relatively fixed position. Very slight changes to the angle and shape of the osculum are possible, but on a much greater time frame than that of the amorphous sponges.

Unfortunately, the heavily structured sponges, such as vase sponges, are unable to change their form dramatically, and therefore are at a disadvantage husbandry-wise. Careful placement is a necessity with the highly structured sponges, and a few general rules can be applied. Current should not be applied directly into an osculum; typically the best growth rates will be seen by placing the osculum perpendicular to or exactly opposite the flow. For this reason in particular, structured sponges can be considered more difficult in general, as compared to amorphous sponges.

Several species of sponge will have both an amorphous form and a structured form. In these species the amorphous form proves to be the default form, but if environmental parameters prove suitable the structured form will begin to take shape.

Collection

There are many factors to consider before collecting wild sponges. Size, location, collecting materials, local restrictions, and species should all be taken into account before collecting sponges.

Collection of very large sponges should be considered with some care. In many respects, sponges can be victims of their own success, with very large sponges being unable to meet their nutritional demands in a captive environment. In general, the smaller the specimen, the easier it is to maintain it in captivity. Small sponges will gain mass and grow as nutritional and environmental demands are met within the captive environment.

Ideally, sponges should be taken that are attached to a manageable-sized piece of rock or shell. If the sponge's attachment point to the rock is left intact, it will be prove much easier to place the sponge in the aquarium without it being uprooted by currents or other animals within the system.

Collection of sponges with intact anchors can prove difficult depending upon local restrictions on the collection of invertebrates and/or live rock. Many collection sites, including Florida and the Bahamas, restrict the collection of live rock and/or shells. With the vast majority of sponges being anchored to rock and shells, this can prove problematic.

If local restrictions or logistics do not permit the sponge to be taken with anchor intact, structured sponges can be severed at or near the base and reattached once in an aquarium environment.

Encrusting sponges can be somewhat more difficult to obtain and stay within regulations. Due to their intimate connection with substrate, it is exceedingly difficult to sever the sponge from the substrate without causing catastrophic damage to the sponge. A small piece can usually be cut from a large encrusting sponge with little effect on the health of the sponge.

Once the sponge has been freed of the substrate it is essential that the sponge be kept out of contact with air. Plastic containers, preferable with a twist lid, are ideal for this purpose, as they can be easily opened and closed underwater, and as opposed to snap-lock lids, do not run the risk of opening under pressure changes or due to improper closure.

Packing and Shipping

Special care must be taken in the packing of sponges. There must be absolutely no air in the packing bag, or there is high probability of the sponge contacting air. Sponges, especially large specimens, can begin to foul during transport, which can corrupt water quality within the shipping container. Limiting specimen size is one way to counteract the fouling of sponges during shipment. Transportation duration is also an important factor in receiving healthy specimens.

Animal distributors are beginning to understand the needs of sponges in terms of collection and packing, however not all take adequate care in keeping them submerged at all times. It is recommended that a discussion be had with distributors to explain the methods that you would prefer to see in the collection and packing of sponges, in order to ensure the greatest possible chance of survival.

Feeding

Sponges are able to trap and consume phytoplankton, bacteria, protozoans, and dissolved organic particles. Sponges are capable of trapping particles as small as 0.1 microns (Frost 1976) within the collars of their flagellated choanocytes. Sponges are further able to capture picoplankton as small as 0.2 microns and microplankton up to about 200 microns (Reiswig 1985).

A mature system is ideal for the husbandry of sponges. With the absence of a raw-water line, supplemental feeds will probably be necessary. We feed a variety of suspended unicellular algae, in sizes ranging between 2-9 microns. We feed a mixture of *Nannochloropsis oculata*, *Stephanoptera sp.*, and *Tetraselmis chuii* to all of our sponges with good results. All algal feeds are supplemented with dry baker's yeast, *Saccharomyces cerevisiae*, with a size of 5-10 microns.

Sponges need to filter bacteria and unresolvable materials from the water. For this reason, minimal external filtration is desired on systems containing sponges. The sponges themselves will consume a great deal of bacteria within a system, so ultraviolet sterilizers are usually unnecessary. In fact, an ultraviolet sterilizer will kill a great deal of the bacteria and particulates that a sponge requires, resulting in slow growth rates and possibly starvation.

Position

While it may seem convenient to wedge a sponge into the cracks of a rock, it can lead to problems. Typically there is little flow through a small crack, especially if a sponge further arrests the flow. Oftentimes a sponge anchored as such will begin to necrose at the base where the sponge is unable to feed and eliminate waste.

Nylon monofilament line serves as an excellent tool for anchoring sponges to substrate. Monofilament is fairly smooth, and will not tear the sponge upon removal even if is has overgrown the line to a small degree.

Some sponges, particularly heavily structured ball and rod-like sponges, do not take well to attachment via monofilament line. The very structure of the sponge either demands a specific attachment point, the base of a rod sponge, or is incompatible with holding a loop of line, ball sponges. In both of these cases small stainless steel insect pins can be used to attach the sponge to structure.

A hole is drilled into a suitable piece of reef-rock. The hole is then filled with Devcon® putty and allowed to harden momentarily. Finally a 00 stainless steel insect pin can be pushed through the Devcon®, so that a small ½" spike protrudes from the opposite side. The sponge

can then be gently impaled upon this spike. The sponge will, with all other factors considered, take root on the rock.

The sponge should be observed carefully for signs of necrosis at the penetration site, as well as signs of growth onto the anchor-rock. Once rooting is observed, the pin should be removed promptly. Even with a stainless steel pin, corrosion invariably occurs which may have negative effects on the sponge's health. Be sure to note original location and orientation before moving the sponge and rock around, and try to return the sponge to the exact same location with the same orientation to the current.

Figure 2. Yellow Ball Sponge, *Cinacyra alloclada*, exhibits walking legs shortly after attachment to live rock panels.

Once a sponge has established an anchor to the substrate, many species will choose to change their position within the tank to some degree. Sponges can grow "legs" which radiate out from the sponge, as seen in Figure 2. These legs will continue to grow in the direction of travel, until they reach a piece of substrate. These legs will become the new anchor for the sponge, as it moves into a more desirable position.

Most sponges do not require light and will flourish under low-light conditions. Many sponges are light tolerant and will control alga chemically, but will often thrive without the competition of algae. Some sponges, however, contain photosynthetic bacteria and will decline in health if not exposed to light. Experimentation with multiple specimens will often prove the best method for determining the needs and preferences of a given sponge species.

Figure 3: A. The red tree sponge, *Ptilocaulis spiculifera*, exhibits exposed spongin skeleton and necrotic tissue. B. *P. spiculifera* with exposed skeleton and necrotic tissue removed. C. Same specimen, *P. spiculifera*, after fragmentation and anchoring onto rock.

Damage and Disease

Sponges typically display distress in a through tissue decay and discoloration. The two most common sources of distress for sponges in captivity are exposure to air and improper environmental conditions. If sponge health is not addressed, dying sponges are legendary for their ability to release chemicals into a system, which can have disastrous results.

The tunnels and canals of a sponge are heavily lined with flagella and are always filled with water. These flagella provide active currents that draw in nutrients and expel waste. If these internal spaces become filled with air, the sponge will no longer be able generate currents, and will therefore become unable to feed or excrete waste. These air-exposed cells will quickly expire, and will often poison adjacent cells.

Dead and dying sponges will typically display localized discoloration that increases in area over time. This discoloration will often be accompanied by irregularities in tissue density, sloughing of tissue, and exposure of the sponge's spongin skeleton.

Not all discoloration is indicative of disease; experience will help to distinguish between healthy individual variations and diseased tissue. In general, the uniform tissue density of the discolored area is a good indicator of tissue health. If inconsistent density or soft spots are detected, more drastic steps may need to be considered. Any mucus or tissue sloughing should be addressed immediately. Due to poriferans' chemical complexity, necrotic regions can expand rapidly as dying cells poison adjacent healthy cells.

A great deal of ailing sponges have been compromised at some point by exposure to air. While exposure to air is not necessarily a death sentence in all cases, it should be avoided at all costs. It is possible to clear moderate amounts of air from sponges, depending a great deal upon the sponge's tissue density and the severity of the air blockage. A one sixteenth inch piece of airline hose can

be used to draw air from a loosely structured sponge. It is important to be gentle with the sponge while siphoning the air from its tissues, as undue pressure can damage the sponge.

Fragmentation

Sponge fragmentation, similar to coral fragmentation, is an effective method for disease/damage control and propagation. Unhealthy sponges can be fragmented to remove dying tissue, and preserve the still-living sponge. Healthy sponges can be fragmented in order to provide multiple specimens, or reduce the overall size of an individual sponge.

In the case of ailing sponges, diseased or dying portions of the sponge can be removed with a sterile razor blade, as seen in Figure 3. It is important to use minimal pressure with the blade, as pressure damage can further exacerbate the damage.

Unless the healthy portion of the sponge contains an anchor, fragmentation should be accompanied by anchoring. Once the ailing portions of the sponge have been removed several techniques can be employed to anchor the sponge to substrate, where it should be closely monitored for further signs of degradation. Fragmented and anchored sponges should be treated as a new sponge, and closely observed for oscula formation.

Fragmentation also serves as a useful tool in the captive care of sponges, as it provides multiple individual specimens to compare against one another. It is recommended to separate the fragmented sponges and place them in diverse locations. The individual sponges can then be observed and compared in order to determine environmental parameters that are best suited for sponge health.

Reaggregation

Reaggregation is the most invasive of the restorative techniques, and should be considered with some caution. If done correctly, reaggregation can be an effective technique for disease control, propagation, or individual-specimen size reduction.

A sponge that is scheduled for reaggregation can be removed from the water, as air-filled canals are not an issue during reaggregation. The sponge should only be removed from the water immediately preceding the reaggregation procedure. Diseased tissue should be carefully cut from the healthy portion of the sponge and discarded. The healthy tissue is then placed in a blender and thoroughly blended. The resulting slurry can then be set aside.

A still tank should be prepared, containing media for newly aggregated sponges to attach. The blended sponge can then be gently squeezed through the cheesecloth and into the tank. Alternatively, the slurry can be gently squeezed through cheesecloth into a mature system with active currents. The sponge cells will often reaggregate within the system. If this method is employed, it should be expected that no sign of the sponge may be observed for weeks or months. The sponge cells, will join together with their flagellum, and recombine into multiple small sponges. Separate reaggregated sponges will further recombine with sponges from the same procedure once their growth puts them within direct contact.

Figure 4: A Juvenile Short Bigeye, *Pristigenys alta*, finds refuge within the osculum of a vase sponge, *Niphates digitalis*.

Discussion

With the advent of more advanced poriferan husbandry, a new realm of exhibitry and animal interactions can be considered. Many species, including several species of amphipods, shrimp, and zoanthids, are considered to be obligate sponge commensals. Sponges serve as reef hotels of sorts, with many animals living in and around their tunneled bodies.

While not commensal by necessity with sponges, countless animals associate heavily with this underrepresented phylum. Small fish, and many fishes in their juvenile phase, seek out the shelter provided by sponges, as represented in Figure 4. Longlure frogfish, *Antennarius multicellatus*, associate heavily with brightly colored sponges, as do many reef invertebrates.

Sponges, with their minimal to nonexistent light-demands, are ideal candidates for twilight and deep reef exhibits; a realm often limited to artificial replicas and inserts. Coldwater reef tanks also become a possibility, where sponges are amongst the most colorful and intricate animals.

As sponge husbandry matures and more is known of their basic needs, larger and more ornate specimens should be available for public display. Distributors are beginning to meet the demands associated with collecting and shipping sponges, which in turn provides healthy, viable specimens for the aquarium industry. Where once small incidental species were the extent of sponges in aquaria, now large, elegant sponges have become a reality.

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TARGET TRAINING AND TACTILE CONDITIONING OF TWO ZEBRA SHARKS, *Stegostoma fasciatum*

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Abstract:

Classic operant conditioning techniques were used to train two female Zebra sharks, *Stegostoma fasciatum*, housed in a 340,650 L mixed species Indo-pacific exhibit. Target training and desensitization to tactile stimulation were employed via successive approximations with positive reinforcement to better facilitate their husbandry needs. These techniques allow for the easy moving of feeding stations and allow for medical procedures to be accomplished with much less stress to the animal. The target training has allowed staff to tend to their day-to-day needs in an efficient, effective, less stressful way, while increasing the safety of the animals during feeds. The tactile desensitization has made handling the sharks easier during medical procedures and is a by-product of the target conditioning. This training also has an end product of a decrease in stereotypical behaviors for these two sharks and has led to staff being able to accomplish relatively stress free blood draws for physicals. It is hoped that throughout the year, these blood draws can be taken regularly to obtain less-stressed baseline blood chemistry values for these two Zebra sharks. This could lead to a better understanding of Zebra Shark health in captivity leading to better husbandry practices in the future for all captive Zebra Sharks.

Introduction:

The Zebra Shark, *Stegostoma fasciatum*, is a tropical marine species of shark that is native to the Indo-West Pacific oceans of the world and is commonly reef dwelling. They are oviparous and are commonly maintained in public aquariums (Kunze and Simmons, 2004). These sharks are benthic dwelling and feed primarily on crustaceans, mollusks, and other benthic dwelling invertebrates and vertebrates. They can obtain sizes of 8 feet or more (Zebra Shark, *Stegostoma fasciatum*, 2006). Their common name in the United States stems from the fact that as juveniles their coloration is striped, and as they mature and grow, their stripes become spots. In their natural range, they are referred to as Leopard Sharks which is well suited. These sharks, in captivity, spend much of their time on the bottom and usually swim during feeds (pers. obs.). In the past, elasmobranchs were only believed to be driven by basic instinctual needs. It turns out that sharks have been shown to exhibit complex behaviors as forms of communication with one another as well as other species. Some of these behaviors include dominant hierarchies (Sabalones et al., 2004). Training, conditioning, and enrichment are relatively new to the public aquarium community, but are becoming more and more common place with these sharks as well as other species.

The two Zebra Sharks at the Pittsburgh Zoo & PPG Aquarium, Zebra Shark A and Zebra Shark B, were captive bred in Guam. They are housed in our ocean exhibit tank which is approximately 340,650 Liters in volume. It is a mixed species exhibit housing both elasmobranchs and teleost fishes from the Indo-Pacific region of the world. This tank is 9 meters deep which can pose problems if a benthic dweller is supposed to feed from the surface when in

captivity. The sharks were observed to exhibit stereotypical behaviors similar to observations in other aquariums (pers. obs. The Aquarium at Moody Gardens). The behaviors here at the PPG Aquarium were noted in these two sharks by aquarists (Bressler, et al., pers. com.). They were getting stuck in the corals and rockwork and would periodically have to be physically removed from such. Eventually the two sharks were moved to quarantine for observation and possible treatment.

Since this exhibit is a mixed species tank, the training and conditioning of the Zebra Sharks here at the Pittsburgh Zoo & PPG Aquarium stemmed from efforts to get the Zebra sharks to feed from the surface and to keep the elasmobranch feeds species specific and separate. It evolved from there as a way to find less stressful means to conduct physicals and obtain baseline blood values for these two Zebra Sharks. There is a general lack of solid baseline blood data for any sharks in captivity and it is important that these values are obtained. This could be dependant on diets and husbandry techniques, but having something to base blood data on for, at least, individuals could become incredibly useful in the future. It is the lack of data that led to the decision to find a way to obtain blood samples from Zebra Sharks in a voluntary, less stressful way. It is hoped that this will lead to baseline blood data for these two sharks and contribute to an elasmobranch blood database to aid other institutions in the pursuit of the same.

Materials and Methods:

All training and conditioning with the Zebra Sharks, and other animals at the PPG Aquarium, is completed using classic operant conditioning via positive reinforcement and successive approximation. The training started in December of 2005 in the quarantine facility and continued when the animals were moved on exhibit in January of 2006.

Contemplating a Bridge:

There was no bridge used during the course of this training. Sharks do possess an inner ear, and sound waves can play an important role in shark behavior though they have no external ear openings. They also have a lateral line that can detect sound waves via mechanical reception in the range of 10-800 Hz (Sabalones et al., 2004). For the purposes of this training, it would not have been practical to use sound as a bridge. The use of simple target training with no bridge seemed the best course of action for the entirety of their training and conditioning up to this point. The use of a low- frequency bridge may be incorporated at a later time.

Target Training:

The target training was started in a holding tank. This was initiated because of concerns that the sharks would not know where to come and feed in the 9 meter deep exhibit tank. The target was first introduced in the normal proximity of where the sharks would usually eat. It was made of a length of 1.9 cm PVC with a yellow, plastic, circle cable tied to the end. It has been scientifically shown that some species of sharks can differentiate color contrasts and colors (Sabalones et al., 2004). They did not come too close to the target at first, so they were fed anytime they were close enough to the target to reach them with feeding tongs. Slowly the food was drawn closer and closer to the target and the sharks were fed. This was done any and every time the sharks ate. When they were close enough to touch the target, the target was shown to

them and then they were rewarded with food. This was done by putting the target close to one of their eyes. The next step was to touch the shark's rostrum to the target and then immediately reward with primary reinforcement.

The sharks were then moved into the exhibit tank. The target training was continued on exhibit until the sharks were completely target trained. The criteria for the completion of their target training was that they would break their swim pattern to come and touch the target before being rewarded with primary reinforcement (Figure 1). At this point, the sharks would touch the target, stay on target, and follow the target before being reinforced. The duration of the target training was conducted using a constant reinforcement schedule. After they were completely trained with the target, they were shifted to a variable reinforcement schedule. There were no differences noted in behavior between the constant reinforcement schedule and a variable one.

Tactile Conditioning:

These sharks had never been subjected to touching by humans except for when they shifted enclosures. This touching was not voluntary and may have been very stressful for them. It was decided that since they would come to the target, maybe they would begin to accept tactile stimulation if approximated slowly enough. Every time the sharks would come over to the target for primary reinforcement, they would be lightly touched. This elicited a flight response at first. Both animals would immediately swim away upon being touched. Over time they began to accept the touching as a non-threatening stimulus (Figure 2). Through successive approximations, the touching was gradually increased first touching dorsally, then dorsally and laterally, ventrally, rostrum touching, reaching around with arms, impeding swimming progress, stopping motion all together, then stopping all together while rubbing all over, and finally rolling them over and rubbing the ventral surface. When rolled over they would receive their primary reinforcement and be released.

Introduction of the S^D (Discriminative Stimulus):

When the training first began, the target was used to initiate the tactile stimulation, and then an S^D was introduced. Waving the fingers under water in a "come here" motion is the S^D for approach for tactile stimulation. This visual queue was introduced, at first, with the target, and then the target was slowly removed. The sharks quickly and surprisingly caught on. It is believed that both their visual sense and mechanical reception via the lateral line are used by the sharks to detect the S^D.

Obtaining Voluntary Blood Samples from Zebra Sharks:

The only facet of the shark's training that was not approximated was the needle puncture under the placoid scales and into the vein to obtain blood samples. It was planned to approximate this, but the sharks were so cooperative at this point that it was not needed. With the sharks rolled over, the needle was inserted into the caudal vein on the ventral side directly posterior of the pelvic fins (Figure 3). Some samples were also obtained posterior of the anal fin, but the sharks seemed to be more sensitive in that region. The needle was 3.81 cm in length, and both 20 and 22 gauge needles were used to obtain samples.

Voluntary Weighing of Zebra Sharks:

A plan was developed to pull these two Zebra Sharks out of the water via successive approximations to weigh them with little or no stress. This was achieved by lifting them out of the water for gradually increasing periods of time. The sharks were lifted out of the water for two seconds each and returned to the water where they were primary reinforced with food. With a two second interval, the sharks showed no visible signs of stress. The interval was raised to four seconds and maintained there for several sessions. The interval that the sharks were lifted from the water was then raised by two more seconds for several more sessions. The interval that the sharks remained out of the water was approximated in this fashion until the duration out was enough to weigh them. There was no stretcher used when obtaining their weights. The trainer would tare him or herself on a scale then lift the shark from the water and step back onto the scale (Figure 4). The weight was then noted, the shark returned to the water, primary reinforced, and released. Both sharks would return to accomplish another behavior after the weighing behavior was accomplished.

Results:

Since the completion of the shark's training, 8 blood samples from each shark have been obtained. This has contributed significantly to the goal of obtaining base-line blood values for these two sharks (Figures 5, 6, and 7). These sharks do not appear to go into tonic immobility when inverted. It is apparent, however, that they tolerate the tactile stimulation and blood sampling in the hopes of getting primary reinforcement. When inverted the sharks appear to be completely aware of what is going on, and when food is offered, their pectoral fins roll to the anterior. This behavior of rolling the pectoral fins was useful in determining when to insert the needle into the puncture site. When in a less relaxed state, the sharks were observed to roll the trailing edge and ends of their pectoral fins to the anterior. When their fins would settle down and flatten, the needle would be inserted with little or no reaction from the shark. The primary reinforcement is readily taken even when rolled over ventral side up.

In the past, there would have been nothing to compare blood values against and the values that were formed were taken from stressed sharks that had to be removed from the water and restrained. It is also important to note that IM injections, intramuscular injections, have also been tolerated by these sharks by just holding them right-side-up, injecting, and then primary reinforcing. During these injections, it was apparent that there was little or no stress to the animals.

These two sharks, when being handled, show definite signs of competition (Figure 8). When handling one of them, the other will come over to compete for the reinforcement. The sharks appear to feel vulnerable while rolled over, so the competition sometimes becomes problematic. It soon became increasingly important to try and keep the one not being handled a good distance away. When one shark would approach the other, the one being handled would become visibly agitated and attempt to display itself to the other. The shark being held would tense up and shake from side to side. These sharks typically display to one another on a regular basis when swimming in close proximity to one another. They will shake quickly and roll over exposing the counter-shaded, white, ventral surface to the other. They then fall through the water column temporarily upside down (pers. obs.). Since both of these sharks are female, these

behaviors are more of a show to establish a hierarchy between the two of them. It has become apparent that, with these two sharks, Zebra Shark B is the smaller yet dominant animal. Mating behaviors among this species of shark differ greatly from those observed here, and have therefore been ruled out as a reason for the display. Anecdotally, these sharks also spend far less time on the bottom and much more time swimming around the exhibit and exploring than they had done previously in the same exhibit.

The training and conditioning of these two sharks has contributed significantly to educational goals of the facility as well. The tactile conditioning has allowed numerous students, invited guests, and even several media personnel, to be able to touch a live shark and leave with a memorable experience. Instead of touching a piece of dried shark skin, students have been able to come down to a platform stationed approximately 5.0 cm above the water and touch these sharks on the dorsal side, ventral side, and on the fins. This interaction strongly enhances the visitor experience as well as gives the institution another way to show visitors and invited guests the importance of the work being accomplished on a day-to-day basis at the Pittsburgh Zoo & PPG Aquarium.

Conclusion:

The target training and tactile conditioning of these two sharks has greatly enhanced the staff's ability to manage a captive population of sharks. The way that the shark's health is managed has also been raised to another bar. These sharks now come to a target, allow thorough, up close visual inspections both dorsally and ventrally including length measurements, voluntarily allow blood draws from behind the pelvic fins and behind the anal fin, allow dorsal saddle IM (intramuscular) injections, and can be lifted out of the water and weighed with little or no stress to them. It has become apparent that the lives of these two sharks have been enhanced as a by-product of the training and conditioning. The safety of the staff, the safety of the animals being worked with, and the safety of other animals in the tank has also been enhanced. The training and conditioning of these two Zebra Sharks has now made it possible to obtain baseline blood data for them which will help to better assess their health in the future. Target training has since been started with the rest of the elasmobranch population in the tank, (Bonnethead Sharks, *Sphyrna tiburo*, and Blacktip Reef Sharks, *Carcharhinus melanopterus*) and it is hoped that a level equal to that of the current training can be obtained.

Acknowledgements

I would like to thank the Pittsburgh Zoo & PPG Aquarium and Allan Marshall (Curator of Aquatic Life) for supporting my efforts during the course of this training. Many thanks also go to Sarah Schwenzer for her dedication to this cause and her hematology skills. I am also thankful to those that showed me the "training ropes" Diane Olson of the Aquarium at Moody Gardens and Margaret Whitaker. I am very grateful to Jenni Snowden for supporting my efforts, giving professional advice, and tolerating the time that was spent during the course of this training and the writing of this paper.

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THE NATURAL HISTORY AND AQUARIUM HUSBANDRY OF THE ATLANTIC SHARPNOSE SHARK, *Rhizoprionodon terraenovae*

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Introduction

The Atlantic sharpnose shark, *Rhizoprionodon terraenovae* (Carcharhiniformes: Carcharhinidae), is the most common inshore shark species in both the Gulf of Mexico (GOM) and along the U.S. Atlantic coast (Castro, 1983; Hoese and Moore, 1998; Carlson and Brusher, 1999; Thorpe et. al., 2004). The genus *Rhizoprionodon* contains seven extant species at present, with a circumtropical distribution (Compagno, 1999). The genus is characterized by small size, short life spans, rapid growth, early maturity, and high natural mortality (Simpendorfer, 1999). While these characteristics coupled with the natural abundance would seem to make the species a prime candidate for exhibit in public aquaria, very few institutions choose to maintain and display the species, largely due to husbandry challenges. This report will attempt to combine a concise review of the published literature on the natural history of the species with anecdotal and empirical observations on the captive biology of the species to present a more complete picture of the requirements of this species in the aquarium. The literature cited section of this paper should also serve as a thorough bibliography of the literature concerning *R. terraenovae* to aid the aquarist in future attempts to keep the species in both the laboratory and aquaria.

Methods

The observations on growth, disease, survivability, hemodynamics, diet, species compatibility, water quality, tank design, and transport are presented in their respective sections coupled with a review of the literature. For the sake of brevity, information regarding methodology is included within these individual sections where applicable. More detailed information may be obtained by contacting the author at the above email address.

Results and Discussion

Population Demographics and Conservation Status

The U.S. small coastal sharks Fisheries Management Plan (FMP) characterizes *R. terraenovae* as abundant and resistant to fishing pressures, and the IUCN designates the species as lowest risk/least concern; however some doubts persist about the future resilience of the species. Wild populations of *R. terraenovae* have been found to increase at a rate of 4.5%, and decrease by 2.9% annually (Cortés, 1995). It has also been reported that wild populations seem to have increasing fecundity and rates of maturation, likely due to decreased density resultant from fishing pressures (Carlson and Baremore, 2003). These most current models theorize that the population may be vulnerable to pressures imposed by recreational and commercial fisheries, despite the assertions of the FMP (Cortés, 1995). The species is not listed on either appendix of C.I.T.E.S.

Growth

It has been previously noted in this journal that there is a paucity of data on length-weight relationships of sharpnose sharks (Mohan, 2000), and the following summarizes data from numerous in-depth investigations that have been published since that date. The growth of *R. terraenovae* has been investigated in a number of studies, including Parsons (1985), Branstetter (1987), Loefer and Sedberry (2003), and Wigley et. al. (2003). In the most recent investigation, Loefer and Sedberry (2003) provide equations based on empirical studies of wild populations to describe the relationship between total length (TL), fork length (FL), and precaudal length (PCL) in the species:

TL = 29.804 + 1.279 PCL
FL = 11.249 + 1.075 PCL

By re-arranging the equations, and substituting one into another, one can obtain FL from TL thusly:

FL = 0.8405 TL – 13.80127

A table of corresponding FL, and PCL values extrapolated from 200-1100mm TL constructed using these equations is given in Appendix I.

The length-weight relationship has also been established for wild populations of *R. terraenovae,* with Wigley (2003), reporting the standard biometric values for ln$a$, S_a, S_b, S_w, and r^2. These parameters may be used to calculate weight from a given length (L) when entered into the standard linear length-weight equation:

$$\mathbf{\ln W = \ln a + b \ln L}$$

When one applies a set of arbitrary lengths to this equation from 250 to 1100mm we obtain a set of corresponding weights from 59 to 5,858g, as represented in table 1.0, and plotted graphically in figure 1.1.

Table 1.0 Length-Weight Relationship of *Rhizoprionodon terraenovae*, Adapted From Wigley et. al. (2003). Representative Lengths are Given as Fork Length (FL).

L (mm)	W (kg)	L (mm)	W (kg)
250	0.059	700	1.438
300	0.103	750	1.784
350	0.167	800	2.178
400	0.253	850	2.630
450	0.365	900	3.140
500	0.506	950	3.715
550	0.680	1000	4.357
600	0.891	1050	5.070
650	1.143	1100	5.858

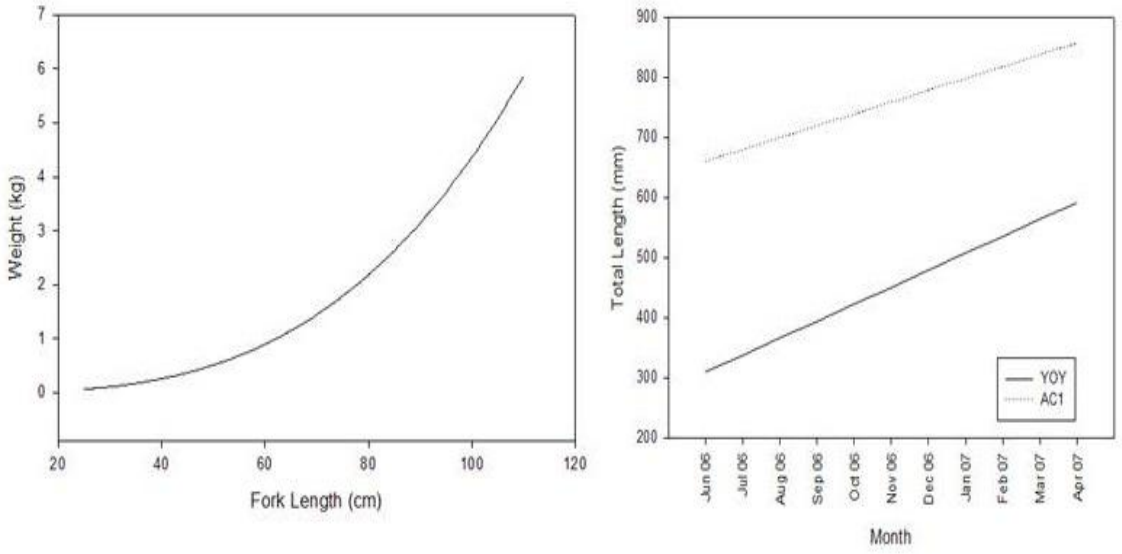
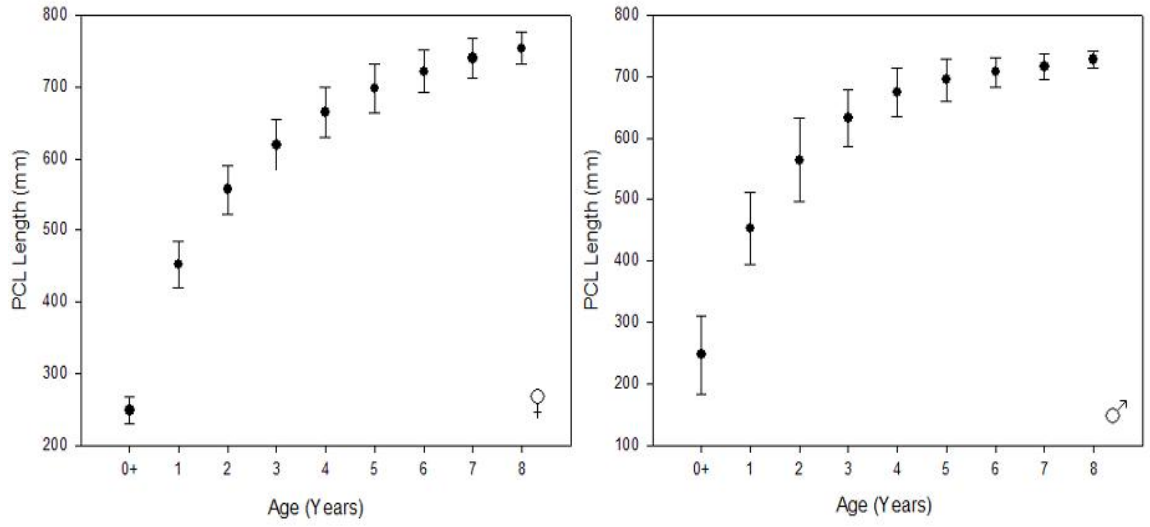


Figure 1.1, 1.2 Length-Weight Relationship of *Rhizoprionodon terraenovae,* Adapted from Wigley et. al. (2003) (left). Growth of captive specimens housed at Moody Gardens (right) (n=7); note that the growth shown here appears artificially linear as it is based on two measurements rather than a series of data points over the study period.

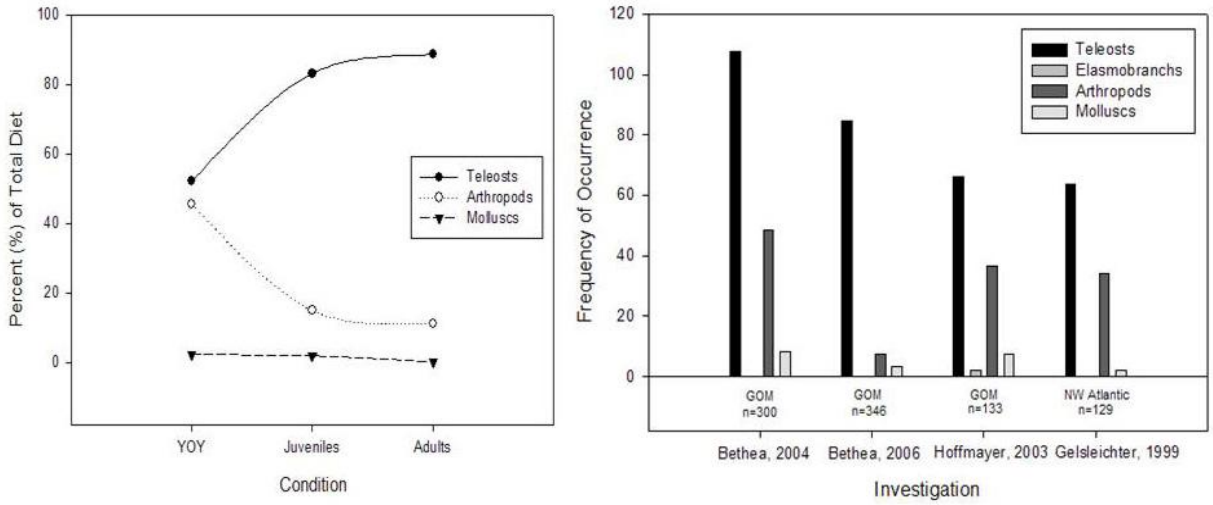
Females typically give birth to juveniles at 279-406mm in size (Parsons, 1983). Parsons (1985) also found that young of year (YOY) *R. terraenovae* grew 5cm month^{-1} in the summer months, and 0.9cm month^{-1} in the winter; age class 1 (AC1) specimens grew an average of 1.0cm month^{-1}; and adults grew considerably slower at 1.7cm year^{-1}. Loefer and Sedberry (2003) also provide age-length relationships for both male and female *R. terraenovae* as illustrated in Figures 2.1 and 2.2. A standard Von Bertalanffy growth model has been constructed by Branstetter (1987).



Figures 2.1, 2.2 Length-age relationship for female and male *Rhizoprionodon terraenovae* from Loefer and Sedberry (2003). Length expressed as PCL, error bars indicate standard deviation.

Diet and Metabolism

Wild specimens of *R. terraenovae* typically consume between 52 and 66% teleosts, 0.15-10% mollusks, and 11-45% arthropods (Gelsleichter et. al., 1999; Hoffmayer and Parsons, 2003; Bethea et. al., 2004, 2006; Bowman et. al., 2000). In all of the above studies, the bulk of fishes found in the gut were sciaenids, most of the arthropod species were decapod crustaceans, and cephalopods comprised the bulk of the molluscan fauna reported. Bethea et. al. (2004, 2006) also noted that there were definite shifts in the preferred prey items as the species grew, with the trends indicating heavier fish consumption and decreasing invertebrate consumption with increasing size (fig. 3.1). It is also interesting to note that despite the fact that the characteristic diminutive size *R. terraenovae* makes it vulnerable to predation by larger congeners, it still occupies a position in the 4th trophic level within its respective ecosystems alongside most other carhcarhinid sharks (Cortés, 1999). In captivity the specimens observed by the author have shown no predilection towards any specific food item, taking a wide variety of commercially available frozen seafood including shrimp (frozen and live), clams, squid, smelt, silversides, capelin, herring, mackerel, bonita, salmon, live blue crab, and blue runner. It is worth noting that even in large numbers the species is not nearly as aggressive towards one another when feeding as larger carcharhinids, though adult specimens have been observed nipping at YOY specimens. For detailed reports on the feeding of captive elasmobranchs the reader should consult Janse (2003) and Janse et. al. (2004).



Figures 3.1, 3.2 Diet Preferences of *Rhizoprionodon terraenovae.* Trends in diet preferences with age of the specimens, adapted from Bethea et. al. (2004) (left). Comparison of diet composition adapted from multiple investigations (right).

Metabolism in *R. terraenovae* has yet to be defined in the literature, though specimens maintained by the author, and by Branstetter (1987) have been successfully kept in captivity on a feeding regime of 10-15% body weight per week (BWW). Specimens in both instances grew at rates approximately equal to their wild counterparts (see fig. 1.2 and Branstetter, 1987). This would seem to indicate that the metabolic rate for *R. terraenovae* is close to that of many other inshore carcharhinid and sphyrnid shark species, and that feed rates of 10-15% BWW for juveniles and 4-6% BWW for adults are appropriate for the maintenance of the species pending more exhaustive investigation of species-specific bioenergetic properties. See Appendix II for a concise review of metabolism in selected inshore shark species.

Reproduction

Mature female *R. terraenovae* give birth in the spring and early summer in the GOM, and give birth to an average litter size of 7 juveniles, with litter size being dependant on the size and age of the female (Parsons, 1983). Mating typically occurs from May to July, and the gestation period is 10-11 months (Parsons, 1983). The average length of juveniles at birth is reported between 297-406 mm TL by Parsons (1983), and 197-465 mm TL by Loefer and Sedberry (2003). Figure 5.4 shows sexual dimorphism in mature adult specimens of *R. terraenovae*; the species matures at approximately 730mm TL and 760mm TL for females and males, respectively (Carlson and Baremore, 2003). The species tends to migrate inshore to mate and give birth (Parsons, 1983), and tagged specimens have been documented moving distances of over 300km (Tyminski, 2006). Interestingly, the energetic condition of the species as determined by hepatic lipid reserves has been documented to fluctuate with this migratory pattern and likely has very little influence on buoyancy as has been traditionally thought (Hoffmayer et. al., 2003). It is unknown at present if this variable energetic condition persists once the species is in captivity. At present *R. terraenovae* has not yet bred in the aquarium, though five other Carcharhinid sharks have been documented as breeding in captivity (Henningsen et. al., 2004), including four species of the genus *Carcharhinus* and *Negaprion brevirostris.* The species has, however, been observed giving birth in captivity following the stress of capture. In June 2005, a large female was collected from the Galveston, Texas beachfront, and transported back to the Aquarium at Moody Gardens Quarantine Facility and Laboratory by truck. En route the female gave birth to 3 juveniles, of which 2 survived, immediately began swimming, and readily adapted to living in a captive population which included numerous conspecifics, as well as other carcharhinid sharks.

Survivability

The specimens are quite resilient to being collected and handled compared to other carcharhiniform shark species, with survivability of hook-and-line captured specimens being reported as high as 90% when immediately re-released into the wild (Gurshin and Szedlmayer, 2004). As in all sharks, survivability post-capture is closely linked to the condition of the animal at time of capture (Hueter et. al., 2006), thus survival rate may be maximized by utilizing collection gear so as to minimize exhaustive effort once hooked, reduce hook damage, and selectively choosing specimens for transport based on their condition at time of capture.

Between 2005 and 2007 as many as five visiting zoological institutions collected a total of 90 *R. terraenovae,* which were staged at the Aquarium at Moody Gardens Quarantine Facility and Laboratory for up to 30 days prior to being transferred to various facilities throughout North America. Of these 90 specimens, only 5 died after collection (within 5 days), giving a captive survival rate of 94.5%. This stands in stark contrast to many of the anecdotal reports of attempted *R. terraenovae* husbandry from other facilities, and from the work of Branstetter (1987), which reported a captive mortality of 63% (n=19) in the first week post-capture in a laboratory setting. These preliminary findings would seem to be attributable to the great advances in shark husbandry made by public aquaria in the past few decades.

Physiological stress is arguably the greatest obstacle the aquarist faces in the husbandry of sharks. The most acute effects of stress induced by collection and handling are defined in the literature, though the specific biochemical changes are not well defined for all species of sharks.

For *R. terraenovae* the physiological responses to capture and handling stress have been well defined by Hoffmayer and Parsons (2001); changes which have obvious implications on captive husbandry. Hyperglycemia is a well documented stress response in fishes, as is the rise in lactate in sharks as the animal enters an oxygen debt following exhaustive exertion. Blood pH in *R. terraenovae* was found to be stable at 15 min after capture, but dropped steadily at 30, 45, and 60 min; with a corresponding increase in glucose and lactate (Hoffmayer and Parsons, 2001). Charbeneau (2004) reports that hypercoloration, hypocoloration, and erratic swimming may be used as indirect indicators of stress in elasmobranchs, all of which have also been observed by the author in *R. terraenovae* after collection, handling, or in response to poor water quality. Interestingly, Hoffmayer and Parsons (2001) found that increasing shark size does not increase the stress response with handling, thus it may be inferred that while there is no physiological advantage in working with smaller specimens, the increased ease of handling smaller specimens may be beneficial in field collections in terms of limited out-of-water time and ease of restraint.

Collection and Transport

The species is caught regularly inshore in the GOM and on the Atlantic US coasts by anglers, and all of the 90 specimens collected to date though the efforts of the Aquarium at Moody Gardens and other facilities have employed this method. Young of the Year specimens are most common, and may be easily overpowered with light saltwater tackle (12-20 lb test line and appropriate leader), adults may also be caught on similar weight tackle, though slightly heavier tackle would afford an obvious advantage in reduced exhaustive effort with larger specimens. Marshall (2004) also reports that the species has been caught using longline techniques.

Between 2004 and 2007 the author has conducted or observed the transport of 90+ specimens for short distances in both simple round, 1.75m diameter HDPE tanks with oxygen aeration, and in 2m round HDPE tanks with recirculating water and oxygen aeration. Most of these specimens were subsequently transported to other facilities using the latter transport system. Marshall (2004) reports that the species has been successfully transported restrained for 6-8 hours, free swimming for up to 30 hours, and in sealed bags for up to 10 hours. In August of 2006 three simulated transports were conducted by placing *R. terraenovae* of approximately 60-70cm in transport systems constructed following those described by Marshall (2004). The specimens were fasted for 24 hours before being placed, one at a time, in the transport rig with recirculating seawater, oxygen aeration, mechanical filtration, and activated carbon filtration for 24 hours. During this time the dissolved oxygen, ionic ammonia, ionic nitrite, temperature, and pH were monitored closely to ensure the health of the animals. Given the success of these simulated transports, the three *R. terraenovae* were transported in excess of 2 days by truck from Galveston, Texas to St. Louis, Missouri, by way of Oklahoma; extending the previously recorded free-swimming transport time for this species. This demonstrates that *R. terraenovae* is a relatively robust species in terms of enduring transport stress, and may be moved great distances by employing proven elasmobranch transport techniques.

Tank Design and Water Quality

Observations from the captive specimens held at Moody Gardens show that like most species of Carcharhinid sharks, *R. terraenovae* require ample swimming room, and do best in

circular tanks that prevent the excessive energy expenditure associated with turning in squared tanks. Specimens have been maintained in 78,000 L, 117,000 L, 156,000 L, 3,950,000 L tanks with diameters of 6, 9, 12, and 30m, respectively. All systems employed appropriately sized rapid sand filters, protein skimmers, and biological filtration. While there have been some unsubstantiated claims that tanks less than 12m in diameter are unsuitable for housing the species, *R. terraenovae* have been housed long term (6 months+) in tanks of lesser diameter (as described above) without incident or mortality, as well as in 5.5m tanks under laboratory conditions as described by Branstetter (1987). The species tolerates a wide range of water quality conditions in nature, being found in the inshore GOM which is known for low dissolved oxygen levels, fluctuating salinity, and high turbidity. Carlson and Parsons (2001) report that the obligate ram-ventilating *R. terraenovae* responds to hypoxic conditions by increasing swim speed, though hemoconcentration (increased hematocrit) in periods of anaerobic metabolism does not occur in the species (Hoffmayer and Parsons, 2001). Specimens collected in the current study have been collected from temperatures ranging from 20-35ºC, and salinities ranging from 16-29‰. These specimens have been maintained in captivity at salinities from 18-35‰, temperatures from 19-29ºC, and pH levels of 7.87-8.30. Branstetter (1987) maintained specimens from 18-27ºC, citing 18ºC as the minimum temperature for the maintenance of Carcharhinid sharks (see also Young et. al., 2001). It appears that the species is relatively tolerant of variable water quality conditions, both in the wild and in captivity, though as in the husbandry of any elasmobranch species, water quality should be considered the most important factor in ensuring animal health.

Diseases

It has been noted that there are few documented cases of bacterial diseases in sharks, save for *Vibrio carchariae* infections, though prudence dictates that shark specimens be considered susceptible to a variety of bacterial infections (Stoskopf, 1993a). For the most complete and recent reviews of elasmobranch diseases, the reader should defer to Smith et. al. (2004), though a brief review of the diseases documented in the literature and by the author in captive *R. terraenovae* follows. Stamper et. al. (1998) reported that captive specimens of *R. terraenovae* were potentially exposed to *Eimeria southwelli* (Protozoa: Coccidia) during an outbreak in *Rhinoptera bonasus*, though none apparently became infected. Christie and Henderson (2007) reported that specimens that were housed with *Carcharhinus limbatus* infected with a presumptive severe *Fusarium sp.* fungal infection for over 30 days did not become infected. Karsten and Rice (2006) found antibodies to *Vibrio anguillarium, V. cholerae, V. parahaemolyticus, V. carchariae, Escherichia coli, Mycobacterium marinum, and M. fortuitum*; suggesting a prior exposure to the pathogens in their estuarine habitats, and increased antibody response to the pathogens in the future. Incidences of thyroid hyperplasia (goiter) have yet to be documented in the species, but have been documented in at least 5 other carcharhinids (Crow, 2004). It is, of course, logical to assume that the species has similar susceptibility to the condition if not properly supplemented with standard shark vitamins. The most common condition anecdotally reported for this species are nonspecific bacterial infections of the rostrum resulting from chronic abrasion (see fig. 4.2). These bacterial infections have yet to be documented thoroughly, though they have been observed both by the author, and by other aquarists who have attempted to keep the species. The author has also observed severe ocular infections in two instances, arising from bite wounds from tankmates during feedings (see fig.

4.1 and 4.5). Cerebral hemorrhage has also been observed in one instance, when a specimen of *R. terraenovae* impacted a wall with extreme force while being chased by *Carcharias (=Odontaspis) taurus* (see fig. 4.4). Death has also been observed in the species resulting from an amputated spiral colon (fig. 4.6) that was bitten during an intestinal eversion event similar to those described by Crow et. al. (1990, 1991).

Parasites

Nine species of metazoan parasites have been reported from *R. terraenovae*. For the sake of brevity, these species are listed in table 1.2 and illustrated in figures 4.3 and 6.0, and only a brief discussion of the more pathogenic parasites with husbandry implications is given here. Bullard et. al. (2006) report that *R. terraenovae* has a 17-33% rate of infection in the GOM of *Selachohemecus* blood flukes, along with a key to these haemoparasites of the Condryichthyes. It is interesting to note that only 3 cestode parasites have been reported for the species (Palm and Overstreet, 2000; Healy, 2003; Beveridge and Campbell, 1993), despite the high rate of infection (up to 98% infection and 4.4 helminths per fish) of cestode pleurocercoids in sciaenids (Overstreet, 1978) and the predilection of *R. terraenovae* for feeding on these fishes (Gelsleichter et. al., 1999; Hoffmayer and Parsons, 2003; Bethea et. al., 2004, 2006; Bowman et. al., 2000). Also of note are the two monogenean parasites reported (Kohn and Portes-Santos, 1990; Hendrix, 1994); though despite the reputation for this class of parasites to induce pathogenic effects, no captive infections have been reported to date. The aquarist should also take care to observe normal coloration patterns of individual specimens, as the species is well documented as having quite variable spotting patterns (Castro, 1983; Hoese and Moore, 1998) which may be confused in captivity for the characteristic signs of ectoparasite infection (see fig. 5.1, 5.2, 5.3).

Pharmacology

A review of drugs used in elasmobranch medicine is beyond the scope of this paper, the reader should refer to Stamper et. al. (2004) for a thorough treatment of the subject; though a brief summary of the treatments used on *R. terraenovae* at the Aquarium at Moody Gardens from 2005-2007 is listed in Table 1.1.

Compatibility

The 90 specimens that have been kept at the Aquarium at Moody Gardens, both on display and in quarantine have frequently been maintained with other species of elasmobranchs and teleost fishes. In general the diminutive size of these sharks dictates caution in exhibiting or holding these species with larger, more aggressive sharks, as the potential for aggression and predation is high. Ideally the species would be displayed in a species-specific exhibit, though when creating a mixed-species shark display the author has found that keeping the sharks in a quarantine or holding tank until they reach approximately 65-75cm TL is advantageous. This grow-out period should take approximately 1 year for AC1 specimens, or 1-2 years for YOY specimens (see fig. 1.2). These larger specimens are much stronger swimmers, likely have greater energetic reserves, and are less likely (in the authors experience) to fall prey to larger sharks or fishes. The use of a floating acclimation pen for 1-2 weeks prior to introduction into a large exhibit has also been found to aid in initial survival in mixed species tanks. Specimens of *R. terraenovae* have been kept with 9 other shark species since 2005 at Moody Gardens, largely

Table 1.1. Chemotherapeutic Agents used on Rhizoprionodon terraenovae at the Moody Gardens Aquarium

Drug	Target Pathogen	Dosage	Administration	Number Treatments	Schedule	Sensitivity (Y/N)	Efficacy (Y/N)	Side Effects
Enrofloxacin (Baytril™)	Bacteria	5 mg/kg	IM	3	q48h	N	Y	None Observed
Enrofloxacin (Baytril™)	Bacteria	10 mg/kg	IM	3	q48h	N	Y	None Observed
Enrofloxacin (Baytril™)	Bacteria	10 mg/kg	PO	3	q48h	N	Y	None Observed
Oxytetracycline	Bacteria	100 mg/kg	PO	10	S.I.D.	N	Y	None Observed
Metranidazole	Protozoa, Anerobic Bacteria	50 mg/kg	PO	1	1x	N	Y	None Observed
Praziquantel	Helminths	2 mg/l	Bath	3-5	1x Week	N	Y	None Observed
Praziquantel	Helminths	4 mg/l	Bath	3	1x Week	N	Y	None Observed
Praziquantel	Helminths	5 mg/l	Bath	2	1x Week	N	Y	None Observed
Praziquantel	Helminths	10 mg/l	Bath	1	1x	N	Y	None Observed
Praziquantel	Helminths	50 mg/kg	PO	2	1x	N	Y	None Observed
Trichlorfon (Dylox™)	Helminths, Hirudinea, Crustacea	0.50 mg/l	Bath	3-5	1x Week	N	Y	Hypersensitivity
Trichlorfon (Dylox™)	Helminths, Hirudinea, Crustacea	0.65 mg/l	Bath	3-5	1x Week	N	Y	Hypersensitivity
Trichlorfon (Dylox™)	Helminths, Hirudinea, Crustacea	0.75 mg/l	Bath	3	1x Week	N	Y	Hypersensitivity

Table 1.2. Parasite Fauna of Rhizoprionodon terraenovae

Species	Class	Family	Microhabitat	Location	Reference
Loimos scitulus	Monogenea	Loimoidae	Integument	Brazil	Kohn and Portes-Santos, 1990
Loimos scoliodoni	Monogenea	Loimoidae	Integument	NW Atlantic	Hendrix, 1994
Selachohemecus olsoni	Trematoda	Sanguincolidae	Heart	GOM	Smith, 1997
Dasyrhynchus giganteus	Cestoda	Dasyrhynchidae	GI Tract	W. Africa	Beveridge and Campbell, 1993
Platybothrium sp.	Cestoda	Onchobothriidae	GI Tract	NW Atlantic	Healy, 2003
Otobothrium cysticum	Cestoda	Trypanorhyncha	GI Tract	GOM & NW Atlantic	Palm and Overstreet, 2000
Perissopus dentatus	Copepoda	Pandaridae	Integument	NW Atlantic	Hewitt, 1967
Nesippus orientalis	Copepoda	Pandaridae	Integument	NW Atlantic	Hewitt, 1967
Kroeyerina deetsorum	Copepoda	Kroyeriidae	Olfactory Sacs	NW Atlantic	Benz et. al., 2001

Figures 4.1, 4.2, 4.3, 4.4, 4.5, and 4.6 (A-F, respectively) Diseases and trauma in the Atlantic sharpnose shark, *Rhizoprionodon terraenovae.* A. Non-specific bacterial infection of the eye (left) compared to normal eye (right). B. Severe rostral abrasion of a large adult specimen, note the cartilage in the wound. C. Parasitic copepods, *Perissopus dentatus* on the pectoral fin, note the umbilical scar denoting a YOY specimen. D. Cerebral hemorrhage in a large adult specimen that struck a wall while being chased by *Carcharhias (=Odontaspis) taurus*, note the pooled blood mixed with clear cerebrospinal fluid within the braincase. E. Severe trauma to the eye and head causing secondary bacterial infection; the specimen was bitten by *Carcharhinus limbatus* housed in the same tank. F. Amputated spiral valve protruding through cloaca, specimen was bitten during intestinal eversion by a tankmate. All photos by the author.

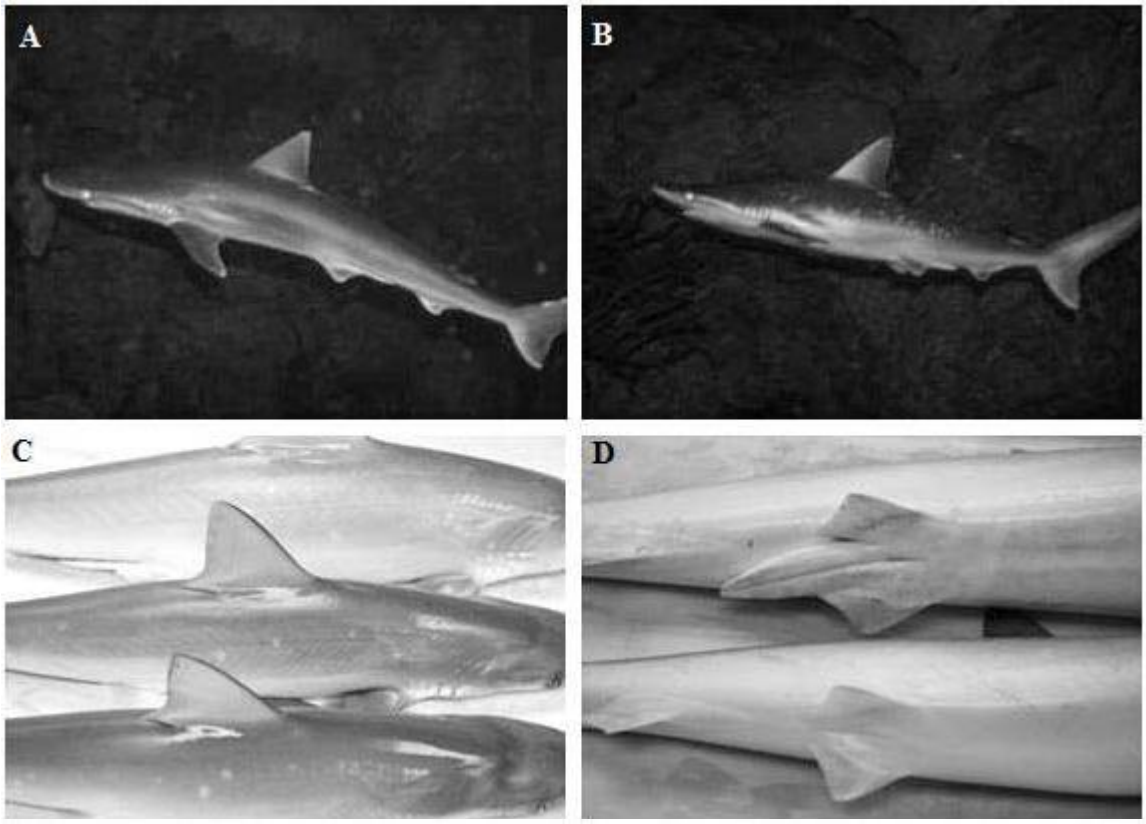


Figure 5.1, 5.2, 5.3, and 5.4 (A-D, respectively) Figures A-C show the variation in spot patterns in the species, aquarists should take care to observe individual normal coloration so as not to confuse heavier white spots, as in fig. 5.2 (B), with monogenean or other ectoparasite infection. Figure 5.4 (D) shows sexual dimorphism in mature adult specimens; of special note is the clasper size, which is approximately 5 times larger than YOY and AC1 specimens. All photos by the author.

Table 3.0. Potential compatibility issues of selected elasmobranch species as observed from specimens kept in captivity at Moody Gardens 2005-2007

	Potential Compatibility Issues		Observed Compatibility Issues	
Species	Harassment	Predation	Harassment	Predation
Carcharhinus limbatus			X	X
Carcharhinus acronotus	X	X		
Carcharhinus plumbeus	X	X		
Carcharhinus isodon	X	X		
Carcharhinus brevipinna		X	X	
*Rhizoprionodon terraenovae**		X	X	
Sphyrna lewini	X	X		
Sphyrna tiburo	X			
Carcharias (=Odontaspis) taurus			X	X
Ginglymostoma cirratum	X			
Rhinoptera bonasus				
Dasyatis sabina				
Dasyatis americana				

**Refers to conspecifics of markedly larger size class*

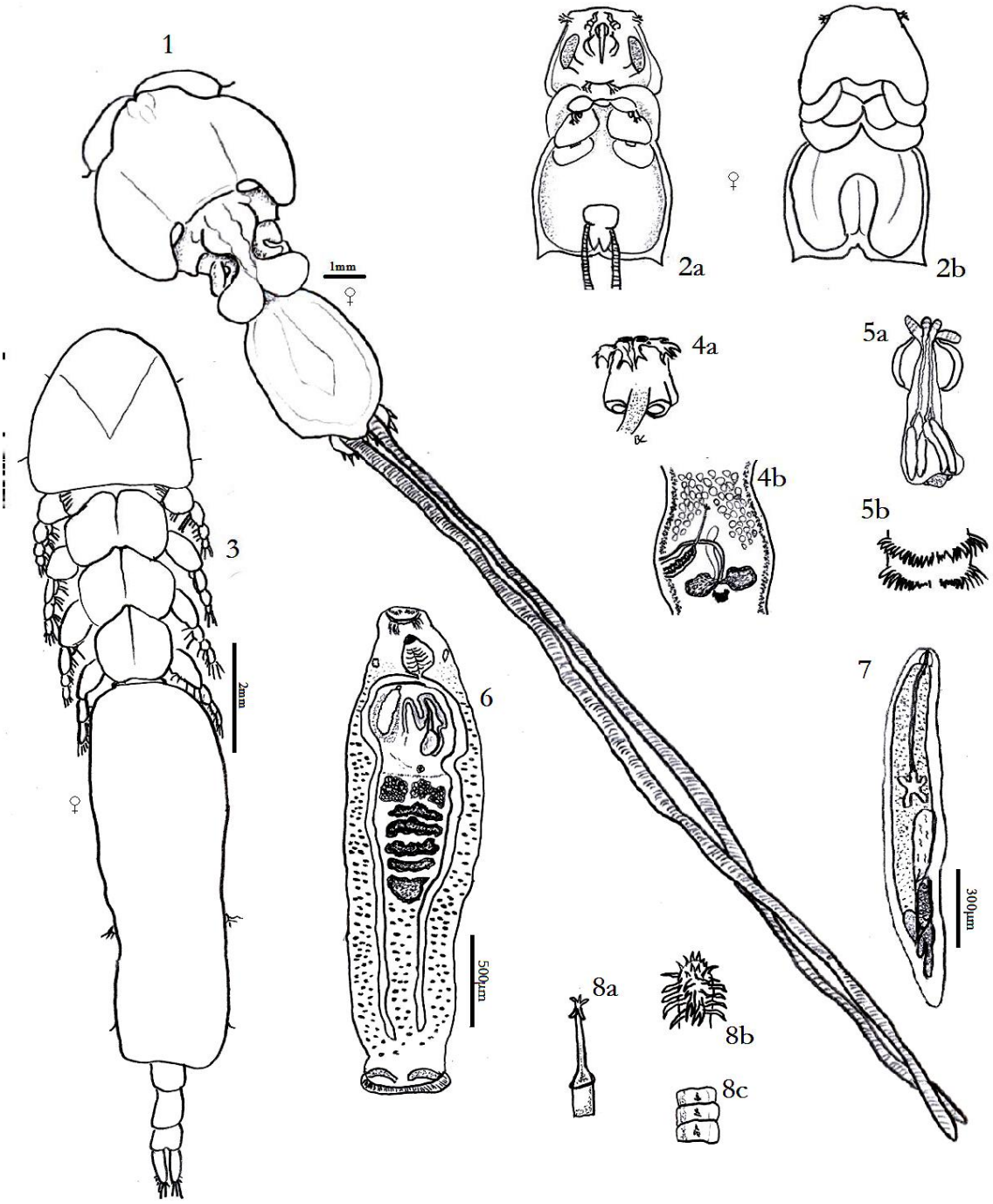


Figure 6.0. Representative metazoan parasite fauna of the Atlantic sharpnose shark, *Rhizoprionodon terraenovae.* 1. *Nesippus orientalis*, female, redrawn from Dippenaar and Jordaan (2006). 2. *Perissopus dentatus,* female, 2a. ventral view, 2b. dorsal view, redrawn from Yamaguti (1963a). 3. *Kroyerina deetsorum*, female, redrawn from Benz et. al. (2001). 4. *Platybothrium sp.* 4a. scolex 4b. proglottids, redrawn from Yamaguti (1959). 5. *Otobothrium sp.* 5a. scolex 5b. proglottids, redrawn from Yamaguti (1959). 6. *Loimos scoliodoni*, redrawn from Yamaguti (1963b). 7. *Selachohemecus olsoni,* redrawn from Schell (1970). 8. *Dasyrhynchus sp.* 8a. scolex 8b. detail of anterior proboscis 8c. proglottids, redrawn and adapted from Yamaguti (1959). Scale bars indicate relative size of selected figures. All figures redrawn from their original sources by the author.

without severe aggression issues. It should be noted, however, that any larger predatory species poses a potential threat in terms of predation or harassment. The results of observed incidents of aggression or predation by tankmates from the author's observations are presented in Table 3.0.

Hematology

There has been a growing interest in the collection and analysis of blood from aquatic specimens in recent years. Most modern public aquaria are maintaining their own in-house databases of this information so that it may be used as baseline data in the future. The public aquarium community at large has also been quite open in sharing information with other facilities, and efforts to construct a comprehensive elasmobranch blood database have been undertaken at the Virginia Aquarium and Marine Science Center. Historically the hematological parameters of *R. terraenovae* have gone undocumented despite the large amount of anatomical and physiological research into the species, though Stoskopf (1993b) provides a complete set of serological values obtained from wild specimens, and Saunders (1966) provides an early description of blood cell sizes and erythrocyte counts. Elasmobranch specimens brought into quarantine at the Aquarium at Moody Gardens now routinely have hematology and serum chemistry analyzed as a matter of procedure, including the wild collected *R. terraenovae* observed in this study. Blood was taken by caudal venipuncture; and total erythrocyte count, total leukocyte count, and hematocrit are quantified using Natt-Herrick solution and an improved Neubauer chamber following the procedures outlined by Walsh and Luer (2004). Serum biochemistry was analyzed by the Texas Veterinary Medical Diagnostic Laboratories. Site of blood collection was recorded, as recent studies have shown that hematocrit varies with the site chosen (MyIniczenko et. al., 2006). Serum biochemistry values from 8 captive specimens are reported in table 4.0. Total erythrocytes were found to have a mean count of 15.1×10^8 cells ml^{-1} with a range of 12.4×10^8 cells ml^{-1} - 22.6×10^8 cells ml^{-1}. Total leukocytes had a mean of 11.1×10^7 cells ml^{-1}, with a range of 0.9×10^7 cells ml^{-1} to 1.6×10^7 cells ml^{-1}. Microhematocrit had a mean of 27.8% PCV with a range of 25-31% PCV.

Table 4.0 Serum Biochemistry of the *Rhizoprionodon terraenovae*, n=8.

Parameter	Unit	Mean	Min	Max
Total Serum Protein	g/dl	3.11	2.8	3.6
Albumin	g/dl	0.2	0.1	0.4
Calcium, serum	mg/dl	15.25	0.2	15.6
Phosphorus, serum	mg/dl	8.93	7.69	11.13
Glucose	mg/dl	132.12	109	143
BUN	mg/dl	352.35	173	519
Creatinine	mg/dl	0.08	0.02	0.16
Total Bilirubin	mg/dl	5.41	0.1	0.41
ALP	U/l	7.5	1	9
CK	U/l	2205	72	5383
AST (SGOT)	U/l	43.13	20	83
ALT (SGPT)	U/l	3.75	3	4
Globulins	g/dl	2.59	0	3.2
A/G Ratio		0.059	0	0.13
GGT	U/l	23	3	21
Amylase	U/l	933.38	41	2456
Cholesterol	mg/dl	108.14	39.7	176.3

Acknowledgements

The author wishes to thank Greg Barord and David Brosette of the Aquarium at Moody Gardens for their assistance in the husbandry and observation of captive specimens. Joshua Frey of Landry's Downtown Aquarium Houston, David Mahlmann of the Florida Aquarium, and Rebecca Leitner of Moody Gardens have also helped the author with the husbandry of this species in the past. Bob Snowden of the Pittsburgh Zoo and PPG Aquarium, as well as G. Barord, Jay Reynolds, and Roy Drinnen of the Aquarium at Moody Gardens spent many long nights with the author fishing for sharks. Library services of Texas A&M University at Galveston provided numerous papers on the subject, without which this work would not have been possible. All specimens were collected under Texas Parks and Wildlife Zoological Permit issued to the aquarium. Animal Capture Equipment of Bacliff, TX designed and fabricated the floating acclimation pen. G. Barord provided critical review of an earlier draft of the manuscript.

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Appendix I

Relationship Between Total Length, Fork Length, and Precaudal Length in *Rhizoprionodon terraenovae* from Equations Given by Loefer and Sedberry (2003)

TL (mm)	TL (cm)	FL (mm)	FL (cm)	PCL (mm)	PCL (cm)
200	20	154.3	15.4	133.1	13.3
250	25	196.3	19.6	172.2	17.2
300	30	238.3	23.8	211.3	21.1
350	35	280.4	28.0	250.3	25.0
400	40	322.4	32.2	289.4	28.9
450	45	364.4	36.4	328.5	32.9
500	50	406.4	40.6	367.6	36.8
550	55	448.5	44.8	406.7	40.7
600	60	490.5	49.0	445.8	44.6
650	65	532.5	53.3	484.9	48.5
700	70	574.5	57.5	524.0	52.4
750	75	616.6	61.7	563.1	56.3
800	80	658.6	65.9	602.2	60.2
850	85	700.6	70.1	641.3	64.1
900	90	742.6	74.3	680.4	68.0
950	95	784.7	78.5	719.5	71.9
1000	100	826.7	82.7	758.6	75.9
1050	105	868.7	86.9	797.7	79.8
1100	110	910.7	91.1	836.7	83.7

Appendix II

Metabolic properties of selected inshore carcharhinid and sphyrnid shark species from the literature.

Species	Temp °C	$mgO_2kg^{-1}h^{-1}$	$kJ\ kg^{-1}\ d^{-1}$	$kcal\ kg^{-1}\ d^{-1}$	% BW Day^{-1}	Reference
Sphyrna lewini		160.9	**115**	12.6		Duncan, 2006
*Sphyrna lewini**	21	**161**	52.7	12.6		Lowe, 2001
*Sphyrna lewini**	29	**203**	66.5	15.8		Lowe, 2001
Sphyrna lewini		**105**	34.4	8.2		Sundström and Gruber, 1998
Sphyrna tiburo		**296**	96.9	23.2		Carlson et. al., 1999
*Sphyrna tiburo***		977.3	**320**	76.5		Sundström and Gruber, 1998
*Sphyrna tiburo****		1114	**365**	87.2		Sundström and Gruber, 1998
Carcharhinus plumbeus					**1.0**	Cortés and Gruber, 1990
Carcharhinus leucas		72.8	23.8	**5.7**		Schmid and Murru, 1994
Carcharhinus acronotus		**395**	129.4	30.9		Carlson et. al., 1999
*Negaprion brevirostris***		**152**	49.8	11.9		Carlson et. al., 1999
*Negaprion brevirostris****		**382**	125.1	29.9		Carlson et. al., 1999
Negaprion brevirostris					**1.5-2.0**	Cortés and Gruber, 1990
Rhizoprionodon terraenovae					**1.4-2.1**	Branstetter, 1987

Numbers in **bold** indicate original values as reported by the cited references, all other values calculated using oxycalorific values reported by Eliot and Davidson (1975). Many of the values from original sources were averaged from multiple values reported. This table is for general reference use only, the reader is encouraged to consult the original sources for detailed metabolic information.

*Denotes varied temperature

**Adult specimens

***Juvenile specimens

THERAPEUTIC PRESSURE CHAMBERS FOR FISH

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Introduction

Sea World Durban was a public aquarium which was created to provide a base and a revenue source for marine research. It was owned and operated by a non-profit company, the South African Association for Marine Biological Research. Starting with a single 800 cubic meter oceanarium tank in 1959, it grew to a 4500 cu. m. complex by 1976; featuring fish, sharks, dolphins, fur seals and penguins. In addition to aquarium staff, 25 full time scientists and assistants conducted research, which focused on marine conservation and fisheries management. This continues today. In 2003 our institute moved to a new facility 4km away, named uShaka Marine World. uShaka is a theme park with a total exhibit volume of 17,000 cu.m.; divided equally between aquarium and mammals.

Over the years Sea World and uShaka staff evolved collecting and husbandry techniques for a wide variety of creatures. An item which we developed and found to be valuable, which does not seem to be in general use, is the oxygen pressurized therapeutic chamber. We use these to treat barotrauma in newly captured fishes; and to remedy certain other problems which can appear in captive specimens.

Collecting

A wonderful variety of fish species is found in our region. 300km to the north of Durban coral reefs occur, with an associated Indo Pacific fish fauna. To the south a more temperate fauna occurs, with a number of endemic species. Some of these grow to a large size.

Our coastline lacks shelter and the sea is often rough. We try to make optimum use of favourable conditions. Our fish collecting methods consist mainly of diving and angling on reefs. To a lesser extent we use small long-lines and seine netting. We work from surf launched ski-boats and rigid inflatables. In the past we shared research vessels with our scientists, collecting while they conducted surveys. We presently hire a small trawler, which can carry two oval 4 tonne specimen tanks plus one 8 tonne tank. Off Durban angling predominates on reefs from 20–70 meters. Our northern coral reefs lie mainly between 15–25 meters, where we use hand and barrier nets. Captured fish are placed in underwater cages.

Barotrauma

For many years barotrauma was a major obstacle to fish collecting at depths exceeding 10 meters. As fish are brought to the surface pressure decreases by roughly 1 bar per 10 meters. Swim bladders expand and fishes' stomachs may be forced out of their mouths. Rapid remedial treatment is required. When angling on deep reefs we once relied entirely on hypodermic needles to deflate distended swim bladders – a well known technique. This is effective for many species but some are sensitive and high mortalities can occur. Needles are often only partly successful, with treated fish remaining over-buoyant.

When conducting diving collecting from anchored ships we raised the specimen cages very slowly to allow the fish to adjust to the pressure change. We found that 6 hours were needed to raise a cage from 20 meters. This is impractical in small craft in rough sea conditions. Elsewhere divers put in needles before ascending. Our cage can contain over 50 fish of very different sizes after a barrier net dive. It is not possible to place needles in all of them.

Development of pressure chambers

In 1976 we made a 120ℓ steel chamber which was pressurized with compressed air. It was not successful and was discarded. Between 1990 and 1996 we built four prototype fiberglass chambers, ranging in volume from 50ℓ – 500ℓ. We pressurized them with oxygen and saw greatly improved results. The prototype chambers were laid up by hand on inflatable moulds. Shapes were cylindrical with hemispherical ends. In routine use 70% of their volume contains seawater. All were tested to twice their working pressures of 1.5 bar. Our prototype chambers gave us very encouraging results but they were not legal pressure vessels. We approached a local company which specializes in such work, with a view to acquiring legal chambers. Many design calculations and technical drawings were required, which were beyond our capability. We provided conceptual drawings and liaised during design and construction. The end result was that the chambers were approved and are Lloyds registered pressure vessels. These are our 1200ℓ and 250ℓ chambers, built in 2005 and 2006. We will shortly order a 50ℓ chamber. Although not officially classified as a pressure vessel due to its small size and low working pressure, it still needs to be professionally designed to be legal. We have scrapped three of our original prototypes.

Locks

When diving for fish at 15 - 25 meters on coral reefs our normal procedure is to set a barrier net with a holding cage positioned at one end. Divers work in successive pairs, using hand nets against the barrier. Captured fish are placed in the cage. At the end of the operation the cage is raised to the surface, where the fish are quickly transferred to the chamber. This is pressurized to 1.5 bar with oxygen, and a decompression schedule begins. The boat beaches and decompression proceeds at our field base. Eventually the treated fish are transferred to transport tanks. Simple chambers without locks work well when the specimens arrive in batches at the surface.

Light tackle angling can be very productive, but the fish arrive at the surface at irregular intervals. We developed entry locks through which new specimens can be introduced into chambers already under pressure. The lock is a tube through the chamber side, with an inner and an outer door. The inner door is operated by means of a pushrod. A captured fish is quickly unhooked and placed head forward into the lock, which contains some water. The outer door is closed and the lock pressurized with oxygen. When the lock pressure equals that of the main chamber the inner door opens and the fish slides into the water. An experienced operator can put a fish through the lock in 10 – 15 seconds. The inner door is then closed with the pushrod, and the lock is depressurized. The outer door opens, ready for the next specimen. It is possible to achieve partial water changes by introducing new seawater through the lock. Excess water can be drained via the bottom drain valve.

The slinger, *Chrysoblephus puniceus* (fam. Sparidae) is an important linefish on which we once conducted extensive research. Small specimens angled from 20 meters exhibited an 80% mortality rate when deflated with needles. Using a chamber a 90% survival rate was achieved.

Efficacy of oxygen

To demonstrate the effect of oxygen in pressure chambers we conducted the following experiments:

We acclimatized a shoal of Olive grunter, *Pomadasys olivaceum* (family Haemulidae) of similar size to aquarium conditions over a period of 4 weeks.

Experiment 1:

16 fish, total weight 1kg, were placed in 35ℓ of sea water (T22^{0}C, initial D.O. 6.2 mg/ℓ, pH 8.15, salinity 35$^0/_{00}$) in a 50ℓ chamber. This was pressurized to 1.5 bar with compressed air. After 20 minutes all the fish had died. Residual oxygen in the water was 0.25mg/ℓ.

Experiment 2:

On the following day 16 fish from the same batch, total weight 1kg, were placed in 35ℓ of water in the same chamber. It was pressurized to 1.5 bar using oxygen. After 12 hours no fish had died. The chamber was depressurized and the fish were transferred to a holding tank. Residual oxygen in the chamber water exceeded 20mg/ℓ (off scale on the meter). No fish died subsequently in the holding tank.

These experiments showed us that a considerable biomass can be maintained in a small water volume, in an oxygen pressurized chamber, without a continuous oxygen supply.

Decompression schedules

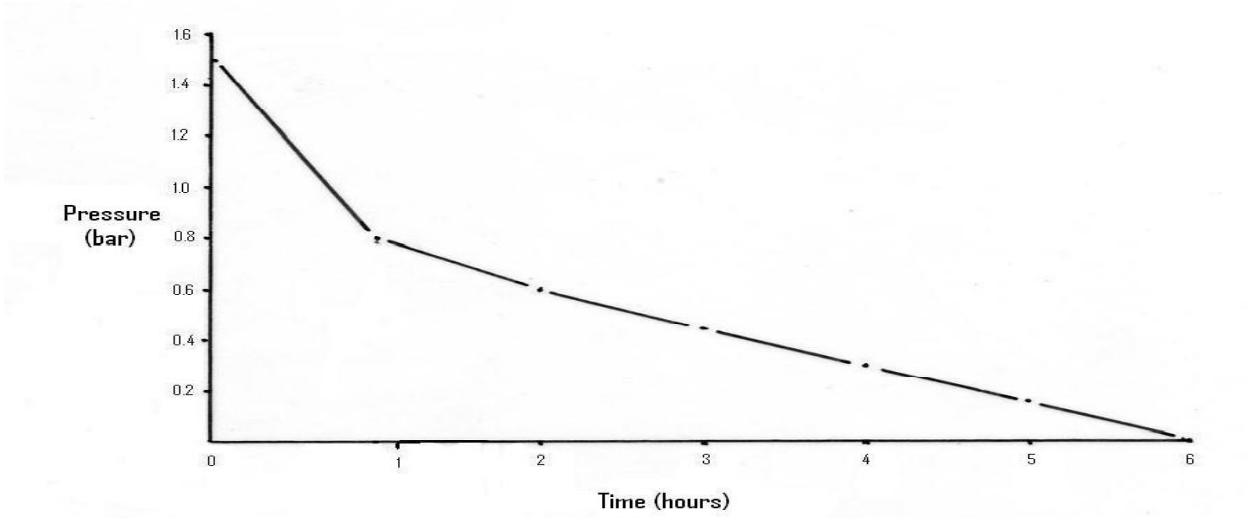
We found that it is not necessary to re-pressurise a fish to the full depth at which it was captured. A fish taken from 40m is fine when re-pressurised to 1.5 bar (equivalent to 15 meters). Pressure can be reduced quite quickly to 0.8 bar, after which one must slow down. The specimens are regularly observed through viewing ports. The decompression rate can be varied. Should a fish still be struggling with over-buoyancy at the end of a procedure, it can be individually re-pressurised after a quick water change. At the pressures at which we work we have not encountered oxygen toxicity.

Types and sizes of chambers

We found that we need 3 sizes of chamber:

a. A simple 50ℓ chamber without a lock. Because it is light, convenient and uses little oxygen it is our most frequently used chamber. It is often employed for low-key local collecting. For example, the shrimpfish *Aeoliscus punctulatus* can be collected by divers on a reef 500 meters from our uShaka aquarium. Shrimpfish are very sensitive to pressure changes and can exhibit symptoms when raised from only 8 meters. They cannot tolerate needles. The small chamber solves the problem. This chamber is often used to treat individual small fish in quarantine.

Figure 1. Typical decompression schedule.



b. A 250ℓ chamber with a lock is used on small craft for diver collecting and light tackle angling. This chamber is classified as a pressure vessel.

c. A 1200ℓ chamber with a lock is used aboard ship, mainly when heavy tackle angling on deep reefs. In addition to the lock there are two doors, configured so that the chamber does not drain when a door is opened. A small person can enter this chamber to facilitate the removal of big specimens. It is bolted to a steel frame for lifting (empty) by crane. This chamber can take a grouper-shaped fish of 50kg. (Figure 3) In our quarantine facility it is fitted with wheels so that it can be moved easily.

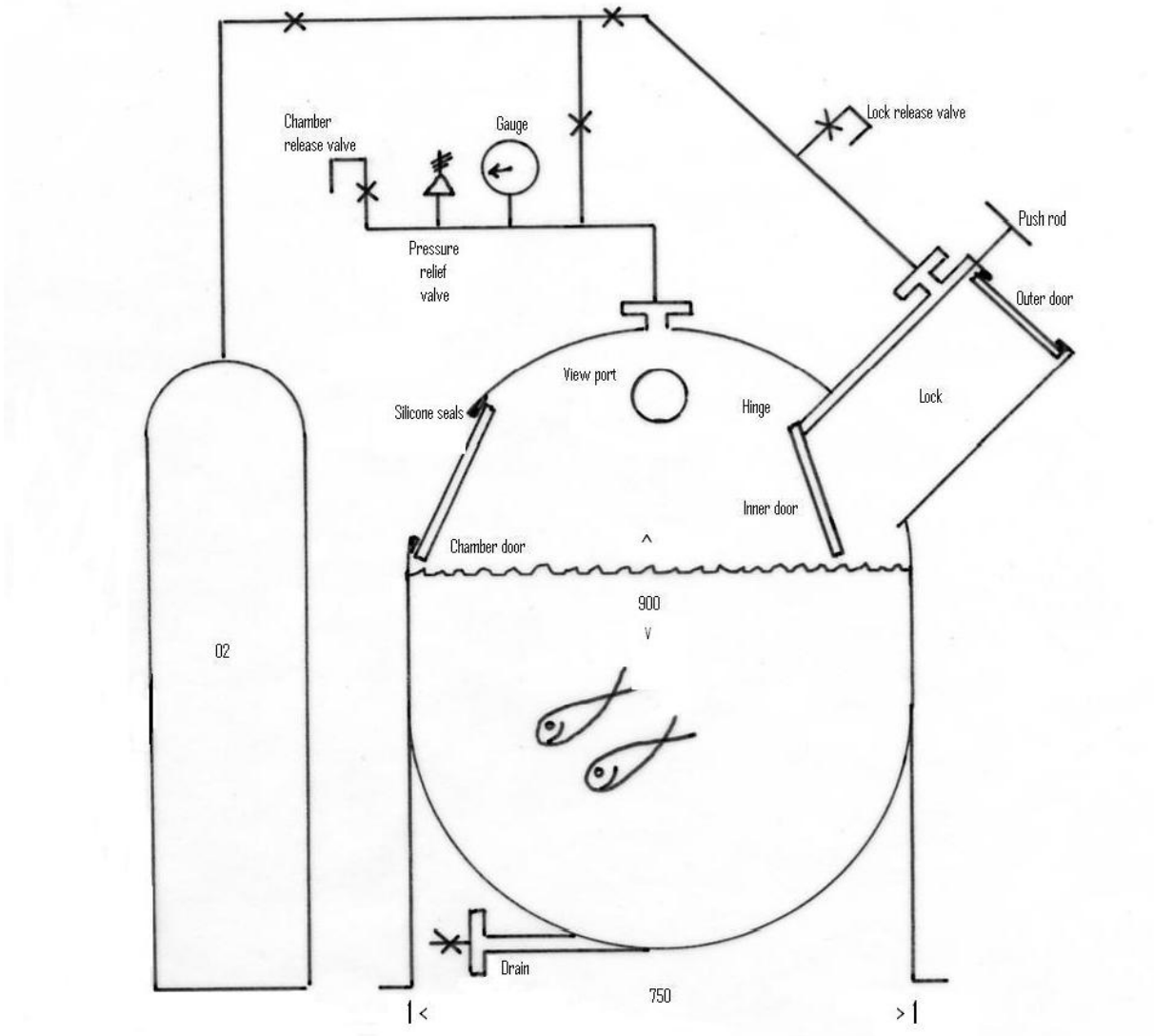
The Black musselcracker, *Cymatoceps nasutus* (family Sparidae), has a bizarre facial appearance when adult hence is valuable for display. Big specimens captured at depth do not survive needles. We presently have three up to 25kg in weight on display. All owe their survival to the chamber.

Extended treatments are being increasingly used in quarantine, during which the water can become murky. We are presently designing an external filtration system for the big chamber.

Medical use of chambers

When they are not in the field our chambers are housed in our hospital / quarantine facility. They are useful for treating the effects of supersaturation and other problems, e.g.: Exopthalmia can be reversed if treated promptly. One of a breeding pair of clownfish was recently saved from severe exopthalmia.

Figure 2. 250 litre pressure chamber. Working pressure 1.5 bar.



A batch of baby seahorses was accidentally exposed to fine bubbles, and floated helpless at the surface. Most recovered under pressure.

A big Red Steenbras, *Petrus ruprestris* (family Sparidae) (15kg), exhibited unusual behaviour in quarantine, "spy hopping" with its head above water and generally disoriented. We suspected neurological problems, possibly caused by a bubble. After a 3 day treatment in the big chamber behaviour reverted to normal. The fish is now on display.

Our quarantine staff regularly use the chambers.

Figure 3. 1200ℓ and 250ℓ pressure chambers

Safety

There are three main safety issues:

a. Structural failure. Although pressures are less than those used in automobile tyres, considerable forces result as surface areas increase. The force on a door of our big chamber is roughly equivalent to the weight of a small truck. Our professionally built chambers are annually inspected by a certified agency and hydrostatically tested every four years. We visually check them before use. Certified pressure relief valves are fitted. Our legal chambers were also tested to twice their working pressures.

b. Fire. Fire is possible in an oxygen enriched atmosphere. Glass reinforced plastic structures can burn. Glass reinforced plastic was selected because of weight and corrosion considerations. We assessed the possibility of fire as very low, because chambers contain 70% of their volume as sea water. Everything inside is wet and the atmosphere is saturated. When decanting oxygen we observe similar procedures to nitrox cylinder filling i.e. no oil or grease, flexible hoses, no smoking. We use a flame arrestor on the hose to prevent flash-back to the cylinder.

c. Training. Although procedures are simple we enforce safety procedures. Only staff who have received training operate the chambers. Access to them is controlled.

Conclusion

The use of oxygen enriched pressure chambers has opened new avenues for our aquarium operations. We can target certain species formerly considered difficult when captured at depth. We can rectify certain physical ailments which we were previously unable to treat.

Speculating on other possibilities:

Exploring the effects of pressure on fish medication could be rewarding.
Our chambers are often used as low pressure transport (0.2 bar) tanks when returning from remote field trips. Extending this to purpose designed transport vessels for active creatures such as sharks and tuna could be interesting, because such high levels of dissolved oxygen occur in the water. Once the tank has been pressurized, a continuous oxygen supply should be unnecessary.

Acknowledgements

Thanks are due to George Buckland who assisted with the construction of the prototype chambers; and to S.A.A.M.B.R. which carried the cost of these developments.

Disclaimer

Because of the possibility of injury, therapeutic chambers should be professionally designed and constructed.

THE EFFECTS OF BACKGROUND COLORS IN THE TODDLER TANKS AT THE MONTEREY BAY AQUARIUM

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Introduction

Within the Splash Zone exhibit at the Monterey Bay Aquarium there is an area called the Coral Cove (figure 1). This area is designed for children 3 years and younger and their parents. The area has four main areas that are designed to entertain and educate young children. The four main areas are called Triangles, Squares, and Circles (Blocks), Feel the Waves (Water Bed), Sea Babies, and Waterplay, There is also a sitting area with cubby holes where shoes and gear can be stored (Shoes).

The Feel the Waves area is usually referred to as the Waterbed area because it has a large industrial waterbed that toddlers can play on. It is surrounded by a lighted bubble wall. The Sea Babies area is for children that are still crawling and their parents. Here adults can sit on padded benches. Babies can crawl on a padded floor and there are several sea oriented tactile interactives that they can play within their reach. Another popular area is the Waterplay area. Here there are three fountains of water that arc into a shallow basin and several floating fishes for toddlers to experiment with.

Within the Block area the triangles, squares, and circles are bright primary three dimensional shapes that can be picked up and physically placed in the corresponding shape at a bench. Along the wall of the Block area there is a 50 gallon tank system with 3 viewing areas commonly referred to as the Toddler Tanks. Each viewing area exhibits a single species. The three species exhibited are Scarlet cleaner shrimp (Lysmata deblius), Green Chromis fish (Chromis viridis), and Blue Sea stars (Linckia laevigata).

Figure 1. Coral Cove

There were a number of subjective observations of the Coral Cove made over the past five years. It was observed that children and there parents seemed to be focus on the Waterbed area of the Coral Cove more than any other area and that very few children took an interest or even noticed the toddler tanks. From a Husbandry standpoint one of our goals is that visitors focus on the live exhibit component of each exhibit. Husbandry strives to make each live exhibit as attractive, appealing, and engaging as possible.

With this in mind, an attempt was made to bring increased visitor focus on the toddler tanks which is the only live exhibit component within the Coral Cove. Observations of children's toys and clothes, as well as direct observation of children revealed that bright primary colors tended to attract their attention and be a very integral part of their world. The toddler tanks have a pastel blue Kydex® background. This background was chosen so that the children could focus on animals in the tanks without being distracted. However, general observation showed that very few toddlers noticed these tanks even if they were playing within the block area. It was hypothesized that adding a bright color background to each of the viewing areas of the toddler tanks might attract more direct observation of the live fish and invertebrates within the Coral Cove. A bright primary green, yellow, and red were chosen because they matched the triangles, squares, and circles that were used in the block area. It was also hypothesized that if subjects spent more time at the block area they would spend more time directly and/or passively observing the live animals in the toddler tanks (since these viewing areas were along the wall directly in front of the block area).

At the Monterey Bay Aquarium husbandry works closely with the exhibits division and changing the background color within the tanks needed validation and proof that such a change would be beneficial to the Coral Cove as a whole. In addition, a cost estimate of changing the backgrounds was made and the estimate was excessive. While only a few square feet of each new color was needed for the background, the colors were unusual enough for the manufacturer that their distributors do not keep them in stock. Such bright primary colors such as red, green, and yellow needed a special run by the manufacturer and therefore, the aquarium needed to order an entire case of each color, an amount that was in excess of several thousand dollars. For these two reasons a low cost experiment to test whether brighter color backgrounds was devised.

Methods

This test compared the effect that bright color backgrounds placed within the toddler tanks had on the length of time children spent at each area in the Coral Cove (Test) in comparison to the standard pastel blue background (Control) in the toddler tanks (figure 2).

Three different colors of poster board were laminated and placed as the test background in the toddler tanks (figure 3). The cost of materials and lamination was approximately $30 which met the initial need of an experiment that was low cost. Colors were chosen so that the animals could be easily observed within each viewing area. For the Cardinal shrimp a standard additive primary green was selected to bring out the shrimp's red color. A primary yellow was selected to focus on the Green damselfish. And a primary red color background was selected to highlight the Blue sea stars. The number of animals was not changed and there was no additional aquascaping done within the viewing area for the test.

Figure 2. Control

Figure 3. Test

For the test and control, the researcher stood outside the Coral Cove and observed the actions of a single child in the Coral Cove. Each child's approximate age (although it was not possible to use this data since the age was never substantiated) as well as their sex was recorded. The time was recorded once the subject entered through the gate of the Coral Cove and each time s/he move to another area of the Coral Cove. The beginning and end time was recorded for each of the following areas: Waterplay, Sea Babies, Waterbed, Blocks, and the time spent in the Shoe area. The time was recorded once the child exited through the gate of the Coral Cove. It was also recorded whether the test subject observed the toddler tanks at all by a yes or no.

In general, subjects visited at least two different areas of the Coral Cove. Some children moved methodically from one area to another exiting after they or their parents decided to leave. Other children bounced back and forth from area to area returning multiple times to the same area. For instance it was very normal for a test subject to keep returning to the Waterbed area. No distinction was made between parents directing there child or letting them wander around by themselves. And once the test subject had exited through the gate she was no longer recorded even if she re-entered later (this was never directly observed with the control or test subjects but was noticed on several occasions by non-test individuals).

29 subjects were observed for the control and 26 test subjects were observed using the bright color backgrounds. The total time spent at each area (multiple visits to one area were added together) as well as the percentage of total time at each area per individual was calculated.

Once the data was collected a focus of the Block and Fish area was also calculated. The number that noticed the toddler tanks, visited the blocks, and visited the blocks first was recorded for the entire data set and for each gender. A Two-tailed Chi-square test was used to determine whether the test results were significantly different.

An Unpaired T-test was used to analyze the total time spent at each exhibit and the percentage of total time per individual.

In general statistical significance was based on whether two populations had a p-value of .05 or less for both the Two-tailed Chi-Square and Unpaired T-test.

Results

Table 1 shows the number of the individuals that noticed the toddler tanks, visited the blocks, and visited the block area first for both males and females. 35% of the control noticed the toddler tanks while 46% of the test noticed them. 10% more individuals visited the block area when bright colors were added to the tanks. However, neither revealed a significant difference. But a two-tailed chi-square test revealed that the difference between the control and the test was significantly different when the number that visited the blocks first was compared.

Table 1. All Data: Males and Females

Variable	Control	Test	Two-tailed p-value
Number of Subjects	29	26	
# that noticed Toddler Tanks	10 35%	12 46%	0.2792
# that visited Blocks	17 59%	18 69%	0.5753
# that visited Blocks First	8 28%	10 38%	0.0298* *significant
Ave time before visiting Blocks	3 min 29 sec	1 min 58 sec	0.1229 Two-tailed t-test
Ave time spent in Coral Cove	2 min 34 sec	4 min 19 sec	Same as total time t-test

Each control and test group were then divided into females only and males only to see if that affected the significance of the findings (Table 2 and Table 3, respectively). However, gender examination of who noticed the toddler tanks, who visited the blocks, and who visited the blocks first revealed that there were no significant difference between the control and the test.

Table 2. Males only.

Variable	Control	Test	Chi-square p-value
Number of Subjects	15	14	
# that noticed Toddler Tanks	6 40%	10 71%	0.1394
# that visited Blocks	12 80%	12 86%	1.000
# that visited Blocks First	7 47%	7 50%	1.000

Table 3. Females only.

Variable	Control	Test	Two-tailed p value
Number of Subjects	14	12	
# that noticed Toddler Tanks	3 21%	2 17%	1.000
# that visited Blocks	5 36%	6 50%	0.6922
# that visited Blocks First	2 14%	4 33%	0.3652

The control and test were also combined to see if there were absolute gender differences (Table 4). The percentage of males that noticed the toddler tanks and visited the block area versus the percentage of females was significantly different. However, the percentage of males versus females that visited the block area first was not significantly different.

Table 4. Results from Test and Control.

Variable	Males	Females	Chi-Square p-value
Number of Subjects	29	26	
# that noticed Toddler Tanks	16 55%	5 19%	0.0115*
# that visited Blocks	24 83%	11 42%	0.0024*
# that visited Blocks First	14 48%	6 23%	0.0911

Two-tailed t-tests were used to examine absolute time and percentage of time per individual spent at each major area as well (Table 5). All p-values were less than 0.05 signifying that there was no significant difference between the control and test.

Table 5. All Data: Males & Females

Variable	Control N=26	Test N=29	Control N=26	Test N=29		Control N=26	Test N=29	
Areas visited	# that visited area	# that visited area	% Time/ individual	% Time/ individual	p-value	Total average time	Total average time	p-value
Water Bed	21 72.4%	22 84.6%	48.6%	38.7%	0.366	0:04:33	0:04:03	0.460
Waterplay	20 70.0%	18 69.2%	44.8%	37.9%	0.366	0:02:23	0:04:36	0.078
Blocks	17 58.6%	18 69.2%	28.7%	30.8%	0.326	0:02:48	0:04:19	0.085
Sea Babies	6 20.7%	8 30.8%	21.1%	16.8%	0.259	0:02:41	0:02:47	0.259
Shoes	13 44.8%	15 57.7%	14.9%	11.3%	0.400	0:01:09	0:01:21	0.185

After looking at the raw data it again appeared that there might be a difference between females and males so the data was split to see if there were any significant differences for total time and percent of the individual's time spent at the three most popular areas for each gender: Waterbed, Waterplay, and the Blocks. In table 6 the percentage of time spent per male went from 52% with the standard backgrounds to 32% when bright primary color backgrounds were tested. In table 7 the percentage of time spent per female went from 67% for the control to 34% for the test. However, neither percentage drop was significant (p-values equaled 0.066 and 0.061, respectively)

Table 6. Males: Control = 15; Test = 14.

Variable	Control: % Time/ individual	Test: % Time/ individual	T-test p-value	Control: Total Time	Test: Total Time	T-test p-value
Water Bed	51.6	31.7	0.066	5:49	3:06	0.104
Waterplay	27.3	41.4	0.199	1:58	2:45	0.091
Blocks	31.3	36.0	0.129	2:55	2:36	0.110

Table 7. Females: Control = 14; Test = 12

Variable	Control: % Time/ individual	Test: % Time/ individual	T-test p-value	Control: Total Time	Test: Total Time	T-test p-value
Water Bed	44.6	47.0	0.173	2:51	5:00	0.062
Waterplay	66.7	34.4	0.061	3:03	5:08	0.190
Blocks	19.2	20.4	0.440	2:11	2:46	0.192

Last a statistical test of the total time spent by the control and test for the entire data, males only, and females only was calculated (Table 8). The entire combined time spent during the control was 3 hours 48 minutes while the entire combined time of the test was 5 hours. There was a significant difference (p= 0.035) when an unpaired t-test was performed on total time. The combined time spent by the females in the control group versus the combined time of the females in the test group also showed a significant difference (p= 0.019)

Table 8. Total Time spent in Coral Cove.

Variable	Control	Test	T-test p-value
All data	3:48:24	5:00:42	0.035*
Males only	2:33:45	2:44:25	0.299
Females only	1:14:39	2:16:17	0.019*

Discussion

It was surprising how much statistical evaluation could be done with this data set. Overall adding bright color backgrounds to the toddler tanks caused about 10% more individuals to notice the toddler tanks as well as visit the block area (table 1). In fact, bright color backgrounds resulted in approximately a 10% increase in the number of individuals that visited all areas except for the Waterplay area (table 5). Interestingly, when each test was divided into males and females there was actually a decrease in visiting certain areas. Males spent approximately 20% less time at the Waterbed area (table 6) and females spent approximately 32% less time per individual at the Waterplay area (table 7). However, none of these comparisons were statistically significant. This could be because there no significant difference or that the sample size is too small. Especially when the data sets are divided into males only and females only, the sample size is fairly small (12-15 per data set). The smaller the sample size the bigger the difference has to be, commented Steven Yalowitz (Yalowitz, 2007) He felt that larger sample sizes could tell if you had a statistical difference much easier. When he does research he usually tries to get a sample size of 100 or so per data set.

In summary the only comparisons that revealed statistical significance of 0.05 or less were:

1) The number that visited the Blocks area first - Control versus the Test for all individuals (Table 1).
2) The number of males versus the number of females that noticed the toddler tanks- Total Males versus Total Females for both data sets (Table 4).
3) The number of males versus the number of females that visited the block area- Total Males versus Total Females for both data sets (Table 4).
4) The total time spent in the Coral Cove for all individuals - Control versus the Test (Table 8).
5) The total time spent in the Coral Cove for females only –Control versus the Test (Table 8).

An attempt was made to draw young visitors to the only live exhibit in the Coral Cove by adding bright color backgrounds that matched the large triangles, squares, and circles that the visitors played with in the block area. The addition of these backgrounds caused more individuals to visit the block area first. It also changed the absolute length of time that each visitor spent in the Coral Cove altogether. However, when dividing the data sets up into males and females it appeared that it was the females that were spending a significantly longer length of time in the coral cove then the males (Table 8).

What was interesting about this study is that it also revealed certain gender differences. Overall, only 19% of females (versus 55% of the males) even noticed the toddler tanks regardless of what was done to the backgrounds. Also, only 42% of the females (versus 83% of the males) went over to play in the block area. There was another interesting gender dynamic that resulted that would need a larger sample size to validate. Adding the bright color backgrounds tended to pull more individuals over to the block area at the expense of the Waterplay area for females and the Waterbed area for males.

Conclusion

Changing the toddler tank backgrounds to bright primary colors draws more young visitors to that area of the Coral Cove and caused more female visitors to spend more time in the Coral Cove.

However, a larger sample size is probably needed in order to pinpoint the dynamic changes such as changes in areas visited and time spent at each play area that it may cause within the Coral Cove.

This research also was very helpful in that it provided a low-cost way to not only initiate discussion within the aquarium but also to evaluate whether the cost of such a change would be enhance the visitor experience in the Coral Cove Exhibit.

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IMPROVING LARVAL SURVIVAL AT THE NATIONAL LOBSTER HATCHERY THROUGH LIVE FEED DISINFECTION

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Introduction

Results of testing at the National Lobster Hatchery (NLH) in Padstow, Cornwall, provide evidence that the pharmaceutical treatment Pyceze®, was able to reduce mortality rates of larval European lobsters, *Homarus gammarus*, when added as a disinfectant to the culture of *Artemia* nauplii feed. Pyceze® bactericidal efficacy is achieved by a 50% solution of the chemical Bronopol (2-bromo-2-nitropropane-1,3-diol). Bronopol is a broad spectrum antimicrobial agent that has been used extensively as a preservative in toothpaste, hand creams and many other household products. Pyceze® is also licensed as an anti-fungal treatment for the salmon industry, where it has been found to be successful in reducing mortality in many marine animals, including other fish species and juvenile scallops. This is thought to be the first trial of the product with larval lobsters.

Much larval mortality is classed as unavoidable (e.g. larval cannibalism and intra-population aggression). However, mortality still occurs as a result of 'avoidable' trauma, such as infection by viral, fungal or bacterial diseases. Although disease problems occur in the wild, epidemia in hatchery conditions can be very problematic. Any increase in larval survival rates will increase the efficiency of the stock enhancement project as a whole.

Photo 1. A post larval lobster ready to be released

Early-stage *H. gammarus* larvae have a greater chance of successful development if fed on live food. However, the cultivation of *Artemia* nauplii can allow extensive growth and diversification of the microbial community within culture water. By ascertaining the degree of microbial colonization in a variety of hatchery locations, the likely source of pathogenic introductions could be identified for further isolation and treatment. INVE© BDS-G® dip slides

incorporating specialised marine agar were used to determine concentrations of heterotrophic and *Vibrio* spp. bacteria in cultures of *Artemia* nauplii and *H. gammarus*, and in the hatchery seawater supply. As predicted, the live feed culture had by far the highest bacterial loading, especially of *Vibrio* spp.

Materials and methods

Prior to embarking on the main larval investigation, the optimum concentration of Pyceze® application to the *Artemia* culture was determined. Bronopol at 150mgL^{-1} (300 mgL^{-1} of Pyceze®, the highest dose administered during tests) was determined to be the most promising concentration, providing an 89.4% reduction in the population of *Vibrio* spp. without significant *Artemia* mortality.

For the larval lobster trials, two 150 litre re-circulation systems were utilised, each with a pair of 33 litre kreisel cones containing approximately 750 larvae, suspended above an 85 litre reservoir. Seawater flow rate was 1,060L/hr. Water quality parameters were measured throughout trialling; Salinity (36‰, ±2‰), Temperature (19.5°C, ±1.5°C), pH (8.0, ±0.2), Nitrite (0.0‰, ±0.4‰) and Ammonia (0.0‰, ±0.25‰).

Artemia were cultivated in 10L vessels, with 50,000 nauplii cultured at 20°C (±2°C) and 35‰ (±2‰). Both cultures were administered with 600mgL^{-1} of Selco®, 7.2mgL^{-1} of Bio-Moss® and equal aeration, but, while the Control culture had no Pyceze® added, the Test culture was supplemented with 3000mg of Pyceze® (to yield a solution with 150 mgL^{-1} of Bronopol). The nauplii were then enriched for 24 hours before being fed to the larvae.

Photo 2. Artemia culture vessels

Results

Figure 1 shows there was a distinct difference in the degree of bacterial loading between the Control and Test systems. *Vibrio* spp. CFU/ml was higher in the Control than the Test in every sample, and on Day 8 the Control system's *Vibrio* spp. concentration was almost triple the levels sampled from the Test system. So throughout the larval rearing trials, the degree of culture water contamination in the Control system was consistently greater than it was in the Test system.

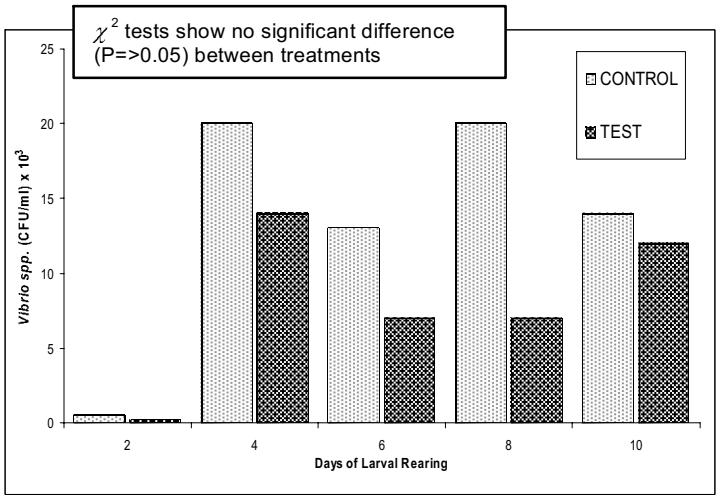

Figure 1. A histogram showing fluctuations in the levels of *Vibrio* spp. within the culture systems throughout the 10 days of the investigation.

The level of larval mortality was consistently higher in the Control system to the Test system, while the rate of increase in mortality also accelerates faster in the Control system, as can be seen in Figure 2 (below). Between Days 6 and 10, the level of cumulative mortality was consistently reduced by >22% in the Test system. By Day 10 a total of 240 larvae had died from the Control system and 168 mortalities were recorded from the Test system, a difference of 72 individuals.

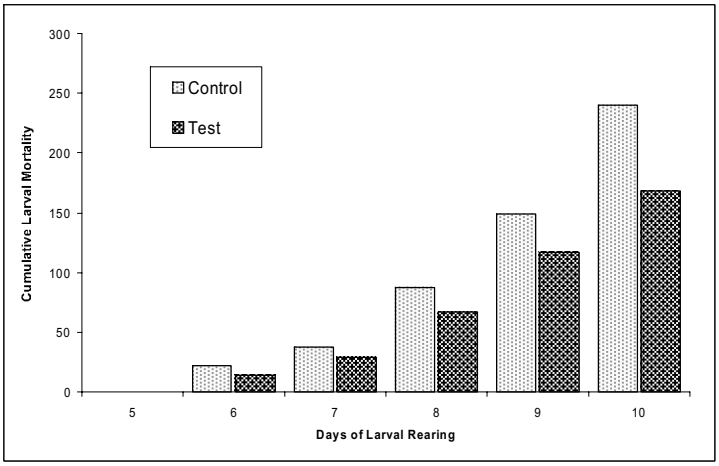

Figure 2. A histogram showing the total cumulative *H. gammarus* larval mortality within the Control and Test systems.

This difference represents a 30% decrease in the occurrence of larval mortality in the Test treatment. Given that each system began with 1500 larvae, the overall survival rate to Day 10 was 84% for the Control and 89% for the Test. However, this assumes that losses from cannibalism were insignificant whereas at these stocking densities this is not a safe assumption and the difference between the treatments in overall survival rate is likely to be considerably greater.

Discussion

Vibrio spp. colonisation was reduced in the Test system in every count. This provides a strong indicator that Pyceze® is effectively controlling the proliferation of these microbes, and the extent to which it is able to do this (Figure 1 shows that *Vibrio* colonisation in the Test is only 35% of the level seen in the Control on Day 8) suggests that treatment is causing the observed reduction in larval mortality.

The difference between treatments in *Vibrio* colonisation was not found to be statistically significant (P>0.05) by χ^2 test though, even after the data had been naturally logged. However, this result could be misleading in terms of the ability of pathogens to cause death by the continual weakening and cumulative overpowering of the lobster's immune defence. Like most

marine invertebrates, lobsters have reduced phagocyte activity of their haemocyte cells during larval stages and their ability to fight pathogens. at concentrated levels during a first exposure is unspecific and severely limited. Over the course of the investigation, larvae in the Test tank were exposed to less than 60% of the numbers of *Vibrio* spp. that Control larvae encountered.

Of all the measured variables in the larval rearing investigation, the reduced bacterial population, seems the most likely source of the statistically highly significant ($P<0.01$) larval mortality reduction. Although mortality doesn't correlate directly with the highest concentrations of *Vibrio* spp, the difference in the rate of larval deaths between the two trials may still be attributed to the degree of bacterial colonisation. Days 4 and 8 show the greatest influx of heterotrophic and *Vibrio sp.* bacteria in the Control system, yet more larvae perished in both control cones on Day 10. Likewise, Day 4 saw the most concentrated colonisation of the Test system by both heterotrophic bacteria and *Vibrio* species, yet Days 9 and 10 saw the most larval mortality. This suggests that *H. gammarus* larvae were not killed directly or immediately, and that mortality is likely to occur as a result of prolonged contact with pathogens, or because of continual weakening that leads to mortality during a stressful event, like handling, exoskeleton moulting between larval stages, or exposure to environmental stressors.

Overall this investigation provides a valid and encouraging assessment of Pyceze's® performance as a feed disinfectant for use in the culture of *H. gammarus*.

SCHEMATICS FOR AN IN-HOUSE BUILT CALCIUM REACTOR

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The purpose of this build was to provide calcium supplementation to a 350 gallon coral reef exhibit renovation. The calcium reactor was constructed and first put on a 325 gallon pre-existing coral tank in an alternate gallery. This allowed the design of the calcium reactor to be small, portable, and able to adapt to a variety of pre-existing life support systems. The following section includes the methods of construction. All parts were obtained from Aquatic Eco-Systems Inc. and a local hardware store. Special consideration: you will need two electrical outlets for the CO_2 solenoid and for the QuietOne 1200® recirculation pump.

Figure 1. Actual Calcium Reactor

A clear 4" PVC cap was glued onto the bottom of a 2' section of clear PVC. A 4" section of 3" PVC was cut with a 1.5" vertical slot cut out. The latter was placed inside the 4" section of PVC so that it rested on the bottom cap. A 4" circular piece of a non-toxic scrub pad, approximately ½" deep, was cut and placed on the inner 3" cut PVC. An appropriate sized hole saw was used to cut a hole approximately 1" from the top of the cap for a ½" Uniseal®.

Only ½" PVC was used to construct the recirculation life support. A ½" union was placed right after the Uniseal® followed by a ¾" cross. The bottom of the cross was for the carbon dioxide (CO_2) injection. A ¾" to ½" reducer was glued into the bottom of the cross, and a ½" to 3/16" nipple barb was glued into the reducer. Standard CO_2 black tubing was used to run from the nipple barb to the bubble counter attached to the CO_2 regulator. The regulator was hooked up to a filled 5lb CO_2 bottle. An in-line check valve was used to prevent water from back siphoning towards the CO_2 bottle. The right side of the cross allowed for the injection of aquarium water. A ¾" ball valve was glued into the right side of the cross. A ¾" union was placed after the ball valve to enable the entire apparatus to be movable. To connect the calcium reactor to the system a ¾" to ½" hose barb was glued into the union. This tapped to a pre-existing Iwaki 20 recirculation pump running a protein skimmer and a fluidized bed filter. The top of the cross followed the recirculation of the calcium reactor and was driven by a QuietOne 1200®. The pump was placed in line with ½" unions on either side to aide in maintenance issues. The effluent of the pump was placed so that it would directly face the top of the 4" clear PVC pipe. A hole was cut approximately 18" from the top of the bottom cap for another ½" Uniseal®. Again, ½" PVC was used to go from the effluent of the pump into a ½" union and, finally, through the Uniseal®. A ½" elbow/90 was used to direct the water downwards inside the 4" pipe.

A 4" flange, with corresponding gasket and bolts, was glued to the top of the 4" clear PVC pipe. A 4" to 2" reducer, followed by a 2" to ¾" reducer, followed by a ¾" to ½" female threaded reducer were glued together and inserted into the top of the 4" flange. A male threaded ¼" stopcock valve was attached to the top of this feature and a ¼" hose barb was attached to the effluent of the valve. Regular air line tubing was used to run from the barb as a drip line into the reservoir. Finally, media was placed inside the reactor. The whole apparatus was attached to a piece of pre-existing ¾" plywood using two 4" pipe hangers.

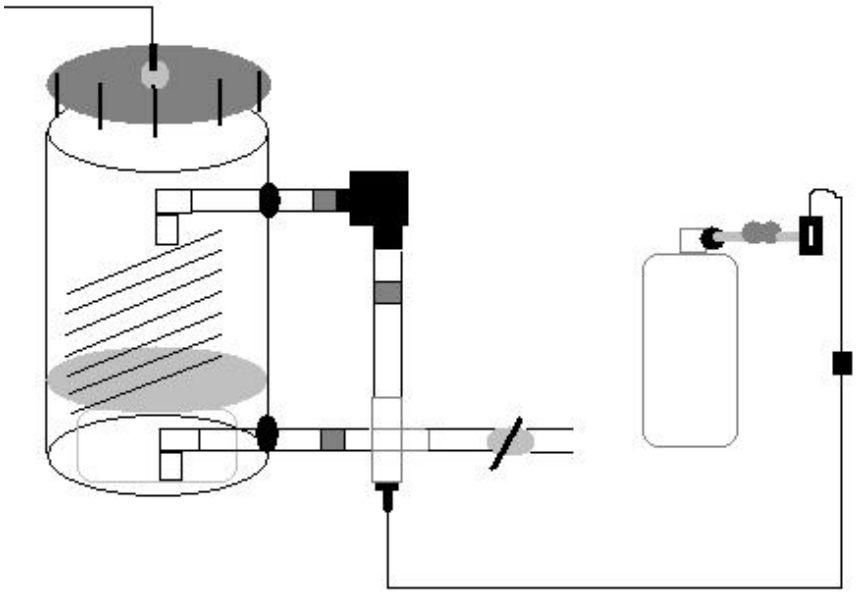

Figure 2. Schematics for Calcium Reactor

Parts List:

- (2') Clear 4" PVC Pipe
- (1) Clear 4" PVC Cap
- (2) 4" Flange
- (1) Gasket and Bolt Set
- (2) 4" Pipe Hanger and Screws
- (2) ½" Uniseal®
- (1) ¾" Cross - slip
- (2) ¾" to ½" Reducer - slip
- (1) ½" Male Adapter to 3/16" Nipple Barb
- (1) 4" to 2" reducer - slip
- (1) 2" to ¾" reducer - slip
- (1) ¾" to ½" female threaded reducer
- (1) ¼" Stopcock valve with male threaded adapters
- (1) 3/16" CO_2 In-line Check Valve
- (10') 3/16" Black CO_2 Tubing/Airline
- (1) CO_2 Regulator w/ Bubble Counter
- (1) 5lb CO_2 Bottle – filled
- (1) QuietOne 1200®
- (1) 5kg Calcium Media

The overall dimensions of the calcium reactor are 33"H by 22"W by 4"D. General hand tools, a drill, and a hole saw kit were needed for this build.

NOVEL FOODS AS ENRICHMENT FOR GIANT PACIFIC OCTOPUSES (GPOs)

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Anderson & Wood (2001) stated categorically that giant Pacific octopuses (GPOs – *Enteroctopus dofleini*) as intelligent animals (Anderson, 2005) deserved enrichment in captivity, just like charismatic megafauna such as mammals are now receiving (Shephardson, 1998). Since their treatment of octopus enrichment, other researchers have described the benefits of enrichment on captive cephalopods (Dickel, et al., 2000; Biegel & Boal, 2006) in terms of exhibiting increased natural behaviors and better performance in intelligence tests given to the octopuses. Anderson & Wood (2001) described 5 reasons for giving enrichment to octopuses,

- To bring behaviors to normal
- To decrease unnatural behaviors
- To enhance reproduction
- To prepare an animal for release into the wild
- To meet the expectations of the public

and Mather & Anderson (2007) further posit that enrichment should be considered for all captive animals on the basis of ethics.

There are several methods of achieving enrichment for octopuses (Anderson & Wood, 2001). Environmental enrichment such as providing naturalistic habitats in an octopus enclosure allow it to explore such as it does in the wild. Behavioral enrichment such as training, cleaning tanks, or feeding in a puzzle box (Rehling, 2000) gives them an added ability to develop mental skills such as those needed in the wild to survive (Beigel & Boal, 2006).

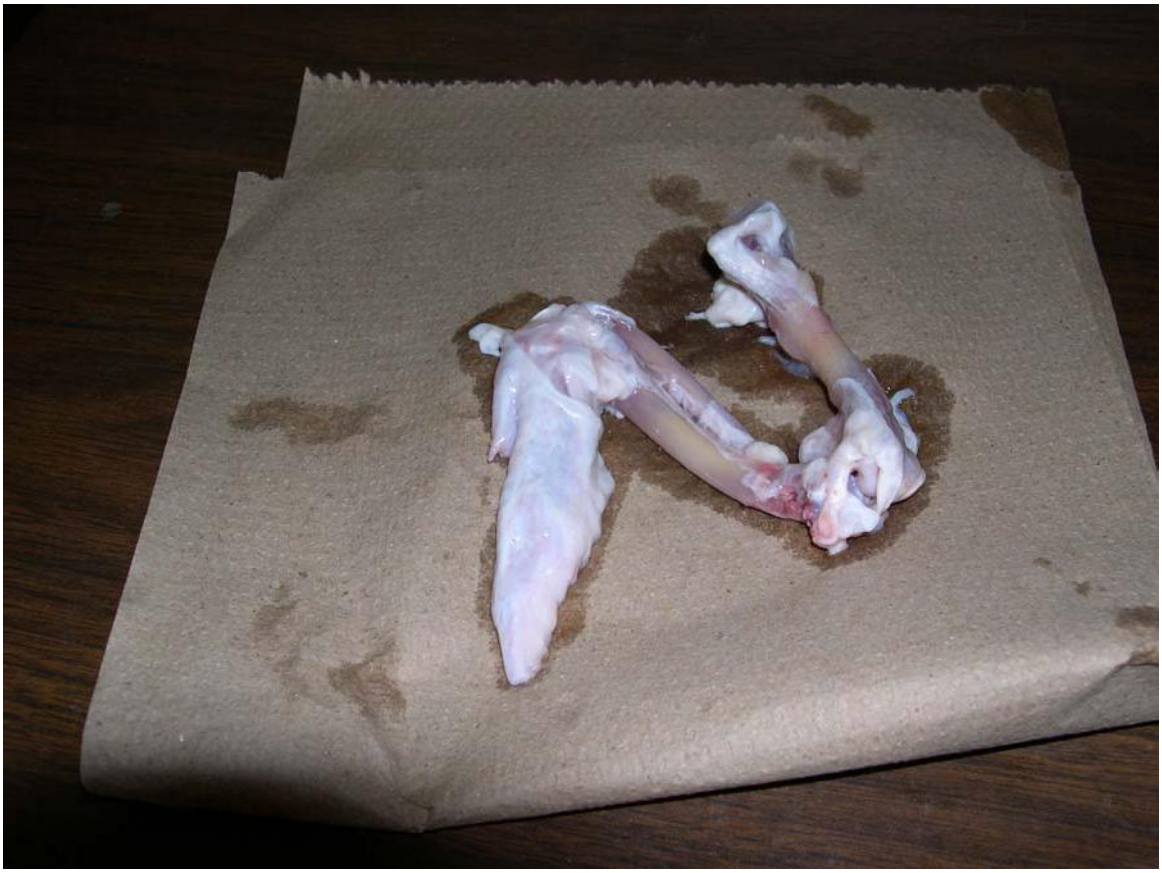
One of the enrichments I highly recommend for captive octopuses such as GPOs is to occasionally give them new and novel food items, such as live food, food they haven't encountered before, food that they have to figure out how to catch, subdue, open and eat, or food that takes a long time to eat. Such food may take a long time to eat, much as some wild foods may require ingenuity to open and eat (Anderson & Mather, 2007). My colleague and I liked to call this aspect of opening food such as live clams "The Packaging Problem," (Anderson & Mather, 2007), when octopuses had to open different species of tightly-closed live clams. Depending on the species of clam, GPOs may pull a clam open, break it with its beak, chip a shell's edge and inject a paralyzing venom, or it may drill a tiny hole in the clam's shell at a predetermined location on the clam's shell using a rasping tongue and then inject venom through the hole. All these behaviors may change with different species of live clam. Although the locations for drilling are predetermined and are probably learned based on previous experience, the method of entry and location of entry vary with different bivalve species. Thus feeding live clams to GPOs provides a very good opportunity for enrichment.

The Seattle Aquarium Enrichment Manual suggests that GPOs should receive some sort of enrichment three times a week and feeding a new and novel food to a GPO once a week can thus supply a substantial portion of the weekly enrichment for a captive GPO. Some of the foods listed here (Table 1) are quite novel for an octopus and would probably not be nutritious in the long term if fed as a steady diet, much as they wouldn't be healthy for humans to eat every day (food such as hot dogs). I wouldn't suggest feeding a GPO a hot dog more than once week, if that often. But these foods provide something different for a GPO to do and may even keep a GPO occupied for hours after a feeding. As an example, our GPOs take up to three hours to eat a raw chicken wing (Photo 1) so I would suggest trying a new and novel food such as these maybe once week as part of a continuing enrichment program for captive GPOs.

Table 1. Unusual foods given to GPOs at the Seattle Aquarium, raw unless otherwise specified. Portions were appropriate for the size of the animal.

Live FW crayfish	Cheese - Sharp cheddar
Live lobsters	Swiss
Live shrimp	Jarlsburg
King crab legs, cooked	Pepper jack
Scallop meat (muscles)	Low cal, low cholesterol
Live fish Canary rockfish Buffalo sculpin Red Irish lord sculpin Greenling Live salmon fry Live herring	Hard aged parmesan
Hard-boiled eggs – shell must be cracked a bit	Bleu, Gorgonzola, Stilton
Salmon meat – filets, steaks, chunks, and carcasses	Brie
Tuna	Edam
Sturgeon	Poultry – chicken parts
Sole	Livers
Cod	Gizzards
Black cod	Cornish game hen
Mahi mahi	Turkey leg
Halibut	Turkey breast
Steak – several kinds, low fat	Bar S jumbo franks^{R}
Pork chop – not eaten, may be too fat	Spam^{R}
Krill in gelatin	Artificial crabmeat in gelatin

Photo 1. Raw chicken wing eaten by a GPO. This kept it busy for about 3 hours. Photo by Leo Shaw, Seattle Aquarium



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A LARGE MOBILE FLUIDIZED BED FILTER

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The initial purpose of this fluidized bed filter was to aid in the seeding of a renovated 10,000 gal marine exhibit. The filter was designed to be mobile, and moved to marine systems requiring an established biological filter. The design took into account the height of tank sides, the ease of mobility, and the height of an existing elevator.

After design and construction, the filter was plumbed into an established marine holding system and allowed to seed for approximately 30 days. The initial system was selected due to its high bio-load (sea turtles), life support design, and system volume (~8,000 gal). Once the exhibit renovation was completed, an appliance dolly was used to move the filter to the new system. The materials and in-house construction allowed for an efficient custom design on a small budget.

Construction

The main body of the filter was constructed of 8 inch PVC pipe 78 inches tall. The bottom of the pipe was capped and sunk in 7 inches of concrete as a base. The influent pipe was constructed of 2 inch PVC capped at the bottom with ½ inch holes drilled into the cap. The effluent was 2 inch PVC, and was 9 inches from the top of the filter. A 2 inch Uniseal® created a water tight seal between the 2 inch effluent line and the 8 inch PVC filter. A check valve and union were used to prevent back siphoning. A 3 inch x 3 inch acrylic window was placed approximately 19 inches from the top of the filter. The window was made of 3/16 inch acrylic molded with a butane torch to fit the inside of the main filter body. The window was held into place using 6 bolts with rubber washers. The window was sealed using Dow Corning® 1200 Primer and 100% silicon. This window was used primarily as a site glass to gauge the medium height. Large gravel was used for the first 3 inches of medium, and then approximately 150 pounds of _ sand were used as the remaining medium. Water flow was controlled by a 2 inch ball and the filter was run with medium approximately 1.5 inches above the bottom of the site glass.

Sources For Materials

2 inch Uniseal®: Aquatic Eco-Systems Inc., Apopka, FL.
(877) 347-4788. #U200

Dow Corning® 1200 Primer: McMaster Carr, Atlanta, GA
(404) 346-7000. #7442A11

Figure 1. Front view of fluidized bed filter with influent pipe and site glass.

Figure 2. Side view of fluidized bed filter with influent and effluent lines shown.

TREATMENT OF NEGATIVE BUOYANCY IN A CAPTIVE CHAMBERED NAUTILUS, *Nautilus pompilius*

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Abstract

A single *Nautilus pompilius* was observed to have problems maintaining neutral buoyancy. This phenomenon is not well understood and not detailed in the literature. As a result of this condition, diagnostic techniques and treatments were employed that are not commonly performed on *Nautilus*. Procedures such as anesthesia and hemolymph removal were not recorded in the literature but necessary in order to properly care for the specimen. Anesthesia was obtained in an immersion bath via a 3% ethanol solution in seawater. While the animal was sedated, skin scrapings, tissue biopsies, and hemolymph were collected. Subsequent analysis of these samples revealed bacterial and parasitic infections. Radiography revealed no abnormalities of the shell structure, but an anomaly in the first chamber that appeared to be fluid filled as a reasonable cause for the negative buoyancy. The techniques used in this case, such as anesthesia and aspiration of hemolymph, were repeatedly performed with increased efficiency, and may be used in further diagnostic evaluation and treatment of *Nautilus*.

Introduction

The successful exhibition of cephalopods requires two primary components: good water quality and constant monitoring (Oestmann, 1997). The immune system of Cephalopoda is comprised of one cell type, a hemocyte, which functions in cellular immunity, but is not phagocytic (Ratcliffe and Rowley, 1981). Foreign antigens are instead bound by lectin-like proteins (Scimeca, 2006). For these reasons, as well as their microvillus epidermis, an increased bacterial load on the system and/or poor water quality conditions can compromise the cephalopod immune system easily, and quickly (Scimeca, 2006). It is imperative that optimal tank conditions are maintained throughout the life of a cephalopod because the basic task of diagnosing disease may inherently cause negative stressors on an already unhealthy animal.

Most cephalopods are highly active and employ defense mechanisms when disturbed. Therefore, anesthesia is necessary when handling and transporting them. Magnesium chloride is a common chemical used when anesthetizing cephalopods. Messenger (1985) found that magnesium chloride was an effective alternative to other chemicals available at the time, such as urethane and ethanol. Scimeca and Forsythe (1999) state that magnesium chloride is the most effective means of anesthetizing cephalopods. Urethane is a carcinogen that requires careful handling. Ethanol can be expensive depending on the source and quantity required, especially for use on large octopuses (Anderson, 1996). Larger animals require a greater volume of water, thus requiring a greater volume of ethanol. Ethanol is used as both an anesthetic and a euthanasia drug. Scimeca (2006) suggests a concentration of 10% ethanol in seawater in order to

euthanize cephalopods, though 5% concentrations of ethanol in seawater are also utilized for euthanasia (Anderson, 1996). *Sepia officinalis* have been anesthetized for surgical procedures using ethanol concentrations in seawater of 1.5%, re-circulating maintenance, and 3%, for induction, (Scimeca 2006). Additionally, concentrations of 3-5% ethanol have been used to anesthetize squid and cuttlefish for surgical procedures (R. Smolowitz, personal communication, 2007). Roper and Sweeney (1983) list five methods for anesthetizing cephalopods: ethanol, cold water, fresh water, magnesium chloride, and urethane. Fresh water has been known to cause agitation in octopuses (Anderson, 1996). Benzocaine has been used as a euthanizing agent in fishes (Brown, 1988), and more recently has been shown to also work as a euthanizing agent in *E. dofleini*, with possible anesthetic effects as well (Barord and Christie, 2007). *Nautilus* has been anesthetized via urethane on numerous occasions (Hurley *et al*., 1978; Bourne *et al*., 1977; Ward *et al*.; Ward and Martin, 1978) but due to its carcinogenic risk, it was not utilized for this procedure. Magnesium chloride was not readily available at the time and was not administered. Ethanol was chosen as the anesthetic agent after consulting with staff at the Marine Biological Laboratory at Woods Hole and identifying an inexpensive and convenient source.

Hemolymph can be utilized as an effective sample in the determination of diseases in cephalopods, as well as other invertebrates such as crustaceans (Noga *et al*., 2000). When analyzing hemolymph superficially, Noga (2000) describes three C's to follow: color, clotting, and clarity. A healthy individual may have hemolymph with an intense blue color, indicating high levels of hemocyanin, while unhealthy individuals may have hemolymph that is both cloudy and white, with low levels of hemocyanin which may be indicative of disease. While these guidelines are not uniform for every invertebrate species, the basic guideline can be referenced when assessing disease in most marine invertebrates, such as *Nautilus*. The site of hemolymph removal is variable within Cephalopoda. Hemolymph can be obtained from *Sepia officinalis* via the cephalic vein while the specimen is under anesthesia. *Nautilus pompilius* hemolymph can be obtained from either the branchial vein, or one of two sinuses; the first sinus lies above the brain and the second sinus is located below the brain and also referred to as the hemocoel as seen in figure 1; adapted from Brusca and Brusca (2003) and from Ruppert and Barnes (1994).

While *N. pompilius* is common in the aquarium trade, little is known about its biology and behavior. Furthermore, successful diagnosis and treatment of disease is uncommon and not well understood. The initial diagnostic procedures and subsequent treatments of a single *N. pompilius* are presented in this paper.

Methodology

Exhibit

A total of four *N. pompilius* were in the collection at Moody Gardens. Each *N. pompilius* had been on exhibit for a minimum of 660 days and were obtained from La Fountaine, Inc. and from the Aquarium of the Pacific. The *N. pompilius* were fed three times each week. Their diet consisted of shrimp, squid, crab, and various fishes. The nautiluses were housed in a 2138 L tank. The dimensions of the exhibit were 1.22 m long by .91 m wide by .91 m high. The filtration system was comprised of gravity fed overflows supplying water to a polyester mesh particle filtration layer over a vertical flow bio-filter tower that emptied into a sump. There was

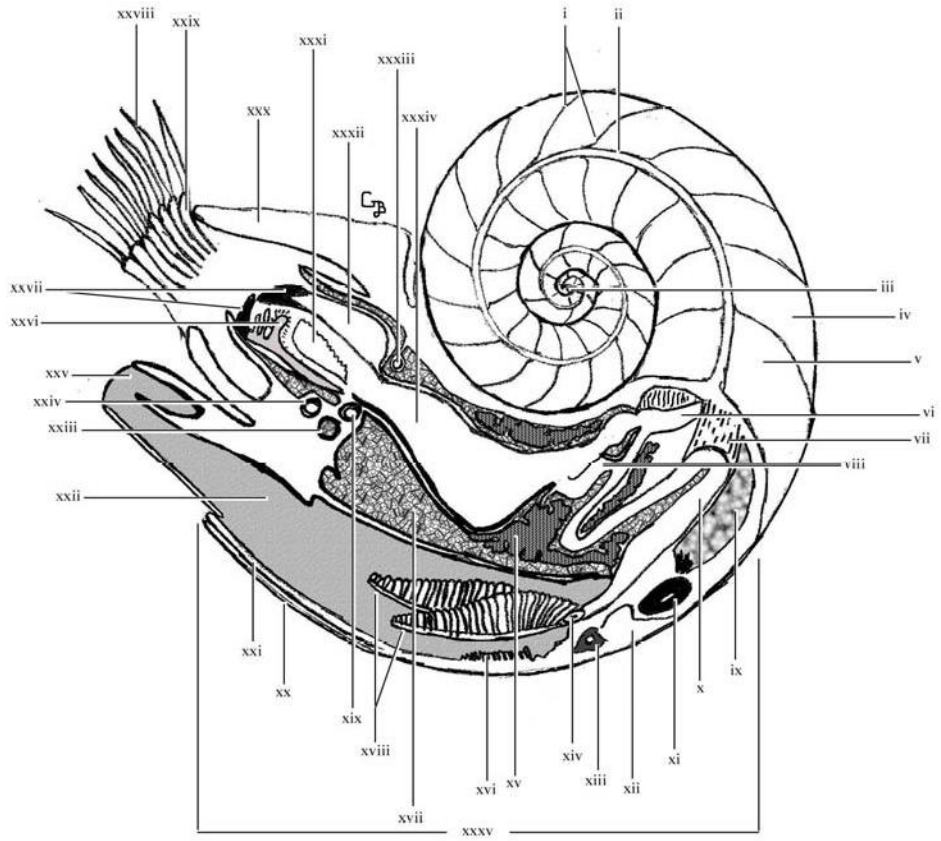


Figure 1. *Nautilus pompilius* internal anatomy
i. septa, ii. siphuncle, iii. umbilicus, iv. 1st chamber, v. 2nd chamber, vi. stomach, vii. genitovisceral ligament, viii. cecum, ix. gonad, x. intestine, xi. heart, xii. pericardial chamber, xiii. kidney, xiv. anus, xv. digestive gland, xvi. nidamental gland, xvii. hemocoel, xviii. ctenidia, xix. pleurovisceral ganglion, xx. shell, xxi. mantle, xxii. mantle cavity, xxiii. rhinophore, xxiv. pedal ganglion, xxv. funnel, xxvi. radula, xxvii. beak, xxviii. buccal cirrus, xxix. tentacles, xxx. hood, xxxi. tongue, xxxii. jaw muscle, xxxiii. cerebral ganglion, xxxiv. crop, xxxv. living chamber.

a side loop protein skimmer that also emptied into the bio-filter. The filtered water in the sump was then pumped through a chiller back to the display tank. Water quality parameters such as ammonia (NH3) and nitrite (NO2) remained within the accepted standards of 0.100 mg/L (Hanlon, 1987; Lee *et al*., 1994; Sherrill *et al*., 2000) although nitrate (NO3) levels exceeded the standard of 20.0 mg/L on average, and by four-fold on some occasions, as shown in Table 1.

Table 1. Average water quality values of the Moody Gardens *N. pompilius* exhibit for 2006 and 2007.

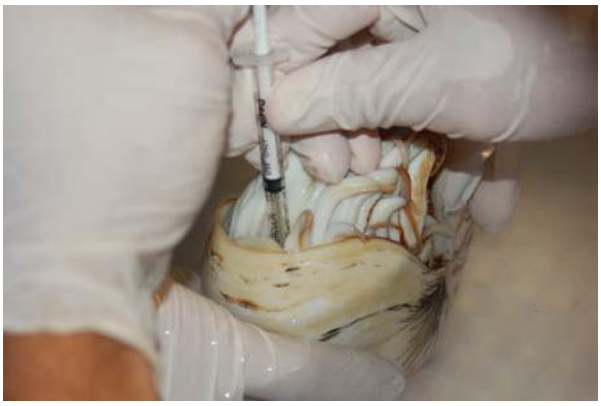
Temperature (°C)	Salinity (%)	Ammonia (mg/L)	Nitrite (mg/L)	Nitrate (mg/L)	pH
18	33	0.00	0.037 (0.137)	59.3 (73.1)	8.14

( ) denotes the highest value reached during the time period tested.

Initial Observations and Management

A single *N. pompilius* was observed to have difficulty maintaining neutral buoyancy in July 2007. The specimen was observed on the bottom of the tank in the same location for weeks. Although the specimen could not move about in the water column, normal funnel activity and tentacle movement by the *N. pompilius* was observed, and it was eating normally when offered food. The following is an account of initial diagnostic procedures and ensuing treatments.

The *N. pompilius* was first observed for 60 days in order to evaluate the full extent of the situation as well as to allow the specimen to recover on its own. In many cases, buoyancy problems are a result of shipping stress. Shipping stress may cause an imbalance in the osmotic/ hydrostatic ratios between the chambers and the surrounding seawater resulting in buoyancy malfunction. While shipping stress was not applicable to this *N. pompilius*, other unknown stressors experienced in captivity may have contributed to the negative buoyancy. Often, given time these buoyancy issues resolve themselves and there are no further problems in the specimen (B. Carlson, personal communication, 2007). No improvement was observed during this time so further treatments were discussed and initiated. The specimen was first placed into a holding cage at the surface of the water in order to alleviate any pressure exerted on the specimen at depth, and to allow for close observation. The holding cage was comprised of plastic mesh with styrofoam attached to the top that allowed the cage to float at the surface. The specimen's uppermost part of the shell was 1 cm below the surface of the water. The condition did not improve and further intrusive diagnostics were initiated. Appendix I presents a chronological account of the events surrounding the buoyancy issue of this *N. pompilius*. The *N. pompilius* was first anesthetized via 95% ethanol, in concentrations of 1%, 1.5%, and 2.0% in seawater. This first attempt was unsuccessful, as the specimen did not reach the desired state of narcosis. The following day, a 3% concentration was employed and the *N. pompilius* was successfully anesthetized. The renal sac was chosen as the first hemolymph extraction site. The location of the renal sac proved difficult to find and this sample location was aborted. The decision was made to draw hemolymph from the hemocoel, while also sampling the epidermis. Hemolymph was successfully removed from the hemocoel via the anterior portion of the animal, as seen in figure 3.

Figures 2 and 3. 2 (left), location of renal sac aspiration and 3 (right), location of hemolymph removal of *N. pompilius*.

Samples of the epidermis were also taken during this procedure to provide additional diagnostic information. Based on microscopic analysis of the hemolymph, an antibiotic course was prescribed to treat a suspected bacterial infection. The first course of enrofloxacin was administered orally twice per day at 11mg/kg. Following the five day run, the efficacy of dosing was questioned with the soluble nature of the antibiotics and the feeding method of *Nautilus*. A second antibiotic regime was prescribed that called for immersion in oxytetracycline baths at 25mg/l to facilitate uptake through the microvillus epidermis. Following the antibiotic treatments, hemolymph was once again sampled and examined microscopically to confirm whether the antibiotic treatments were successful. Hemolymph was sampled from a second, healthy *N. pompilius* at that time to establish reference qualities. Radiography was carried out on the unhealthy *N. pompilius*, as well as a healthy *N. pompilius* for comparison. Based on comparative hemolymph and radiography diagnostics, it was determined that the bacterial infection was still present. Subsequent hemolymph samples were taken specifically to attempt culture and sensitivity to identify the most appropriate antibiotic treatment. Samples were sent to the Texas Veterinary Medical Diagnostic Laboratory (TVMDL) and Micro Technologies, Inc. for bacterial culture and sensitivity testing. Hemolymph was also cultured in house. Attempts at bacterial isolation and identification as well as sensitivity testing have not revealed useful information at this time.

Results

Anesthesia

The primary objective of the treatment was to restore the specimen's ability to maintain neutral buoyancy. This treatment course involved anesthetizing the specimen on multiple occasions. Identifying a safe, effective anesthetic regime was a secondary benefit of the primary diagnostic procedures. Table 2 provides an account of the ethanol concentrations applied to the *N. pompilius* in order to sedate the specimen so that it could be safely handled and notes on the effects at the concentrations. The stage and plane designation of anesthesia is based on classic work by Stoskopf (1985) recently adapted to cephalopods by Barord and Christie (2007) in describing the euthanasia of *Enteroctopus dofleini.* Anesthesia planes progress through sedation (stage I), narcosis (stage II) and anesthesia (stage III), with associated light (plane 1) and deep (plane 2) designations. An initial concentration of 1% ethanol in seawater was the starting dose to first ensure that *N. pompilius* responded well to the chemical. The specimen did not reach any

stage of anesthesia, as it was still behaving normally, ventilating normally, and displayed normal movement of its tentacles and funnel. After 20 minutes, the concentration was increased to 1.5% ethanol in seawater. The specimen progressed to a state of light sedation (stage I/ plane 1). The *N. pompilius* had decreased activity levels, though tentacle movement, funnel movement, and ventilation rates were still normal. After 30 minutes, the concentration was increased to 2.0%. There was no appreciable increase in the anesthetic effects at this concentration and after a total of 40 minutes it was decided to discontinue the procedure.

The second attempt at anesthesia used a 3% ethanol concentration and successfully sedated the nautilus to a state of deep narcosis (stage II/ plane 2). The *N. pompilius* had little to no tentacle or funnel movement and ventilation rates had substantially decreased. This progression took a total of 18 minutes at which point the *N. pompilius* was sedated enough that it was easily handled. Each successive anesthesia with this specimen was accomplished using the 3% concentration and each time the specimen reached the same stage/ plane of anesthesia. As a means to compare diagnostic samples, a second healthy *N. pompilius* was anesthetized using the same procedure. On this attempt, the *N. pompilius* reached a state of light anesthesia (stage III/ plane 1) characterized by further decreases in ventilation rates, muscle tone, and tentacle and funnel movement. Revival of *N. pompilius* was successful and uniform throughout each procedure. The *N. pompilius* were placed into fresh seawater, if anesthetized at the vet clinic, or directly into a holding cage on exhibit if on property. The *N. pompilius* became active after 10 minutes and were released back on to exhibit after one to two hours.

Biological Sampling

Upon reaching a state of deep narcosis, initial attempts of obtaining reliable samples were employed, the first location being the renal sac. The renal sac lies on the ventro-lateral side of the *N. pompilius*, on both sides. A 76 mm spinal tap needle was used for this procedure. The needle insertion was made laterally on the left side of the *N. pompilius* as shown in figure 3. Although the renal sac aspiration was unsuccessful, during the procedure a sample of tissue sloughing off was taken and examined. Microscopy showed multiple organisms present on the tissue including bacteria, nematodes, algae, euglena, ostracods, protozoans, and fungus; though none were positively identified to genus or species level.

Subsequent attempts to aspirate hemolymph concentrated on the hemocoel. A shorter 40 mm needle was used for this procedure, with successful extraction of approximately 2ml of hemolymph on the first attempt. Superficially, the hemolymph color was not characteristic of a healthy sample as suggested by Noga (2000), as it was cloudy with only a slight blue tint. Microscopic examination of the hemolymph revealed high numbers of multiple types of bacteria. A total of 4 more hemolymph samples were taken (3 on the unhealthy specimen and one on a healthy specimen), accessing the hemocoel while fully sedated.

Table 2. Anesthesia on *N. pompilius* via ethanol using the stage/ plane terminology based on Stoskopf (1985), adapted to cephalopods by Barord and Christie (2007).

Date	Ethanol (%)	Time (min)	Stage	Plane
20 September 2007[a]	0.010	20*	0	
20 September 2007[a]	0.015	10**	I	1
20 September 2007[a]	0.020	10**	I	1
21 September 2007[a]	0.030	18	II	2
22 September 2007[a]	0.030	18	II	2
11 October 2007[a]	0.030	17	II	2
11 October 2007[b]	0.030	16	II	2
19 October 2007[a]	0.030	15	II	2
22 October 2007[b]	0.030	17	III	1
15 November 2007[a]	0.030	18	II	2
28 November 2007a	0.030	17	II	2

[a] *N. pompilius* exhibiting negative buoyancy
[b] Healthy *N. pompilius*
* Time elapsed after initial concentration
** Time elapsed after additional concentrations added

Samples of the hemolymph were sent to the Texas Veterinary Medical Diagnostic Laboratory (TVMDL) for culture and sensitivity testing. A total of six colonies of bacteria were cultured from the hemolymph: two colonies of a Gram-positive pleomorphic rod, two colonies of an orange-pigmented Gram-negative rod, and two colonies of a pink-pigmented Gram-negative rod. The colonies were not identified and no useful sensitivity information was gained from this first round of bacteriology. A second set of hemolymph samples were sent to Micro Technologies, Inc. for an additional set of culture and sensitivity tests. Moreover, in-house cultures were maintained on trypticase soy agar (TSA) and dextrose tryptone agar (DTA); two replicates each. At 24 hours, bacterial colonies were visible on the TSA medium. At each successive 24-hour period the colonies had multiplied on the TSA medium. At 72 hours, bacterial colonies were present on the DTA medium. In-house isolation, identification and sensitivity are not complete at the time of this publication.

Radiography

The *N. pompilius* exhibiting negative buoyancy was taken to the Galveston Veterinary Clinic for radiography. The expectation was to learn of any structural problems in the shell that may have caused the negative buoyancy. Both lateral and dorsal radiographs were taken. The

dorsal radiograph did not yield useful data, as the x-ray was unable to penetrate the multiple chambers, as shown in figure 4. The lateral radiograph proved quite effective. All internal chambers and multiple anatomical structures were visible as shown in figure 5. The first chamber was dense, whereas the remaining chambers were not. This increased density may have been a result of a fluid buildup in this one chamber. For comparison, a second healthy, as seen in figure 6, *N. pompilius* was taken to the Galveston Veterinary Clinic for radiography. The first chamber in this healthy *N. pompilius* was not dense and did not illustrate any problems, as the unhealthy *N. pompilius*' first chamber did.

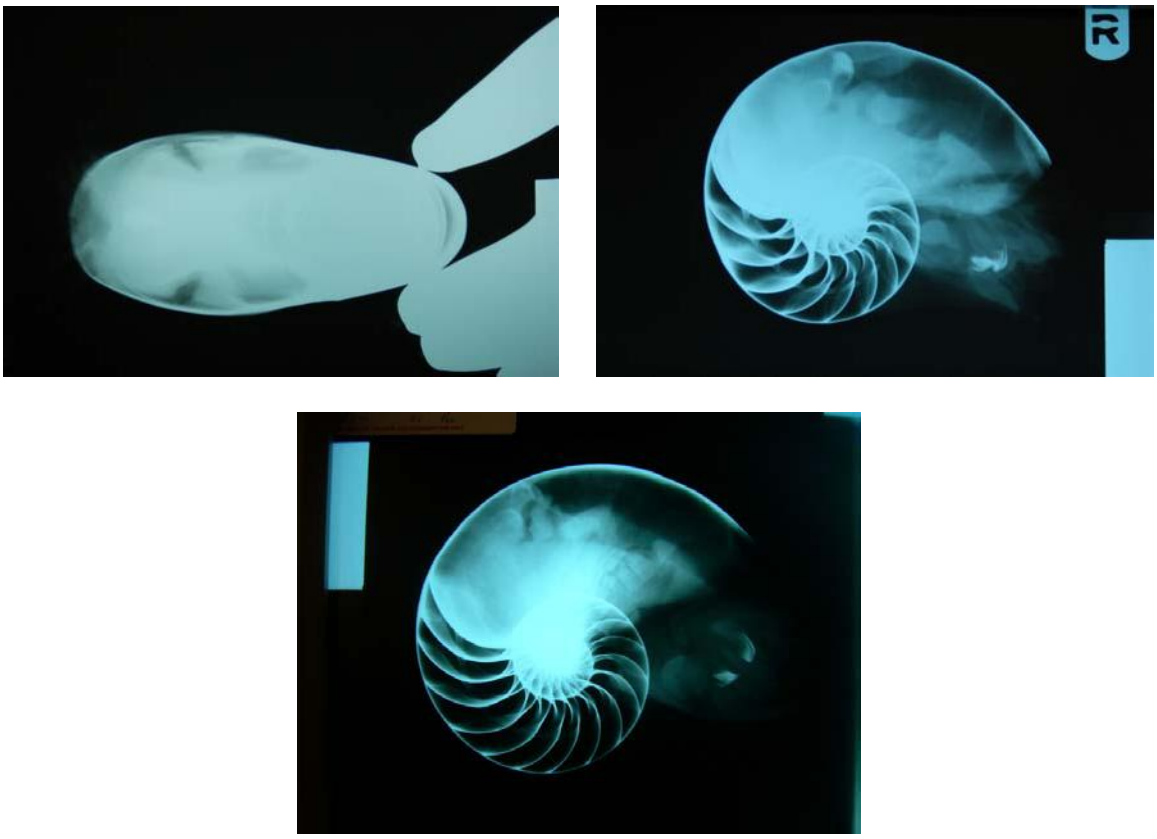


Figure 4, 5, and 6. 4 (top left), dorsal radiograph of the unhealthy *N. pompilius*, 5 (top right), lateral radiograph of the unhealthy *N. pompilius*, and 6 (bottom) lateral radiograph of a healthy *N. pompilius*.

Discussion

Acute observation, constant monitoring, and excellent water quality are critical husbandry components in the captive display of cephalopods. The ability to quickly identify problems allows the individual to manage the specimen with greater ease without the need to resort to extreme measures. While considering which treatment methods to employ, care must be taken in regards to animal health. Treatment protocols and procedures should be expedited to the full extent in order to provide the specimen the best, and quickest, care possible.

While certain fish disease and parasite issues are easily traced to an initial source, cephalopod disease is more difficult. In this case, water quality was not acceptable in regards to nitrates. Poor water quality impacts cephalopods by decreasing the specimen's ability to fight off infections. As a result, multiple internal functions such as buoyancy, and organs such as the siphuncle, may be compromised by the lack of an efficient immune system. While ammonia and nitrite are broken down into nitrate, the nitrate levels will continue to rise unless acted upon by an outside force such as a water change or a denitrification unit. While water quality was not unequivocally determined to be the cause of the negative buoyancy, it may have played a role in the overall condition of the *N. pompilius*. Other variables such as light cycles, noise vibration, diet, the individual specimen's captive history, genetics and age are all contributing factors in the health equation.

While cephalopod anesthesia is a commonly employed routine for multiple species, the sedation of *N. pompilius* is not regular. Much can be inferred from accepted dosages used for octopuses and squid, but the anatomical and physiological differences between coleoids and nautiloids, such as ventilation rates and activity levels must be taken under consideration when manipulating anesthetic concentrations. Squid are fast moving animals that may require a stronger, and fast-acting, dosage while *Nautilus* move about more slowly.

The initial anesthesia attempt using ethanol proved to be ineffective. The *N. pompilius* was at no time sedated to the point that it could be handled. When handled, the *N. pompilius* retracted back into its shell and any attempt at hemolymph aspiration could not be accomplished. The tentacles were still active, making the use of needles around the animal dangerous to both the specimen and the staff. The data suggests that a concentration of 3% ethanol in seawater induced a state of deep narcosis, stage II, plane 2; a state that the animal could easily and safely be handled in. This stage was characterized by decreased muscle tone and ventilation, as well as a decrease in funnel and tentacle movement. At this state the *N. pompilius* was handled with ease and hemolymph was drawn on multiple occasions. The body of the animal did not retract and the tentacles and funnel were slightly spread apart in order to remove hemolymph. It was interesting to note that no excitement stage was observed in the *N. pompilius*, as seen in euthanasia trials of *E. dofleini* (Barord and Christie, 2007). While internal changes may have been occurring that could have been characteristic of an excitement phase, external behaviors did not support this stage. At no time did the *N. pompilius* increase ventilation, funnel, or tentacle movement during the process.

One instance of anesthesia did produce a state of light anesthesia (stage III, plane 1) characterized by further decreases in muscle tone, tentacle movement, and ventilation rates. This procedure was performed on a healthy *N. pompilius* at the Galveston Veterinary Clinic. Two *N. pompilius* were transported in coolers with exhibit water. The unhealthy *N. pompilius* was sedated first and reached the same state of deep narcosis (stage II, plane 2). The second, healthy *N. pompilius* was sedated after the first procedure, and although the same protocols were followed, this animal reached a light anesthesia (stage III, plane 1). The variation in the degree of sedation could be attributed to individual differences between specimens, a lower anesthetic threshold in the unhealthy specimen, or even a slightly warmer temperature of the seawater at

induction of the second *N. pompilius* that changed the rate or resulting degree of ethanol uptake.

The color of hemolymph is often employed as the first diagnostic tool once the hemolymph is drawn. While exact causes must be determined at a later point, the color can quickly alert you that there is a problem. The initial hemolymph sample was viscous and cloudy with little blue coloration. Subsequent comparison to that of a healthy *N. pompilius* showed distinct color differences. The hemolymph removed from the healthy *N. pompilius* was thick and had a definite blue coloration to it. The microscopic analysis of the hemolymph confirmed that the *N. pompilius* was not healthy. The large amounts of bacteria in the hemolymph were characteristic of a systemic infection. Traditionally, bacteria culture, isolation, identification and antibiotic sensitivity provide the best means to treat bacterial infections. In the case of cephalopods, microbiology may pose challenges in this process due to their exotic nature. Broad spectrum antibiotic classes that are frequently used in more traditional aquatics veterinary medicine may not be effective against cephalopod infections to the same degree.

It is difficult to ascertain the effectiveness of these first treatments, as the oral method proved difficult to evaluate. The enrofloxacin pills (11 mg/kg) were effectively inserted into small pieces (2 cm) of shrimp and placed directly into the *N. pompilius* tentacles. The pills proved to be soluble in the seawater, so much so that the pill would degrade in fingertips within 30 seconds. While oral treatments are accomplished with little stress placed on the animal, in some cases they are not effective and should not be utilized. An additional alternative to intravenous (IV) or intramuscular (IM) injections was a bath treatment. Given the *N. pompilius* microvillus epidermis, a bath should allow for maximum uptake potential through numerous channels and pores across the body. Both treatments proved ineffective and the bacterial infection remained.

Radiography is a common diagnostic method for higher vertebrates, principally in humans. While most cephalopods do not possess any hard structures that would materialize in a radiograph, the *Nautilus* shell will show up in a radiograph. The radiograph proved very effective. It showed no structural deficiencies in the shell as well as an abundance of fluid focused in the 1st chamber, and when compared to a healthy *N. pompilius*, this fact was further validated. The radiograph identified a reasonable explanation for the cause of the negative buoyancy. Radiographic analysis will serve as a critical component to future diagnostic treatments of *Nautilus*.

Excellent husbandry, such as good water quality and daily observations on the animal's condition and behavior, will provide the animal the most optimum conditions and reduce stress. The use of sound diagnostic techniques, as described, will improve both the understanding of the specimen's condition, as well as ensure that the animal will receive the best care possible. The diagnostic techniques performed in this case were no different than those employed on elasmobranches or fishes, or even humans. The inherent nature of the exotic *N. pompilius* drew the attention away from this. Skin scrapings, blood draws, and antibiotic administration are all commonly used for a variety of marine animals. The successes of this study should provide a better understanding of techniques that can be used in *N. pompilius* as well as provide the

individuals performing them the proper technique and background for the situation. Further research and treatment methods will continue, as the specimen has still not regained neutral buoyancy.

Acknowledgments

The authors thank Karen Keister and Kristin Shivers for reviewing the manuscript. Special thanks to Roland Anderson, Jennifer Basil, Bruce Carlson, Robyn Crook, Roger Hanlon, Phillip Lee, Janice Simmons, and Roxanna Smolowitz for their advice and assistance throughout the treatment. Gratitude is given to Jay Reynolds, Barrett Christie, and Rebecca Leitner for their assistance during the procedures. Thanks, also, to the entire aquarium staff for their assistance when required.

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Appendix I. Chronological Time Line of Events (as of 01 December 2007)

Date	Account
July 2007	Buoyancy problems were observed in specimen while conducting research study.
August 2007	Staff initiated research into negative buoyancy and contacted applicable experts in the field.
01 September 2007	Initial treatment protocol developed, enlisting the advice of experts in the field.
15 September 2007	Specimen placed in holding cage at surface of water.
19 September 2007	Initial protocol presented to and approved by the veterinarian.
20 September 2007	Anesthesia employed via EtOH; concentrations of 1%, 1.5%, and 2% unsuccessful.
21 September 2007	Anesthesia employed via EtOH (3%) and was successful. Sloughing tissue was removed and analyzed; nematodes, alga, euglena, horsehair worms, ostracods, protozoans, and fungus present; renal sac stick unsuccessful.
22 September 2007	Anesthesia employed via EtOH (3%) and was successful. Skin scraping and hemolymph sample taken; bacteria present in both samples and antibiotics prescribed.
26 September 2007	Specimen given 11 mg enrofloxacin tablet twice-daily via food.
04 October 2007	Specimen given oxytetracycline bath at 25 mg/L for 1 h.
06 October 2007	Specimen given oxytetracycline bath at 25 mg/L for 1 h.
11 October 2007	The unhealthy and one healthy *N. pompilius* bled; bacteria only present in the unhealthy specimen
19 October 2007	Radiography performed on the unhealthy specimen. The lateral view showed an abundance of fluid in the 1st chamber.
22 October 2007	Radiography performed on a healthy specimen. The 1st chamber was clear
13 November 2007	Met with husbandry manager, assistant curator, lead biologist, veterinarian, and veterinary technician to discuss treatment options. Hemolymph bacterial isolation, culture and sensitivity decided as next step.
15 November 2007	Anesthesia employed via EtOH (3%) and was successful. The specimen was bled and hemolymph was sent off to the Texas Veterinary Medical Diagnostics Laboratory for culture and sensitivity testing.
27 November 2007	Final report from TVMDL received; six colonies of bacteria were isolated (two colonies of Gram-positive pleomorphic rod, two colonies of orange-pigmented Gram-negative rod, two colonies of pink-pigmented Gram-negative rod). TVMDL was unable to identify strains.
28 November 2007	Hemolymph samples sent off to Micro Technologies, Inc for bacterial culture and sensitivity tests. Cultures were also taken in-house and plated on trypticase soy agar (TSA) and dextrose tryptone agar (DSA).
29 November 2007	TSA cultures produced colonies at 24-hours.
01 December 2007	TSA colonies multiplied; colonies visible on DSA.

DIVERSITY IN AQUARIUM COLLECTIONS

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Species diversity in a public aquarium collection may be an important measure of how well that facility interprets the natural world for the aquarium visitor. Obviously, a public aquarium with just one or two species could hardly be called an aquarium at all. On the other hand, a diverse "art gallery" collection, (consisting of one or two specimens of a multitude of species) while showcasing diversity, offers little in terms of promoting captive propagation or showing naturalistic assemblages of animals. Some level of diversity is optimal for any aquarium. How this can be measured and then a proper degree of diversity instilled in any given collection can be a difficult task.

In its most basic sense, diversity can be quantified by simply stating the number of species in the collection. An aquarium collection that exhibits 300 different species will offer the visitor a more varied look at aquatic life than an aquarium that houses just 100 species. Observable diversity is also important. If the aquarium that houses the 300 species maintains 200 of them off-exhibit, the observable diversity (for the visitor) of the two collections would then be the same. Likewise, the scope of the diversity is also significant. An aquarium that houses 100 species including such varied taxa as flashlight fish, sharks, seadragons and giant crabs is going to be perceived by the typical visitor as having a more diverse collection than an aquarium that houses 200 species, but of just cichlids and characins. An additional consideration is the question of what species should be counted towards the diversity of a facility. Aquatic exhibits always have a substantial number of microscopic or cryptic species living alongside the display specimens. Should any of these organisms be enumerated as well? At what phylogenetic level does this counting become impractical or counterproductive? Presumably, no public aquarium in North America counts marine amphipod species as part of their living collection, yet these animals are present in many exhibits, and may be noticed by some observant visitors. On the other hand, a comet (*Calloplesiops altivelis*) that hides almost all of the time and is never seen by visitors would still be counted on the species roster of most public aquariums. There really is no standard, but certainly each species that is formally accessioned into the aquarium's collection would be counted.

There are other ways to quantify specimen diversity than just enumerating the species. A simple equation that takes into account the diversity of a collection as a ratio of collection size is N/S = X, where N is the total number of specimens in the collection and S is the number of species. X is then an average number of specimens per species in a collection. The value of X calculated from collection data of some representative facilities holding fishes as published by the Association of Zoos and Aquariums (AZA) ranged from 2.5 (In the case of a zoo with a small collection of fish - 30 animals of 12 different species) to 96.3 for a standalone aquarium that reported housing 20,218 animals of 210 species. The latter case most likely included a large number of animals held in breeding programs.

Some trends can be seen when calculating this value. If the value of N/S for a facility is < 5, this usually indicates that it is a smaller, "postage stamp" collection. Facilities with an N/S value of between 5 and 10 show good basic diversity and are usually medium sized collections (as seen in a zoo aquarium for example). Larger aquariums have an N/S value of between 10 and 20, with the higher number seen in oceanarium-type facilities. An N/S value greater than 20 usually indicates that the facility is involved in captive breeding, where they house huge numbers of just a few species, or its exhibits consist of large numbers of just a few species.

The Simpson's Diversity Index has been used by biologists to measure biodiversity in various ecosystems. It has been applied to zoological collections as well (Willis 1999). This index takes into account the number of species present, as well as their relative abundance in the ecosystem. It represents the probability that two randomly selected individuals in the ecosystem will belong to the same species. With this index, zero indicates infinite diversity and 1 results if there is no diversity. One way to express this is in the equation:

$$\mathbf{D=\Sigma(n/N)^2}$$

Where n = the total number of organisms of a particular species, and N = the total number of organisms of all species. This equation can be applied to single multi-species exhibits or to an entire collection.

To show this in an example, in an exhibit that has 5 species in it and 25 specimens total, N=25. In this case there could be the following numbers:

10 specimens of species A, so $(n/N)^2 = .16$
5 specimens of species B, so $(n/N)^2 = .04$
5 specimens of species C, so $(n/N)^2 = .04$
4 specimens of species D, so $(n/N)^2 = .0256$
1 specimen of species E, so $(n/N)^2 = .0016$
N=25 Diversity index = .2672

In a second example, an exhibit only has 3 species in it, but 50 individuals, so N=50
30 specimens of species A, so $(n/N)^2 = .36$
15 specimens of species B, so $(n/N)^2 = .09$
5 specimens of species C, so $(n/N)^2 = .01$
N=50 Diversity index = .46

The first example would be a more diverse exhibit. Interestingly, if the values in the first example are changed to 5 specimens of each of 5 species, the calculated diversity is then a bit higher at 0.2. There are still 5 species of 25 specimens, but in this case, there is a slightly better chance of seeing any one species, so the diversity index is higher. If the exhibit was a "postage stamp" collection of 5 species with one specimen of each, there is still the same chance of seeing

any one species, so the diversity index is also 0.2, but there are fewer actual specimens in the exhibit. In an ultimate "postage stamp" collection, where there are 25 specimens, each of a different species, the diversity index would be 0.04

Some people find it helpful to use the reciprocal of the index (1/D). The value of this index starts with 1 as the lowest possible figure. This figure would represent a facility containing just one species. Then, the higher the value, the greater the diversity. The maximum value is the number of species (or other category being used) in the sample. For example of five species in the sample of 25 specimens, the maximum value is 5.

Obviously, the resulting values can only be used as a relative measure of diversity between different aquariums or different exhibits within an aquarium. When calculating the diversity of large exhibits or entire facilities, it is helpful to develop a spreadsheet to handle the summation task.

The reciprocal Simpson Diversity Index was calculated for three public aquariums for the marine fishes in their respective collections and compared to their N/S value:

1) A large coastal aquarium with medium diversity, with many specimens of fewer species.
N/S = 17.7
1/D = 1.7

2) A very large standalone aquarium with high diversity and many specimens.
N/S=10.3
1/D = 9.5

3) A medium-sized zoo-aquarium with very high diversity, but fewer total specimens.
N/S=2.46
1/D = 45.8

There does seem to be a correlation between the N/S value and the Simpson diversity index. You can imagine how these collections differ; Aquarium #1 has large schools of local fishes in their exhibits, Aquarium #2 has large schools of fish, as well as the added diversity of some mixed exhibits, while Aquarium #3 has the typical postage stamp collection with very high diversity, but no large exhibits of single-species schooling fishes.

Many public aquariums operate on the three basic tenets of education, conservation and entertainment. Developing a collection plan with an appropriate level of diversity should address all three components (Hemdal 2006). Measuring the quality of the animal diversity at a given facility is subjective at best. However, the presence or absence of certain keystone species can be identified. A diverse aquarium collection should contain those animals that the public expects to see (primary keystone species), or better yet, some that they may never have seen before (secondary keystone species) but are appreciative of seeing them when given the opportunity.

Table 1 lists some of these potential species. For some regional aquariums, substitute keystone species would have to be identified as it may not be in their mission statement to exhibit the more typical species.

Table 1. Some Possible Keystone Aquarium Exhibit Species

Examples of Some Primary Species	**Examples of Secondary Species**
Anemones / Clownfish	Coral exhibit
Eel (Moray or Electric)	Flashlight fish
Large Fish (pacu, etc.)	Giant Pacific octopus
Piranha	Japanese giant spider crab
Seahorse	Jellyfish
Shark	Seadragon
Stingray	Australian lungfish

A question facing many aquarium curators is, "How can diversity in an aquarium collection be maximized given the existing resources?" The first step is to measure the relative diversity of the exhibits to begin with. The primary tools at the curator's disposal are the specimen inventory and Institutional Collection Plan. Without these documents to use as a roadmap, there is no way for a curator to track species in the collection and compare it to what species will be acquired in the future.

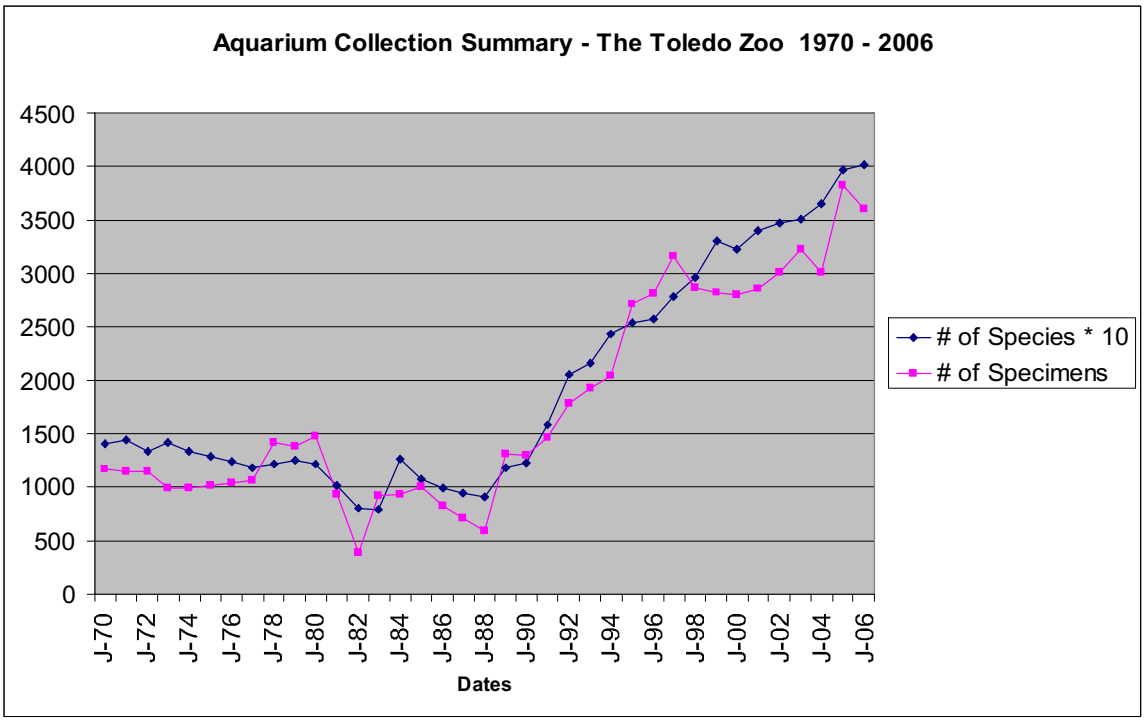

Figure 1. Specimen and species numbers for 36 years at the Toledo Zoo

Figure 1 shows data from the yearly inventory for the past 36 years at the Toledo Zoo. The number of species in the collection was multiplied by ten in order to bring it on to the same scale as the number of specimens. The idea that the aquarium collection was originally rather small and relatively stagnant can be clearly seen during the decade of the 1970's. The results of a tragic building fire and subsequent rebuilding of the collection can be seen before and after November, 1981. A curator who was pressed into service as a general curator can also be seen in the static condition of the collection during the late 1980's. The affects of a new curator and increased resources can be seen starting in 1989. Overlaying this data from that date forward was an increase in the invertebrate collection (both terrestrial and aquatic) as well as the aquarium staff maintaining a more accurate specimen inventory (Early census data may not have been as comprehensive, resulting in lower numbers). By plotting similar data, aquariums should be able to identify trends in collection size and diversity at their own facilities. For example, in Figure 1, the leveling off of the collection size and diversity starting in 2005 was a planned process, as anticipated by the ICP and the collection reaching its limits given the available resources.

One method that may be employed to test the public's acceptance of increased diversity in one group of animals over another is by means of temporary exhibits. A themed exhibit is designed with the (hopefully) tested hypothesis that the public will find the animals interesting, educational or part of an conservation initiative. At the conclusion of the exhibit's run, the most successful living components can be rolled into the facility's permanent exhibits. Recent temporary exhibit ideas at aquariums in the United States have included; seahorses, frogs, sea jellies, crabs, dragons, deadly beauties, butterflies, sharks, stingray touch tanks and turtles. Temporary exhibits may also focus on regions rather than taxa. Examples have included Lake Victoria and "deep sea" exhibits. Appropriate analysis of visitor exit surveys will usually identify which species (if any) from a temporary exhibit has the best attributes for inclusion into the institution's collection plan as a permanent exhibit.

References

Hemdal, J.F. 2006. Advanced Marine Aquarium Techniques. 352pp. TFH Publications, Neptune City, New Jersey

Willis, Kevin 1999. Recent History and Future of Taxonomic Diversity in Zoos: Will there be a Mutiny Over Bounty? Presentation at the National AZA Conference

RAW 2007 ABSTRACTS

Regional Aquatics Workshop, June 18 - 21
Pittsburgh Zoo & PPG Aquarium

Compiled by W.R. Langbauer Jr.
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Abstracts for oral presentations precede those for posters. Within each section, presentations are in alphabetical order, by first author. There was also a ZIMS update by Hans Keller (hkeller@aqua.org), which didn't have an abstract.

Monday, June 18

Pre-Raw AZA Conservation Group Meetings

Contact the *Association of Zoos and Aquariums* aquatic TAG and SSP chairs for more information on these groups, or to obtain copies of meeting minutes or handouts. While AZA is not affiliated with RAW, this informal annual meeting of aquatic professionals has come to serve as the venue of choice for some AZA working meetings. Groups holding meetings included the Marine Fishes TAG, Freshwater Fishes TAG, Aquatic Invertebrate TAG, Coral Reef CAP, and Aquatic Information Group (AqIG).

Tuesday, June 19

Session 1: Project & Workshops reports

Project Piaba: Supporting Environmentally Friendly Aquarium Fisheries in the Amazon. How Your Institution Can Get Involved.

Scott Dowd

New England Aquarium

For the past 15 years, Brazilian researchers and AZA partners have been studying the aquarium fish trade in the Barcelos region on the Rio Negro in the Brazilian Amazon. The conclusion is that the industry has little, if any long-term negative impact on fish populations or the local

environment. In actuality the fishery results in a net benefit to the region in that the resource provides a basis for livelihoods for rural Amazonians that requires environmental stewardship. In recent years however trend have developed that could lead to the collapse of the industry and cause catastrophic results for the economy, the environment, and the people of the Amazon. Project Piaba has been working to develop a full understanding of the fishery locally and in the world market, the threats that faces it, and how strategic adaptations can preserve the fishery and enhance its benefits.

The presentation will provide a detailed description of the fishery, the issues, the plan to save it and how your institution can get involved.

2nd SECORE Workshop: Conserving the Threatened Elkhorn Coral, *Acropora palmata*

Dirk Petersen, Rotterdam Zoo, The Netherlands

Eric Borneman, University of Houston, USA

Mike Brittsan, Columbus Zoo and Aquarium, USA

Mary Hagedorn, Smithsonian Institution, USA

Michaël Laterveer, Rotterdam Zoo, The Netherlands

The 2nd SECORE Workshop was held in Puerto Rico (USA) from the 10th to 17th of August, 2006. Coral specialists from 13 public aquaria and researchers from the U.S. and Europe participated in this training and research workshop. This was the greatest effort ever undertaken to establish an *ex situ* population of the threatened Elkhorn coral, *Acropora palmata*. During the 2nd SECORE Workshop, we applied *ex situ* rearing methods to the broadcast spawning Elkhorn coral, *Acropora palmata*.

Updates of 2006 and 2007 ROV Studies and Technical Diving Proposals to Research and Capture Latimeria sp.

Forrest A. Young & Ben Daughtry

Dynasty Marine Associates, Inc.

Technical diving and ROV are both useful tools for exploration of the deep reef slope. Field application of advanced diving techniques in combination with ROV as a guide and submersibles as support are considered. Included are footage of a live coelacanth discovered at Buol, Sulawesi, Indonesia in 2006. Decompression strategies and risk are considered in depth.

Conditioning of Eagle Ray Pups, *Aetobatus narinari*, for Introduction into a Multi-Species Reef Exhibit"

Angela Hillenbrand, Denise Ashdown-Swider

Discovery Cove

Three eagle ray pups were born at Discovery Cove in the summer of 2006. They were conditioned to hand feed and station on a target in a quarantine holding facility for a quicker reintroduction back into a 1.7 million gallon exhibit. The conditioning used to target train the stingrays proved to be successful. Within a week all three pups were eating with the adult eagle rays at the feeding station and after three months the use of the target was no longer required. By training while the pups were isolated from the rest of the collection and the guests, the acclimation of the pups to the larger exhibit proved to be vital towards their care in all aspects of aquarium husbandry.

A Mouse among Rays!

Allen Wilson, Animal Care Specialist & Jane Davis, Aquarium Curator,

Walt Disney World's Animal Programs

Castaway Ray's Stingray Adventure is an educational in-water guest interaction excursion with southern stingrays which takes place on Disney's own private island in the Bahamas, called Castaway Cay. Our southern stingrays have been conditioned specifically for guest interaction in this unique program. The rays have been conditioned to swim through a floating interaction station and touch a target; guests then have the opportunity to feed them when the rays are targeting and within the floating interaction station. Rays were initially collected by aquarists from the Living Seas in May of 2005. Stingrays are housed in a lagoon measuring 150 feet by 75 feet, with a depth that goes from shoreline to 8 feet with guest facilities that include covered seating for up to 55 guests with props. Conditioning of the rays and training of staff began from the time of collection and continued until opening the on September 21st, 2005. The program operates on the hour, 7 times daily accommodating 50 guests at a time. This presentation outlines the steps involved in the collection, daily husbandry, veterinary care, educational messages shared with the guests, and conditioning of southern stingrays for Castaway Ray's Stingray Adventure.

California Angel Shark (*Squatina californica*) Husbandry at Aquarium of the Bay (with notes on ex-utero care of aborted live embryos)

Reid Withrow

Aquarium of the Bay

Aquarium of the Bay has a history of seven years of California angel shark husbandry. This small, demersal ray-like shark can be difficult to maintain in captivity; it does not usually free feed from food broadcast into the exhibit but has to be trained to accept food offered by exhibit divers. Probably because of its sedentary life style, angel sharks can be monogene and leech magnets that require more hands on maintenance than many other shark species.

We had the opportunity to attempt rearing aborted live California Angel shark pups that probably were conceived in captivity.

***Manta birostris* Release**

Núria Baylina, Gonçalo David Nunes

Oceanário de Lisboa, S.A.

In November 2002 one 1,6 m *Manta birostris* was caught in Algarve in a fishermen net, transported to Oceanário de Lisboa and introduced in the Open Ocean tank. This animal adapted very well to this tank.

In April 2007 this 3,5 m male was released back in the sea . This operation involved several companies and a large number of people due to conditioning factors such as animal size, tides, weather conditions, transport time, etc.

This presentation shows the several phases of this operation: capture, removal from the building, introduction in the transport tank, transport by boat to the location of release, satellite transmitters attachment and the release.

Session 3: Veterinary I

Case Studies of Neoplasia in Fishes with a Review of Tumors in Aquarium Species

Barrett L. Christie and Rebecca R. Leitner

The Aquarium and Rainforest at Moody Gardens

This report documents four case studies of significant neoplasia in both freshwater and marine fishes. In the first case a yellowtail snapper, *Ocyurus chrysurus* (Lutjanidae), was observed with

an obstructed buccal cavity. Cursory examination revealed a large mass on the glossus, which was later diagnosed as a follicular cell carcinoma of the thyroid. The second case was an Asian catfish, *Pangasius sutchi* (Pangasiidae), from which a large ovarian mass was discovered during necropsy. Histopathology determined the mass to be a dysgerminoma. The third presented case is that of a cobia, *Rachycentron canadum* (Rachycentridae), from which testicular and hepatic masses were found post-mortem. The hepatic lesion was determined to be a sarcoma, and was massive in size, having a diameter of nearly 15 cm and a mass of 397.7g. The final case study is that of a black knifefish, *Notopterus notopterus* (Notopteridae), which had a large trans-cranial mass surgically excised and determined to be a fibrosarcoma. The lesion redeveloped quickly and subsequent rapid growth coupled with the malignant nature resulted in the mortality of the animal. The tumors from *N. notopterus* and *R. canadum* are the first recorded from these species, and the latter may prove to be one of the largest fish tumors ever documented. The significance of these and other lesions is presented systematically alongside a review of more than 1500 cases of tumors in aquarium species from the Registry of Tumors in Lower Animals database.

Treatment of a Fungal Infection in the Blacktip Shark, *Carcharhinus limbatus* with Amphotericin B

Barrett L. Christie[1] and Richard Henderson, DVM[1,2]

[1]*The Aquarium at Moody Gardens, Galveston, Texas*

[2]*The Galveston Veterinary Clinic, Galveston, Texas*

Preliminary findings of an experimental trial of the antifungal drug Amphotericin B in five specimens of the Atlantic blacktip shark, *Carcharhinus limbatus,* are presented. Five specimens of *Carcharhinus limbatus* were captured via hook and line in June of 2006 from the beachfront of Galveston Island, Texas. The specimens were housed in a 160,000 L holding tank with several other elasmobranchs until September 2006, when an outbreak of *Dermophthirius penneri* (Monogenea:Microbothriidae) occurred. The monogenean caused skin lesions and loss of placoid scales in *C. limbatus*, opening the sharks to secondary and tertiary infections of bacteria and an unknown fungus. Bacterial overgrowth of the fungus prevented *in vitro* culture for positive identification. The fungal dermatitis was first treated with Itraconazole at 5 mg/kg P.O. S.I.D. for 7 days, though the specimens failed to respond to treatment. A more aggressive course of antifungal therapy was started, employing Amphotericin B given at 0.5 mg/kg I.P. 3x weekly for 3 weeks. The specimens tolerated the treatment, which was extended for 2 additional weeks. Amphotericin B greatly reduced signs of fungal hyphae in the skin scrapings, though *D. penneri* proved resistant to multiple antihelminthic treatments, leading to eventual mortality of the sharks. This report documents the first recorded use of Amphotericin B in elasmobranch fishes, and is the first published record of antifungal chemotherapy in sharks with any measure of success. The lethal nature of most mycotic infections and lack of treatment options available make these preliminary findings noteworthy, though not necessarily definitive.

Successful Resolution of *Fusarium* Infection in a Bonnethead Shark (*Sphyrna Tiburo*) with Anti-Fungal Therapy and Environmental Manipulation

Eric Curtis[1], Michelle Davis[2], Caryn Poll[1], Michelle Sattler[1], and William Van Bonn[1]

1. John G. Shedd Aquarium, Chicago, IL 2. Chicago Zoo and Aquatic Animal Residency Program, University of Illinois, Urbana, IL

"Bonnethead shark disease" is a well described disease of bonnethead sharks (*Sphyrna tiburo*) caused by the fungus, *Fusarium solani*[4]. The syndrome is characterized by the development of white pustules along the lateral line system and bonnet of affected individuals. The disease is progressive and fatal, with hemorrhage into the deep muscle or cartilage and invasion of the fungus into skin, muscle, cartilage, and, occasionally, internal organs[3,4]. Attempts to treat affected individuals have been unsuccessful. The disease has also been reported in scalloped hammerhead sharks (*Sphyrna lewini*)[2]. We describe the clinical course and successful resolution of disease in an adult female bonnethead shark displaying clinical signs and lesions typical of "bonnethead shark disease." Treatment included systemic use of a newer triazole antifungal medication[1] and temperature manipulation.

Hematocrit Differences Between Two Venipuncture Sites in Sharks

Eric Curtis[1], Natalie Mylniczenko[2], Rachel Wilborn[3], and Forrest Young[4]

1. John G. Shedd Aquarium, Chicago, IL 2. Brookfield Zoo, Brookfield, IL 3. University of West Florida, Pensacola, FL 4. Dynasty Marine Associates, Marathon, FL 33050

This talk presents the results published last year in the American Journal of Veterinary Research[1] describing the differences in hematocrit (Hct) values between blood collected from the dorsal fin sinus versus the caudal tail artery in seven species of sharks. A significant difference in Hct was found between the two common blood collection sites. This finding has obvious implications when comparing Hct values between animals or when attempting to establish normal values for various elasmobranch species.

Sponge Worthy? - The Husbandry of Poriferans in Public Aquaria

Peter Gawne and Joe Masi

New England Aquarium

Sponges, phylum Porifera, are the simplest and oldest multicellular animals on the planet. Poriferans are also incredibly varied, compromising in excess of 8,000 species worldwide and holding title as the most chemically diverse phylum on Earth.

Despite the simplicity of these animals, their captive care and basic husbandry needs remain largely a mystery. This discussion highlights the biology, collection, and husbandry for sponges in seawater systems. Restorative techniques, including fragmentation and reaggregation, for treating ailing sponges will also be explored.

Challenges of Keeping a Live Coral Tank, and the Effects of Freshwater on a Reef System

Amy Baynes

Aquarium of the Pacific

Keeping live coral tanks was once thought to be an unattainable achievement and nearly impossible in a closed environment. But, through time, hard work, and experience it started to become more practical, and keeping live coral exhibits in aquariums has become more popular over the course of the years. Even though it has become commonplace, there are still many challenges that come with caring for a live reef system. There are many requirements that are essential to keeping the system healthy and for coral growth. I will be discussing some of the more important aspects that are required, and some functions that I perform in order to help keep our tank running smoothly and aesthetically pleasing. I will also be talking about some of the challenges that I have encountered over the years while caring for a live coral exhibit.

Abiotic Filtration Methods for Live Coral Systems in Public Aquaria

Richard Terrell, Jr.[1], Richard Klobuchar, Jr.[2] and Carrie Pratt[3]

1 Pittsburgh Zoo & PPG Aquarium 2 University of Hawaii - Waikiki Aquarium, 3 Columbus Zoo and Aquarium

rterrell@pittsburghzoo.org

Over recent decades, advances in life support systems for captive corals have increased public aquariums' ability to more closely mimic water quality conditions found on coral reefs. This, in turn, has allowed these institutions to keep, exhibit and reproduce an increasing diversity of coral species. We will discuss abiotic life support systems in terms of mechanical and chemical filtration. Mechanical filtration will focus on such methods as filter floss, cartridge filters, rapid sand filters and bag filters. Examples of chemical filtration included are ozone, ultraviolet sterilizers, carbon and other adsorptive media and ion exchange resins. Perhaps the most significant development in abiotic life support systems for living reef systems is the use of foam fractionation. This unique life support element functions as both chemical and mechanical filtration. We will discuss the advantages, disadvantages and caveats of each filtration device or media as they relate to the husbandry of live corals in public aquaria.

A Survey of Commercially Available Nitrogen Cycling Products for Saltwater Aquaria

Audra Seladi, Water Quality Technician

Newport Aquarium, Newport, Kentucky

aseladi@newportaquarium.com

The effectiveness of several commercially available saltwater cycling products was evaluated based on their ability to speed up the nitrogen cycling process. Product performance might be improved by the addition of various bacterial nutrients. Specific water quality and life support system parameters must be met to ensure optimum performance of any cycling product containing live bacterial cultures.

The Great *Polyorchis* Hunt, or an Exploration into the World of Hydrozoans

Michael J. Howard

Monterey Bay Aquarium, 886 Cannery Row Monterey, CA 93940; p: 831.648.7975; e:

mhoward@mbayaq.org

Historically, sea jelly displays at public aquaria have been hugely popular. The vast majority of species displayed and cultured on a regular basis belong to the class, Scyphozoa. Some facilities display jellies within the class Hydrozoa when they are available seasonally. Even fewer facilities display hydrozoans year-round because very few species have yielded cultures with a viability and production level high enough for year-round display. Their diminutive size also has limited the number of hydrozoan species successfully displayed within the expansive exhibit kreisels most often encountered at public aquaria.

Hydrozoans demonstrate rich diversity in many aspects of their biology and life histories. The study of hydrozoans and their culture offers excellent opportunities for discovery. The hydrozoan, *Polyorchis spp*, is seasonally abundant in the Monterey Bay. The medusae are visually stunning and seem like an excellent candidate for year-round display. However, its polyp remains unknown despite the independent efforts of many scientists to generate cultures in-vitro. I set out to find or produce a culture for this hydrozoan. During these efforts, I have collected many hydroid colonies and medusae from local coastal waters. Some of these specimens are currently growing in-vitro. As they grow, I have identified some to genus or species, while others have only yielded partial stories and remain unknown. The hunt begins.

Wednesday, June 20

Session 5: Veterinary II

Splinting a Fractured Rostrum on a Freshwater Sawfish, *Pristis Microdon*

Eric Castillo

Aquarium of the Pacific

For decades large elasmobranchs have captured the awe and imagination of the public. While the growing demand to view multi-species exhibits have given rise to some impressive displays, the relative close interaction between these animals increases the likelihood that a severe life threatening bite will occur. Our fresh water sawfish, *Pristis microdon,* sustained a severe bite to her rostrum from a sand tiger shark, *Carcharias taurus.* This presentation will focus on the creative husbandry techniques used to capture, restrain and surgically repair a fractured rostrum; video and still footage will be presented.

A Review and Comparison of Treatment Methods for the Control of the Protozoan *Uronema* sp.

Pilar J. Gibson

New England Aquarium, Boston, MA

Outbreaks of infestations by the ciliated protozoan *Uronema sp.* affect systems of captive marine fish and invertebrates, sometimes with disastrous results, and can be a source of frustration for those charged with the care of these animals.

This is an overview of current literature and of current practices employed by some institutions in the US and abroad for the management and treatment of this parasite. Frequently cited therapies, either singly or in combination, are freshwater, formalin, and malachite green, with varying degrees of success reported. The schedules and dosing vary. Other therapies are also discussed.

The historical trend appears to be that, although many therapies and methods have been implemented, resolving *Uronema* infections remains a challenge to aquarists and animal health staff alike. Anecdotal evidence may suggest that yet more resilient strains of *Uronema* and other *Uronema*-like ciliates may be emerging.

The Analysis of Growth Lamellae as They Relate to Age Determination and Stress in *Sepia pharaonis* and *Sepia officinalis*

Gregory J. Barord

The Aquarium @ Moody Gardens

Age is a difficult parameter to calculate in animals whose life span can easily be changed and regulated by environmental factors such as temperature. Statolith analysis is a common method used to determine age in cephalopods. Unfortunately, high-powered microscopes are needed for this method, and these are not always readily available. The ventral side of a cuttlebone is lined with 1mm grooves, called growth lamellae, which are produced every 2-12 days, depending on the temperature. Additional aspects of the cuttlebone are darker growth lamellae, also referred to as scars, which have been attributed to periods of trauma in *Sepia orbignyana* (pink cuttlefish). In captivity, the darker lines can be ascribed to poor water quality conditions, agitation, and many other stressors that can induce erratic behavior and cause scarring along the cuttlebone. Thus cuttlefish with a minimum number of dark lines on the cuttlebone could be deemed healthy. However, other factors such as reproductive activities must be taken into account when examining the cuttlebone. A cuttlefish is more likely to sustain trauma during reproduction due to aggressive behaviors. A healthy cuttlebone should also contain some scars correlated to the reproductive activity of the cuttlefish. Analysis of the growth lamellae allow for a quick and inexpensive method of not only determining age, but also discovering any stress placed on the

animal. The ability to examine the history of an individual cuttlefish, with minimal time and effort, will aid in providing the next generation of cuttlefish the best possible conditions in captivity.

Treatment of Shell Deterioration in Captive *Nautilus pompilius*

Gregory J. Barord

The Aquarium @ Moody Gardens

Shell deterioration has long been observed in captive *Nautilus*, however the cause of this phenomenon is not yet understood. To date, there have been no observations of this anomaly in wild *Nautilus*. Dissimilar bacterial fauna, pressure changes, trauma, and diet are some of the different theories explaining this malformation. In this study, the rate of shell deterioration was measured as a precursor to any treatment. Thereafter, changes in the diet were made in order to analyze increases in calcium uptake. Initial diet changes included the addition of *Pagurus* sp. (hermit crab) and *Callinectes sapidus* (blue crab). Though both were initially collected in abundance, *C. sapidus* became scarce. The *Nautilus* showed no observed hunting behavior towards *Pagurus*. As a result, frozen *Mallotus villosus* (smelt) and *Osmerus eperlanus mordax* (capelin) heads were added to the diet in place of the crabs. Although high in calcium, this new diet corresponded to increases in nitrate values to critical levels (NO3>60mg/l). Thereafter, calcium pills (65 mg) were substituted for fish heads to one nautilus; to first ensure there were no side effects. The initial diet changes did not stop or slow deterioration, although the calcium pill did show signs of improvement throughout the study.

This study will be continued with a new diet comprising only fish heads. One nautilus will be given a fish head, from a freshly collected estuarine fish, at each feeding. Results from this diet change will be analyzed within two months after the start date of 23 March 2007.

Session 6: Coral and Invertebrates II

Some Successes in Raising *Octopus rubescens* at Monterey Bay Aquarium

Sidney Snider

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The raising of octopus species has been varied and limited at best. *O. bimaculoides* and *O. vulgaris* have reports of successes as well as a few others. The raising of *O. rubescens,* however, have eluded aquarists for the most part. On a number of occasions Monterey Bay Aquarium has had the opportunity to raise eggs that have been laid in captivity but with little success. In the

summer of 2006, eggs were laid and handled in such a manner that over 3,000 juveniles hatched, allowing ample opportunity to try various methods at keeping them alive. The eggs incubated for just over four months before beginning to hatch. In prior opportunities when an *O. rubescens* laid eggs they were thought to be infertile and were not given ample time for gestation. In June 2006, the eggs were given more than adequate aeration, flow and time for full development, first by being allowed to stay with the mother and then later being transferred to another tank. Upon hatching into a standard 2 X 3 tank, the juveniles were then divided up into several different tanks. Out of the original 3,000 *O. rubescens* only a handful survived to the nearly 28 day mark. This paper discusses some of the various methods used to keep the octopus alive post-hatching and what successes and failures were encountered with those methods.

Preliminary Attempts on the Capture, Transport and Husbandry of the Humboldt Squid, *Dosidicus gigas*

Steve Blair

Aquarium of the Pacific

Relatively little is known about the biology of the Humboldt Squid, *Dosidicus gigas*, which recently has become seasonally abundant along the Pacific coast. Although a significant population exists in the Sea of Cortez, Mexico the affects of this large predator on more northern ecosystems is unclear. The AOP has been conducting field studies on this large and aggressive squid to determine its basic environmental preferences and biology and to develop capture and transport techniques. We have begun to develop some husbandry techniques and have met with limited success in keeping them in captivity.

Predators and Pests of Corals

Mitch Carl

Omaha's Henry Doorly Zoo

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Coral keeping as a hobby and for exhibits in public aquariums has exploded in popularity over the last decade. Due to this increase, more and more corals are being imported from all over the world and more corals are being traded amongst coral enthusiasts. With this increase in new sites and more aquaculture and mariculture operations in place, many new parasites, pests and diseases of corals are being introduced and spread between persons and groups. The first talk will first focus on the many predators of corals that can inhabit our systems. A summary of the more common and currently important predator species will be covered, including flatworms, nudibranchs and protozoan along with their control and hopeful eradication. The second talk is a

look at the pests of our coral systems. These animals and plants do not directly kill our corals, but are unsightly and hard to control. A summary of animals such as Red Bugs (*Tegastes acroporanus*) and flatworms and the various algae that can plague coral tanks will be given.

A Direct Solution to an Ever-Present Nuisance Anemone: The Ability of *Berghia* to Solve the *Aiptasia* Problem

Ashley Ayres

Dynasty Marine Associates, Inc.

Aiptasia, commonly known as the glass or rock anemone, are a naturally prolific and reproductively successful cnidarian that commonly runs over marine reef tanks by way of waging a physical and chemical war against much of the desirable and sessile livestock. Large public aquariums, as well as, marine reef tank hobbyists have sought to rid their tanks of this anemone, resorting to a number of different methods of removal. Some of these methods include physical, chemical, and biological controls that are usually time-intensive, impermanent, and usually end up creating bigger, more frustrating problems. Nonetheless, a little nudibranch that preys only on *Aiptasia* anemones is fast becoming the ideal way to rid a marine reef tank of *Aiptasia*. *Berghia verrucicornis*, the *Aiptasia*-eating nudibranch, has come to be regarded by many as one of the best all-natural and reef-safe *Aiptasia* controls simply because these nudibranchs will eat the entire *Aiptasia* polyp, are self-maintaining, and will not affect anything else in the tank.

Session 7: Miscellaneous

Where Schooling Really Counts, an Aquarium Science Up-date

Bruce Koike[1], Marion Mann[1], Tim Miller-Morgan[2], Eileen Flory[1] , and Jane Hodgkins[1]

[1]Oregon Coast Community College [2]Oregon Sea Grant Extension, College of Veterinary Medicine, Oregon State University

Expansion (and other factors) in the public aquarium industry, ornamental fish businesses and aquaculture ventures creates a need to identify skilled and qualified aquatic animal husbandry personnel. In 2003, the Oregon Coast Community College was awarded a National Science Foundation/Advanced Technological Education grant to develop and implement the nation's first Aquarium Science degree program in response to this skilled worker shortage. The program has witnessed two graduating classes, consisting of 9 individuals total and each person is employed in an animal care capacity. Animal care businesses/organizations have also hired 7 "non-completers", those who have chosen employment over degree completion.

In addition to the Associates of Applied Science degree, the college offers a 1-year Certificate of Completion to those individuals who already possess a Bachelors degree or higher in a life science.

The presentation highlights:

- How the aquarium industry helped develop, refinement and deliver educational opportunities,
- Survey results of the two graduating classes,
- The construction of a dedicated building to teach Aquarium Science from (completion in late 2008),
- And how the public aquarium industry can continue to be an active partner in support of this educational program to standardize the level of training for entry level aquarists.

Trials and Tribulations of a Mixed Species Exhibit

Peter Kriz and Keri O'Neil

National Aquarium in Baltimore

The *Amazon River Forest* exhibit at the National Aquarium in Baltimore is a visual representation of a blackwater flooded forest ecosystem. In the seven years since the exhibit opened, a variety of terrestrial and aquatic plants and animals have been kept in the exhibit, with both successes and failures.

The goal of this presentation is to discuss the complexities of managing an exhibit that was intended to display terrestrial and aquatic plants, reptiles, birds, fish, and mammals in the same enclosure. We will discuss what species have been attempted and why, what combinations were successful or not, and the methods used to manage species interactions. The animals we will discuss include anacondas, caimans, turtles, red-tail catfish, pygmy marmosets, and silver arowana, among others. Emphasis will be placed on animal management strategies and interdepartmental teamwork and communication.

Development of a Fish Culture Program for Reintroduction of Native North American Fishes and Mussels.

Marc R. Kibbey[1], and Mike Brittsan[2]

[1]*Museum of Biological Diversity, The Ohio State University.* [2]*Columbus Zoo & Aquarium, Shores Department*

In the winter of 2006 we were asked to breed darter fish species for the Freshwater Mussel Research and Conservation Center. Freshwater bivalve mussels of the family Unionidae require a

host fish to provide a traveling meal for the bivalve larvae (glochidia) until the glochidia are well enough developed to drop off, hopefully into suitable substrate. OSU's Mollusc Curator Tom Watters needed a reliable source of captive bred host fishes that have not developed an immune resistance as a result of previous infections by the glochidia. The effort began modestly with a couple of locally abundant darter fish species known to have been successfully cultured and that would enable us to develop suitable equipment and methods. We raised so many rainbow darters that we are hard pressed to figure out what to do with 'em all! While we had less success with the greenside darters, we learned a lot of lessons along the way that will translate into better success in our future endeavors. In collaboration with the Ohio Division of Wildlife we've designated the imperiled eastern sand darter, a species that has been extirpated from some Ohio streams, for propagation and reintroduction in waterways that we will assess for suitability using habitat modeling with the assistance of OSU Prof. Lance Williams' graduate student. Expansion of the fish propagation facilities under the direction of Columbus Zoo and Aquarium's Doug Warmolts will enable us to carry out propagation and reintroduction of more rare and endangered fish species at the center.

Closed System Reproduction of Rainbow Trout
(Or how life thrives in spite of me)

Timothy S. Huebner

Cabela's Fort Worth Aquarium Manager

I was hired in March 2005 to lead the construction, start up, and stocking of trout into two 55 degree ponds totaling 10,000 gallons in the middle of a Cabela's retail outdoor store in Fort Worth, Texas. I had never worked with any kind of trout before but had 10 years public Aquarium experience, so I knew the system would work. Surprisingly and exceeding my expectations I am now having rainbow trout spawn and hatch naturally, catching the fry, rearing them to a safe size and returning them to the exhibit.

Session 8: Culture and Breeding

Breeding Behavior and Captive Propagation of the Ribbon seadragon, *Haliichthys taeniophorus*.

Paula Branshaw, Daryl Richardson, Julie Chompunuchtanin

The Dallas World Aquarium

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Depicted as the rainbow serpent in Aboriginal art, drawings of the Ribbon seadragon, *Haliichthys taeniophorus*, first appeared in Arnhem Land rock art more than 6000 years ago.

Australian researchers originally believed the artwork represented a seahorse, but later decided upon the Ribbon seadragon, found around Irian Jaya and the coast of northern Australia from Shark Bay in Western Australia to the Torres Strait.

In August, 2005, The Dallas World Aquarium became one of the only facilities in the world to acquire these incredible fish. At that time we received three females. In October, 2006, we introduced a male to the collection. Within 48 hours he was brooding eggs in his pouch, and to date has continued to do so every 14 days with little exception.

We have reared one female juvenile to more than 160 days and have several hundred others at various stages of development. Our biggest challenge has been finding appropriate foods that will be accepted by the juveniles at the early stages of their development.

Observations made will prove to be invaluable to future propagation efforts and potential collaborative research programs with other institutions.

This paper will describe the breeding behavior of the ribbon seadragon and the methods used in rearing the offspring.

Spotted Rat-fish *(Hydrolagus colliei)* -Tentative Reproduction at Oceanário de Lisboa

Luisa Moreno

Oceanário de Lisboa, S.A.

Chimera (*Hydrolagus colliei)* is a touching marine animal, because of their fragile and mystic look. They also have been survivors in earth; these animals exist for more than 400 million years. Meantime there is little information about their reproduction.

In 2001, Oceanário de Lisboa opened an exhibit with these animals. Since then egg laying has been observed but only in 2005 the break out of one of those eggs and the birth of the first Chimera happened. Ever since, Chimeras continued to be born, but none survived.

The present work shows the husbandry techniques for this species at the Oceanário de Lisboa that turned possible the break out of the eggs.

Aquaculture in Today's Aquarium

Nate Jaros

Long Beach Aquarium of the Pacific

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In a world growing ever more environmentally conscience, zoos and aquaria are leaders in the conservation effort. As leaders we have a duty to minimize the footprint we leave behind on our world. When choosing animals to become ambassadors of their species, we must consider the impact we place on wild populations. One trend in modern aquaria is adopting methods of aquaculture to create a sustainable source of display animals. In this presentation I will reference my experience culturing syngnathids and sea jellies at the Aquarium of the Pacific. Through the use of life cycle and juvenile displays we justify investing time and resources to this cause. Closing the life cycle in more species becomes a terrific educational tool, inspiration for conservation, and a great resource for animals for our institution and to others through surplus.

Techniques for Hatching Wolf-eel (*Anarrhichthys ocellatus*) Egg Masses at the Oregon Coast Aquarium

Evonne Mochon Collura, Stuart Clausen

Oregon Coast Aquarium

A pair of wild-caught wolf-eels (*Anarrhichthys ocellatus*) shares a den in the Aquarium's "At the Jetty" Exhibit. For the past three years, the couple has produced an egg mass in autumn and in 2006, the aquarium staff was able to successfully hatch and raise the offspring. In previous years, the adult wolf-eels exhibited good parental care for the egg mass however, aquarists were unable to recover any larvae. The 2006 egg mass was left in the den for as long as possible and monitored with minimal disturbance of the adults. After approximately 12 weeks, the egg mass was removed from the den and placed in a separate tank where aquarists monitored progress and cared for the egg mass. Within two weeks, the larvae began emerging and eggs continued to hatch for another six weeks. During this time, a second egg mass was discovered in another exhibit and moved to a holding tank, where it was handled in the same manner. Approximately 15,000 eggs were incubated, however hundreds expired while hatching or within the first two weeks. The hatching of the second egg mass was quite different with respect to larval emergence, developmental abnormalities, and mortality rate. By trying various tank styles, flow patterns, and foods, aquarists were able to eventually reduce mortality in both populations. To date, several hundred baby wolf-eels have been shipped to aquariums around the country and several hundred continue to thrive at the Oregon Coast Aquarium.

Asia's First Sea Jelly Exhibit

David Lai, Walter Tang, Yvonne Lim, Lell Luk, Wong Tin Po, Li Chi Ho and Nikky Chan

Aquarium Department, Ocean Park Hong Kong

Asia's first stand alone sea jelly exhibit, the "Sea Jelly Spectacular", made its debut in Ocean Park Hong Kong on April 14, 2006. It features more than 1,000 sea jellies of over 10 distinct species sourced globally.

This facility was created, designed, and developed in-house, within the constraints of an existing facility after being inspired by aquariums around the world. Equipped with state-of-the-art theatrical lighting, multi-media sound and visual special effects to capture the sea jellies in their pristine natural beauty, this exhibit is also supported by a back-of-house facility, which is also a nursery, housing other species that will be featured on a rotational basis. To date, 10 species of jellies are bred and raised in-house to support the exhibit.

In giving our guests an entirely refreshing experience, the take home message is unforgettable; they learn and remember that each of us can make a difference by using their shadows to 'wipe out pollution' in an interactive shadow game to reveal a clean and healthy ocean. And to fulfill our role to educate through entertainment and interaction, there is also an educational corner, where guests, through special arrangements, can have personal coaching by the Park's education specialists on the caring of sea jellies.

Wednesday, June 20

Session 9: Veterinary III

Treating for Mycobacteriosis

Mark Schick

John G. Shedd Aquarium, Chicago, IL

Mycobacteriosis has long been an issue in the zoo and aquarium industry. Various species of the bacteria can infect terrestrial, freshwater and saltwater animals and can be found as a normal background inhabitant in healthy systems. At this time, positive identification is time consuming and treatment for the infection is inconsistent.

Ongoing research into testing methods show promise in reducing detection time from a month to almost instantly. While tests conducted by Shedd Aquarium with multi-drug treatments were not successful, various husbandry techniques have drastically reduced the prevalence of infections in animal populations.

Endoscopic Evaluation of a Green Moray Eel, (*Gymnothorax funebris*), Suffering from Chronic Regurgitation

Nicole M. Roddy

Mystic Aquarium and Institute for Exploration

The patient, "Termie," an adult green moray eel, was observed to be consistently regurgitating various food items. This eel is housed with two other healthy green moray eels in a 35,000 gallon Atlantic tropical reef exhibit. Termie was isolated in a 300 gallon overflow tank for closer observation. Although appetite and behavior remained normal, whole food items were found in the tank usually 12-24 hours following each feed for a month. At this point the eel was anesthetized in order to perform a thorough diagnostic exam. The initial procedure consisted of radiographs, ultrasound, blood collection, fecal sample, and endoscopy. During the endoscopic evaluation of the gastrointestinal tract multiple areas of cell hyperplasia were identified. Biopsies and a gastric aspirate were collected, but due to insufficient equipment, the sampling was limited. Following the initial procedure, Termie was started on oral metoclopramide to stimulate intestinal motility and prevent further regurgitation. The eel was returned to the overflow tank for continued observation. Although the patient responded well to the metoclopramide and regurgitation resolved, a second endoscopy was scheduled to obtain additional biopsies for a more thorough analysis. Physicians specializing in endoscopy from Westerly Hospital participated in this procedure. Biopsy results revealed gastrointestinal cell hyperplasia, but no evidence of tumors, infection, or inflammation was identified. Currently the patient has been returned to his original exhibit and both appetite and behavior are normal. Termie remains on metoclopramide and the regurgitation appears to have resolved.

Ultra-sound Evaluation of Southern Stingrays, *Dasyatis Americana*.

Rebecca Sinkoff

Downtown Aquarium, Denver

Within five months after receiving a shipment of rays, 4 of our female southern sting rays (Dasyatis americana) and one female cow nose ray (Rhinopterus bonasus) died. Necropsies revealed shrunken blue colored livers and fibrous growths on the ovaries. Further testing of the livers indicated hepatocellular necrosis, a result of a depleted glycogen reserves. Although the animals were being fed a well balanced / vitamin supplemented diet they would still waste away. It is unclear if this condition is found in the wild in a percentage of specimens or if the condition was acerbated during transport or acclimation.

The preliminary study taken up by the staff at Downtown Aquarium and staff at Colorado State University was to look at this situation. The working hypothesis was that the rays were under reproductive stress when captured and rather then aborting or absorbing the eggs they became necrotic. This necrosis caused the fibrous growths found on the ovaries, which then became an

internal infection which impeded the livers capability to maintain or store glycogen. Ultra-sound was used on 20 individual female and two individual male southern rays and two females were found with the shrunken liver and growths on the ovaries. Surgery was performed on one ray to remove the ovaries, which was hypothesized would remove the infection source and thus stop the fatal process. Unfortunately the sutures failed and the specimen died two days after surgery.

During discussions with other facilities within our organization it was also discovered that one female southern ray died after showing the same symptoms, and a male southern ray died after showing the reduced dark colored liver. The source of infection on the male ray could have been the fish hook found internally during the necropsy.

In the future we will be running ultra-sound tests on rays we receive to note any abnormalities and to take corrective action early on. We will also be running tests on other ray species we have in our collection and work with other facilities to obtain more detailed data.

The Eradication of Hyperiid Amphipods from Jellyfish (*Chrysaora fuscescens*) Using Diflubenzuron in a Closed Recirculating System.

Sharyl M.G. Crossley

Tennessee Aquarium

On December 2, 2006, a heavy infestation of parasitic Hyperiid amphipods (*Hyperia medusarum* and *Lestrigonus shoemakeri*) was discovered in the West coast sea nettle (*Chrysaora fuscescens*) exhibit at the Tennessee Aquarium. Toxicity tests that exposed moon jellyfish (*Aurelia aurita*) and sea nettles (*C. fuscescens*) to therapeutic levels of diflubenzuron, as defined by Dr. Noga in "Fish disease: diagnosis and treatment," confirmed that the treatment would be tolerated by these species of jellyfish. The exhibit tank was dosed with 0.03ppm concentration of diflubenzuron for seven days, after which the medication was removed. A randomly chosen subset from the sea nettle exhibit was sampled regularly over the next eight weeks to monitor the parasite population. The average number of amphipods per jellyfish sampled decreased throughout the treatment and sampling period. No live amphipods were observed six weeks after the start of treatment, and the sea nettles remained unaffected. In general non chemical means, such as early detection and physical removal of parasites, remain better methods of control. When applied properly, however the use of diflubenzuron to eradicate Hyperiid parasites from host jellyfish is a safe and useful option.

POSTERS

Red Bug Copepod Infestation of *Acropora* Coral Successfully Stopped by Ivermectin

Mike Henley, Alan Peters, and Suzan Murray

Smithsonian National Zoological Park

Abstract: It has been established that a copepod commonly known as "red bug," *Tegastes acroporanus*, infests hard corals in the family Acroporidae and becomes a debilitating irritant. The established treatment for these parasites is milbemycin in the form of Interceptor tablets. Ivermectin treatments have not been reported to be successful until recently when the Smithsonian's National Zoo Invertebrate Exhibit and Department of Animal Health cooperated in a study comparing the efficacy of both medications. When acroporid colonies were treated in adjacent tanks**,** we confirmed the initial presence of the copepod and the successful elimination of the organisms by both medications, with ivermectin at varied concentrations. The results of the treatments were "red bug" free corals, healing of damaged tissue, fully extended polyps, and enhanced colony growth and coloration. The mode of action for both medications is very similar, and it is beneficial to extend the list of successful alternative treatments. A common veterinary medication available in liquid form, ivermectin has proven to be useful since it is easily and quickly measured and administered into treatment tanks.

Personal Meaning Mapping Methodology Used at the North Carolina Aquarium at Pine Knoll Shores to Investigate the Impact of Sea Turtle Exhibits and Educational Programs on Visitor Learning.

Heather Johnson and Jenifer Hoskins

North Carolina Aquarium at Pine Knoll Shores, Pine Knoll Shores, North Carolina, USA

The North Carolina Aquarium at Pine Knoll Shores (NCAPKS) is one of three state operated aquariums and is part of a division in the North Carolina Department of Environment and Natural Resources. The mission of the three aquariums is to inspire appreciation and conservation of North Carolina's aquatic environments. To educate visitors about sea turtles, the aquarium works with North Carolina Wildlife Resources Commission to rehabilitate loggerhead hatchlings. During rehabilitation and growth, these hatchlings are exhibited and used in educational programs to teach visitors about sea turtles, focusing on local loggerhead nesting sites and conservation issues. Goals of the NCAPKS are to inspire stewardship of sea turtles and their natural habitats and instill conservation of these animals. In an effort to assess the aquarium's ability to achieve these goals through the exhibits and programs, the personal meaning mapping methodology was used to qualitatively analyze the visitors' learning experience. The method has been designed by the Institute for Learning Innovation to assess learning in environments like aquariums. School

groups and general visitors of all ages were interviewed before encountering a sea turtle exhibit or program at NCAPKS and again interviewed after making the journey through the facility. This information is compared to learn what impact these programs and exhibits have on the visitors and their frame of mind regarding sea turtles. Information learned from this study will be used to further develop, improve, and diversify the exhibits and programs to achieve the aquarium's mission.

A Lot of "O Wow!!" Aquariums in Science & Conservation Education

William Langbauer, Mandy Revak, Mollie Devinney

Pittsburgh Zoo & PPG Aquarium

There is a large body of evidence showing that when we provide children with "O WOW!!" animal and nature encounters, we are doing the single best thing to insure that they have a conservation ethic as adults.

Public aquariums are one of the great places to provide our now largely urban population with such magical experiences, because of the:

- Visual majesty of the displays
- Ability for hands-on experiences, such as touch tanks with stingrays, behind the scenes with penguins, etc. (active participation works *so* much better than passive viewing)
- Up close experiences with charismatic species
  both well-loved (e.g. marine mammals)
  and misunderstood (e.g. sharks)

Aquariums have long used this majesty to impart knowledge. While the typical zoo & aquarium visitor knows more about animals & conservation than the general public, this is not necessarily true of school groups. We have the ability

- To create a wide variety of environments provides opportunity to experience rarely-seen environments up-close
- Show ecosystems in action – many species living together in one environment

Finally, aquariums have something that most terrestrial zoo exhibits do not: NUMBERS!

This provides the opportunity for students, as well as professional scientists, to conduct studies with sample sizes large enough to get meaningful results.

Middle School Science: Do Penguins Like Kids?
The Observer Effect in an Aquarium Exhibit

William Langbauer, Josie Romasco, Katy Antkowiak, & Galit Fydman

Pittsburgh Zoo & PPG Aquarium

Aquariums are great places to teach science, because their exhibits often contain a statistically significant number of animals. The KidScience middle-school program used the Zoo's penguin exhibit to test whether penguins prefer familiar to unfamiliar visitors.

We found that 3 of 4 penguins tested moved closer to a student who had been observing and interacting with it from the visitor space of the exhibit, and away from an unfamiliar student. This may be because penguins associate those humans most familiar to them, the keepers, with food and care. This is a more subtle effect than the observer and habituation effects that are typically observed in primates.

The project was halted prematurely due to breeding season, and will resume when the season is over.

The Mind of a Mollusk: Behavioral Management of Giant Pacific Octopus (*Enteroctopus dofleini*) at the National Aquarium in Baltimore

J. Ramsay & H. Hellmuth

National Aquarium in Baltimore

At the National Aquarium in Baltimore, we are continuously developing new ways to enrich the lives of the animals in our collection. Behavior management through environmental enrichment and training husbandry behaviors has been a part of the care of the giant Pacific octopus for many years. By maintaining this enrichment and training program with each GPO we exhibit, we are able to not only improve the quality of life for our animals, but also can facilitate husbandry and medical procedures, reducing stress on both the animal and staff.

Do Penguins Use Smell to Forage Underwater?

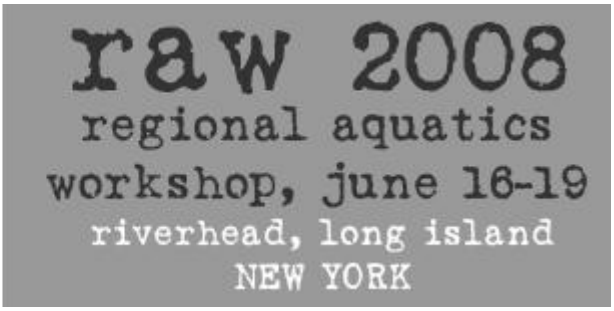
Josie Romasco, Katy Antkowiak, Galit Frydman, William Langbauer

Pittsburgh Zoo & PPG Aquarium

While olfactory foraging strategies are well known for birds of flight (Bonadonna et al 2006), the extent to which olfaction plays a role in penguin food capture is not well know.

We placed in the penguins' pool a permeable cloth sack containing either plain gelatin (control) or gelatin made with 50% fish juice (test stimulus) to test whether penguins preferentially explored either stimulus.

Although the study was halted prematurely due to the onset of breeding season, our preliminary paired weekly trials show that penguins spend more time exploring the test stimulus than the control. This suggests that penguins have the capacity to use chemical senses to forage for food underwater.



Taken from http://www.rawconference.org/ on 1/5/08 (from an email dated 9/25/07):

The new RAW website for 2008 is under construction. However, RAW is all set for 2008. The official dates for the hotel rooms are Sunday June 15th-Friday June 20th. As of now, TAG meetings will take place on Monday, June 16th, with the conference lectures on Tues, Wed, Thurs and Olympics on Thurs.

Hotel space is very limited out in the boonies here, so I ***STRONGLY*** recommend making your room reservations ***NOW***. The hotel is giving me blocks of 40 rooms and once they are taken, they'll give me another 40, etc. The Best Western hotel, http://www.bestwesterneastend.com/, (where the conference will be held) has 100 rooms, and there is one other large hotel (Holiday Inn Express) about 5 minutes from there if needed.

The other reason to get your rooms now is June is the beginning of our summer season out here and the rooms will fill up quickly if we don't grab them, plus there are lots of June weddings which will also take up the rooms.

So, looking forward to seeing all of you but you'll first need to call the Best Western at:

631-369-2200 and reserve your room(s) under "RAW 2008".

We'll be posting more info on the 2008 RAW website soon.
Truly looking forward to it!

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RAW 2008!