DRUM and CROAKER

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(Cover photo by Randy Wilder: Adult *Metasepia* female pulsing hypnotic bands over her mantle)

DRUM AND CROAKER 50 YEARS AGO
(Excerpts from the 1964 issue)

Construction News

From June 1963 to June 1964 we were treated to an unprecedented expansion of the Aquarium - Oceanarium facilities of the United States.

Tacoma, Washington - Cecil Brosseau (Director of the Tacoma Aquarium and Zoo) has put together the finest marine aquarium in the Pacific Northwest. There are about fifty tanks around a central tank some forty feet in diameter. The Tacoma display is limited to the endemic flora and fauna of Puget Sound. Vertebrates and invertebrates are well represented. Utilizing an open, unfiltered system has permitted the maintenance of many filter feeders yet to be displayed elsewhere. Although the open system also creates a tremendous turbidity problem it is one that will be solved shortly and yet permit the keeping of such unusual forms as scallops, feather duster worms, and clams. There are also plans to incorporate a cetacean display. The Tacoma aquarium opened in June 1963 at a cost of about $300,000.

San Francisco, California - Steinhart Aquarium reopened its doors after a complete renewal which closed the Aquarium for fifteen months. Earl Herald's reconstruction and new additions have made Steinhart a new experience from in front of the tanks as well as behind the scenes where most of the improvement dollars were spent. Everything inside the building is new and incorporates the latest materials and techniques. Ultraviolet sterilizers cast their eerie glow over thousands of feet of P.V.C. pipe and valves. For the first time marine mammals have gained access to the classical aquarium and dolphins and seals are displayed in one large tank.

San Diego, California - The world's largest marine oceanarium opened in Mission Bay Park. For details write Mr. Donald Zumwalt, Curator, Sea World.

Honolulu, Hawaii - The world's largest and most complete marine oceanarium opened at Makapuu Point, Oahu.

Niagara Falls Aquarium - Construction of a $1,000,000 aquarium - oceanarium was recently announced. William. E. Kelly, formerly of the Cleveland Aquarium, also announced that the commercial firm, Aquariums, Inc., is backing the Niagara Falls Aquarium, plans to branch out, and will construct several branch aquariums. Construction of the Niagara Aquarium was to begin in July 1964. The new aquarium will include a porpoise tank plus smaller displays of fresh-water and marine life. According to Kelley the displays will extol the natural abilities of the marine inhabitants. A total of fifty displays ore planned for the 30,000 square foot building.

Noteworthy Advances

William Kelly reports that the artificial sea water developed at the Cleveland Aquarium is adequate for the maintenance of invertebrates, i.e. octopus, sea anemones etc., as well as fishes. The cost of manufacturing his formula is only a few cents per gallon.

Statement of Intent Regarding the Coelacanth: *Latimeria Chalumnae*

Earl Herald, Director-Superintendent

Steinhart Aquarium, as part of a recent reconstruction program involving the use of public funds totaling about $1,750,000, has had designed and constructed a special oval tank specifically for display and study of coelacanths. This tank has a capacity of 6500 gallons, and is equipped with two observation windows of 2.5-inch - inch glass, each measuring 5 ft x 5 ft. In addition to the normal turnover as new filtered water comes into the tank, there is a separate system powered by a 4-inch pump, which operates whirlpool circulation patterns in either direction within the tank. The important aspect of this tank is that it works as planned, and in recent months it has provided a variety of habitat conditions for both slow-moving as well as fast-moving fishes. The next problem is to obtain two living coelacanths so that they can be studied in captivity.

BEHAVIORAL CONDITIONING OF THE BROADNOSE SEVENGILL SHARK, (*Notorynchus cepedianus*) AT AQUARIUM OF THE BAY

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Abstract

The broadnose sevengill shark, *Notorynchus cepedianus*, is the only shark in the Order Hexanchiformes to be successfully displayed in aquariums. A variety of training techniques and behavioral conditioning have been used on sharks in captivity to reinforce specific behavior that enhances animal care practices. Although broadnose sevengills are often considered sluggish, primitive sharks, they are a species that has been conditioned to respond to stimuli in captive environments. The Aquarium of the Bay has maintained a large, diverse group of sevengill sharks collected from the San Francisco Bay that vary in age, size, and sex. Over a period of a year, the sharks were conditioned to respond to auditory and visual cues in order to have them accept food with additional dietary supplements such as vitamin C, and voluntarily enter an isolated acclimation pool. This study group also represented an opportunity to test the effectiveness of training techniques on animals in different stages of development that have been in captivity for various time spans.

Introduction

The broadnose sevengill shark, *Notorynchus cepedianus,* is perhaps the most thoroughly studied shark within the Order Hexanchiformes. This shark is readily identifiable due to a combination of unique characteristics such as: seven gill slits, singular dorsal fin, a robust broad snout, a wide mouth, six tooth-rows on the lower jaw, small eyes, and spotted coloration. The broadnose sevengill shark is a distinctly coastal species and has a wide but disconnected distribution throughout the Atlantic, Pacific, and Indian Oceans between 60°N and 59°S (Compagno et *al.*, 2005; Ebert et *al*. 2013). The only conspicuous gap in broadnose sevengill distribution is their absence from the temperate waters of the Eastern and Western North Atlantic as well as the Mediterranean Sea (Compagno et *al.*, 2005; Ebert et *al.*, 2013). Many populations appear to be associated with areas of high biological productivity, such as upwelling zones. They are often caught over sandy or muddy bottoms. However off the coast of the Eastern Pacific, sevengills can also be found in abundance in rocky reef habitats and kelp forests (Ebert, 2003; Stephen et *al.,* 2006). Juveniles and adolescents can be found in bays at depths as shallow as 10 meters (m), while larger sharks frequent coastal bays and estuaries where they move inshore or offshore with incoming and outgoing tides. In offshore waters *N. cepedianus* are usually found near deeper channels to at least 136 m (Compagno et *al.*, 2005; Ebert et *al.*, 2013).

In contrast to other cow and frilled sharks, this species regularly inhabits relatively shallow coastal waters throughout its range over the course of the year and also adapts well to captivity. This has allowed researchers to collect significant information on life-history, ecology and behavior of the species from various locations (Ebert 1986, 1989; Lucifora et *al.*, 2005;

Stephen et *al.*, 2006). The broadnose sevengill shark has been successfully displayed in numerous aquariums around the world, and is highlighted at the Aquarium of the Bay in San Francisco, California (CA) due to its importance as an apex predator in the San Francisco Bay and its impact on the local marine ecosystem. The sevengill shark is often considered a primitive species due to its languid behavior and unique but ancient body design. However, this species has been successfully trained to respond to basic operant conditioning (Daly et *al*, 2007). Several shark species have been trained to respond to operant conditioning for the purposes of public feeding shows, animal health procedures, or to help reduce stress during transport and relocation (Sabalones et *al.*, 2004). The main purpose of this project was to station-train a captive group of broadnose sevengill sharks ranging in size, age, and sex to respond to auditory and visual stimuli. By conditioning the sharks to come to specific areas of the exhibit, several goals could be accomplished. The primary goal was to have the sharks voluntarily enter the acclimation pool during animal health procedures. Additionally, sharks could receive extra vitamins and supplements, or medication could more easily be given to a specific individual. This training would also be used to offer the sharks additional food items during periods of increased appetite without the need of another feeding given by divers. This conditioning would also set the foundation for a training program that could be used on other elasmobranchs in the aquarium collection and modified for specific species or situations.

Materials and Methods

Broadnose sevengill sharks are collected in the San Francisco Bay with an institutional collecting vessel, the *Blue Shark,* using a hook-and-line method. The sharks are caught with non-barbed hooks and placed in a flow-through deck tank supplied by San Francisco Bay water. During transport back to the aquarium, additional oxygen is added to the water to help sedate the animal and reduce stress; the dissolved oxygen (D.O.) concentration is carefully monitored during the transport, and adjusted as needed based on D.O. fluctuations and the shark's condition. Once at the aquarium, sharks are moved from the boat to a quarantine area in a transport stretcher where they are acclimated to aquarium water and placed in a temporary acclimation pool. The sharks' behavior and condition in the acclimation pool are closely observed by staff biologists before they are released into the 360,000-gallon Offshore Tunnel supplied by filtered San Francisco Bay water. A minimum of two staff divers are present in the exhibit when a shark is released to assist the sevengill as it adjusts to navigating the exhibit, and to further monitor its condition. When the shark appears to be navigating well and its stress level has decreased, the divers exit the exhibit.

A total of nine sevengills were on exhibit at the beginning of the training, five males and four females. The sharks ranged in size and development from juvenile to fully mature specimens, and depending on the shark, had been in the aquarium collection for a three month to ten years. The sevengills are usually tong-fed twice a week by staff divers in the exhibit, as part of a scheduled feeding show, for a period of no longer than thirty-minutes. This feeding program represented the initial foundation for the animal training program since the sharks were already exposed to a form of behavioral modification. Basic methods involved in behavior modification typically include using some form of stimulus (visual, auditory, olfactory) to drive a subject's response toward reinforcing a certain targeted behavior. Operant or instrumental conditioning is a type of training that develops a learning association through the use of cues, or stimulus. A subject's behavior is modified by reinforcing actions that follow the cues in order to generate a desired or avoid an undesired response (Ramirez, 1999).

When designing the animal training program for these sharks, the S.P.I.D.E.R method, a six-step process recommended by the American Zoo and Aquarium Association (AZA), was implemented: **S**et goals, **P**lan, **I**mplement plans, **D**ocument results, **E**valuate results, **R**e-adjust (Sabalones et *al.*, 2004). As previously mentioned, the goal of this program was to have the sharks come to specific areas of the tank by responding to auditory and visual cues. Eventually, this training would allow the sharks to voluntarily enter the acclimation pool during animal health procedures instead of using staff divers to herd them into the pool with nets. This behavior would be reinforced by presenting food to the sharks at a specific station.

The planning phase included assembling materials for the training, setting a timeline, and establishing a means of assessing response to the training. First, a 16' section of 1" PVC pipe was modified into a retractable feeding pole that could reach the bottom of the exhibit from a catwalk running along the length of the Offshore Tunnel. A 12" neon green equilateral triangle made from semi-rigid plastic was secured to the feeding pole as a visual cue. Then a two-foot long lead pipe was drilled and outfitted with a carabineer so it could be secured to the side of the catwalk and partially submerged in the water. This pipe would be struck by a large metal wrench in order to generate an auditory cue. The first station would be set at the same location as the underwater shark feeds in order to more easily acclimatize the sharks to being fed from a pole instead of tongs. As the sharks responded favorably to the training, the station would periodically shift several feet further down the catwalk, closer to doors leading into the acclimation pool. The final station would be at the doors to the acclimation pool where the sharks would be trained to follow the feeding pole up the water column and through a narrow causeway into the pool itself. The feeding pole would then be used to help guide the sharks back out of the pool, where they would receive further positive reinforcement as they exited.

The training plan was implemented during the end of October 2012, during a period of seasonally-high sevengill feeding activity. The first year of training would be considered the study period. The sound produced by striking the pipe with the wrench would serve as a start-of-session cue, and would occur at a rate of one strike per-second for thirty-seconds prior to placing the visual target/feeding pole into the water. The sound cue would continue to be initiated for five to ten seconds every minute throughout the feeding session, and every time a shark successfully took food from the pole to act as a bridge between the noise, the target, and food (Sabalones et *al.*, 2004). In order to discourage other animals from also responding to the feeding cues, and to better gauge each shark's response to the training, every feeding session would be limited to a maximum of fifteen-minutes. Training sessions would take place three to five days weekly.

After each session, the sharks' response was documented on a log sheet and rated by how quickly they came to the feeding station and how many pieces of food they consumed. Responses were given a behavioral score between zero and four, with a score of zero representing no response from the shark and a score of four signifying a discernibly successful response (Table 1). The behavioral scores for each shark were averaged at the end of every month. Graphs of average monthly scores with regression analysis over the year were created for each shark. Graphic details of average monthly behavioral scores for the entire group were also generated. Finally, graphic analysis of 'successful responses' (a behavioral score of 4) throughout the year were created. Care was taken to not over-feed the sharks so they would still respond to the twice-weekly underwater feeding shows. The sevengills were usually offered sardines (*Sardinops sagax),* pacific saury (*Cololabis saira*), and herring (*Clupea pallasii*) during the feeding sessions. Since anecdotal evidence suggests 'mega-dosing' of vitamin C may help elasmobranchs heal minor wounds at a faster rate (M. Dvornak, personal communication, November 12th, 2013). To test if this would help heal rostrum abrasions, all food items were supplemented with 1000 mg vitamin C pills.

Table 1: Behavioral Scoring for *N. cepedianus* operant conditioning.

Measured Response Score	Description
0	**No signs of interest in food or response to audio/visual stimuli.**
1	**Shark did not appear to respond to stimuli or showed slight interest over 15 minute time span; incidentally took food item for pole.**
2	**Shark seemed to eventually respond to stimuli, took multiple items from feeding pole or stayed in general feeding area for extended period of time attempting to find food.**
3	**Shark appeared to show discernible response to stimuli and came to target within 5-10 minutes of start cue, took multiple food items during session.**
4	**Shark showed clear response to stimuli and came to target within the first two-three minutes of session, took several items during feeding time, voluntarily entered acclimation pool.**

Adjustments to the training were made as the station location progressed along the perimeter of the tank toward to the doors entering the acclimation pool. The green plastic triangle was removed because it created drag in the water and made the feeding pole less effective; in some cases it actually interfered with the shark obtaining food. The feeding technique was also modified as the stations shifted. Sharks were trained to either feed at the surface or follow the feeding pole up through the water column before being allowed to take the food item (Figure 1).

When the sharks started training at the station leading into the acclimation pool, modifications to the feeding technique were implemented due to the obstacles presented by the doors leading to the acclimation pool and the narrow causeway between the pool and the main exhibit (Figure 2). When the sharks began entering the pool, further adjustments to the feeding technique were made to help guide the animals back out of the pool without elevating their stress level while simultaneously providing positive reinforcement (Figure 3).

Results

Seven of the nine sevengill sharks began taking food from the feeding pole during the first month of training, but the average scores were low, with all sharks receiving averages of less than two (Figure 4). All nine sharks in the collection took food from the pole during the second month of the training, with five of the nine sharks maintaining similar or better averages than the previous month. Due to the increased response during the second month of training, the station was shifted several feet closer to the doors leading into the acclimation pool at the end of December 2012.

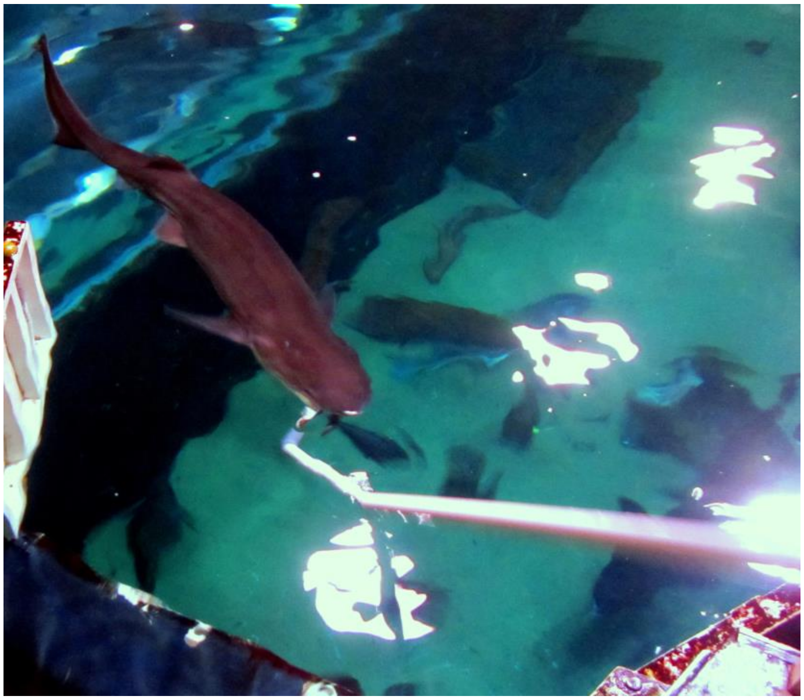


Figure 1: Conditioning shark to feed at the surface.

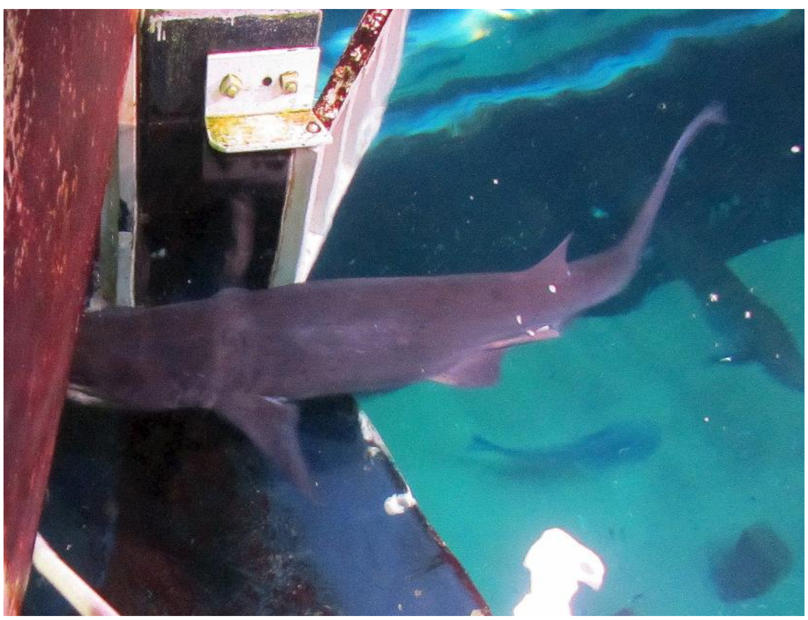


Figure 2: Shark voluntarily entering acclimation pool.

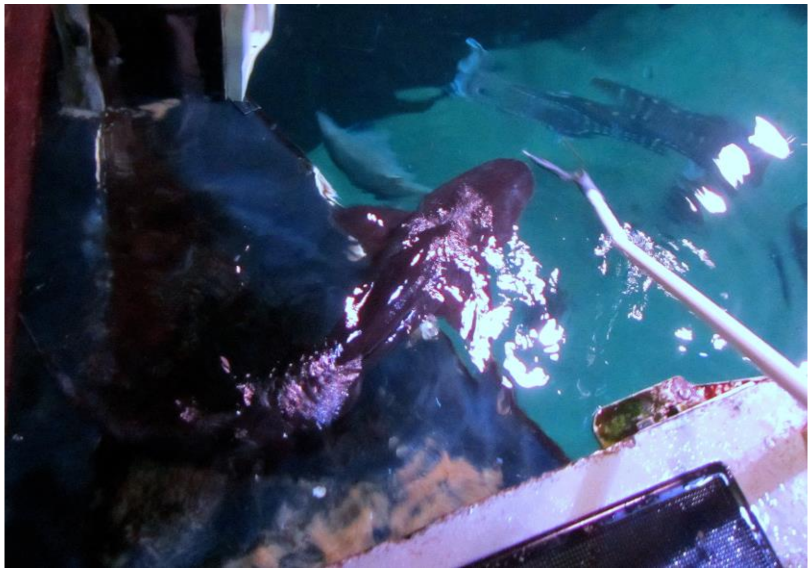


Figure 3: Shark is reinforced upon exiting acclimation pool.

The sharks initially continued to respond to the auditory cue by going to the first feeding station, but they quickly adapted to the second station and showed no appreciable decrease in behavioral scores. During the first month of training at the new station, two sevengills had what was considered a successful response, and received behavioral scores of four (Figure 5). This trend continued throughout February of 2013, so the station was shifted once again at the beginning of March. All of the sharks did not respond as readily to the third station. The behavioral score of five sharks decreased in March of 2013, with one shark not responding at all. Only one successful response was recorded during the first month at the third station. Although the trends remained variable in April it was decided to begin training at the final station, the entrance to the acclimation pool beginning in May. Many of the sharks' average behavioral scores remained approximately the same when introducing them to feeding near the entrance to acclimation pool, but four sharks did show successful responses on more than one occasion during May and June.

In July, the sevengills began voluntarily entering the acclimation pool during the training sessions. Seven of the nine sevengill sharks in the collection responded successfully during sessions and entered the pool at least twice over the course of the month. The average behavioral scores of the seven sharks consistently responding to training also increased to between two and a half and four. The average monthly scores from August-October remained variable among the group, but all seven sharks that began entering the acclimation pool in July continued to do so on multiple occasions. By September, during a typical fifteen-minute training session, an average of three sharks would voluntarily enter and exit the acclimation pool. In September and October, an eighth shark began to successfully respond to the training also, and entered the acclimation pool multiple times during the month of October.

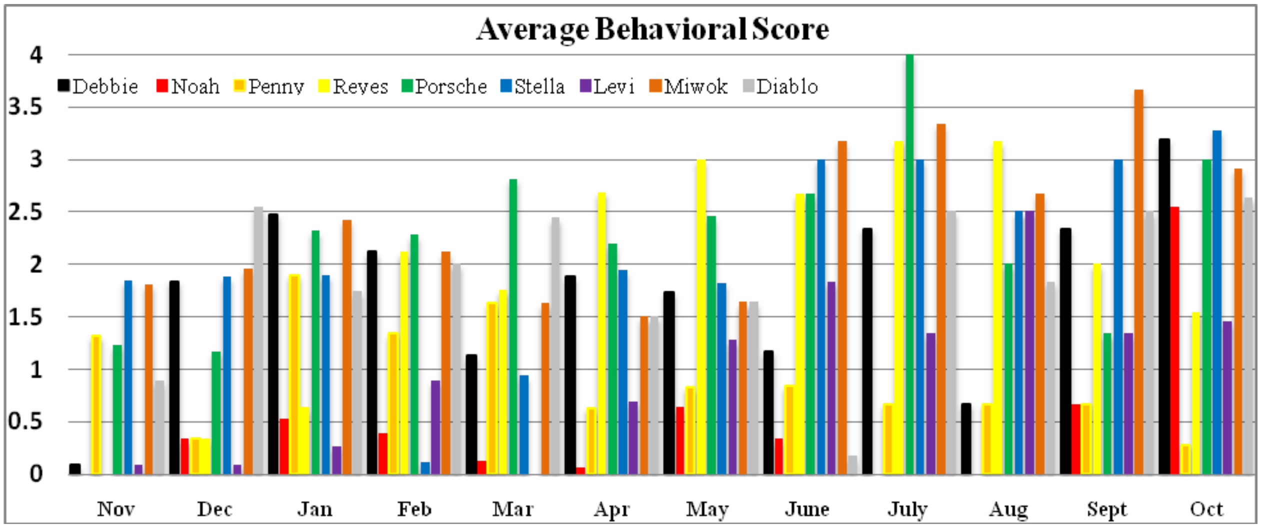


Figure 4: Average Monthly Behavioral Score for Nine *N. cepedianus* at Aquarium of the Bay from November 2012 to October 2013.

Interpretation of the data gathered on average behavioral scores showed no empirical evidence to suggest neither males nor females responded faster or more consistently to the training. Furthermore, regression analysis showed no significant correlation in any individual shark's response (Figures 6.1-6.9). When examining the successful responses of each shark, no appreciable difference could be found between the males and females in the study group. Additionally, time in captivity did not appear to have a clear impact on training response.

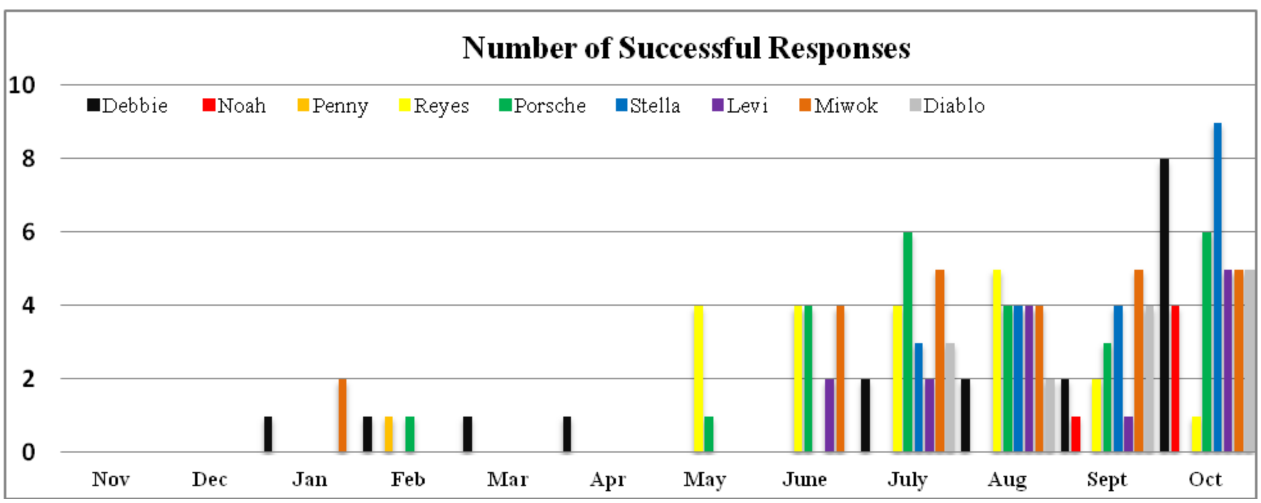
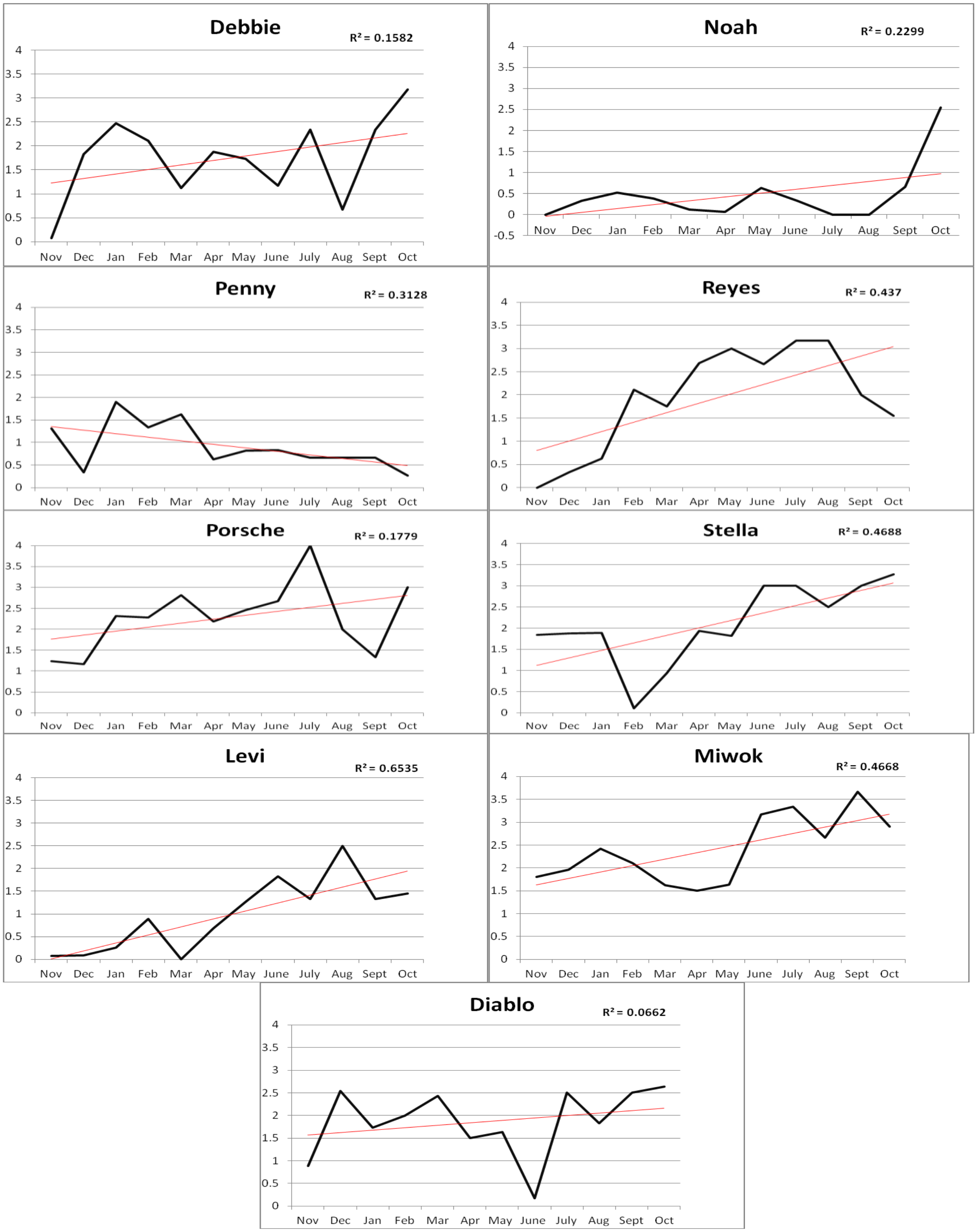


Figure 5: Number of Successful Responses to Behavioral Conditioning of Nine *N. cepedianus* at Aquarium of the Bay from November 2012 to October 2013.



Figures 6.1-6.9: Average Monthly Behavioral Scores for Individual *N. cepedianus* at Aquarium of the Bay with Regression Analysis

Discussion

Although the green plastic target representing the visual cue was removed from the feeding pole in the beginning stages of the training, it did not seem to have an impact. The sevengills would often approach the feeding pole and attempt to take food from it even when no food was present; on two separate occasions two different sharks followed the empty feeding pole into the acclimation pool, suggesting the pole itself represented a sufficient visual cue. Feeding sessions became progressively shorter as time went on and successful responses increased. Decreasing the session time by several minutes became advantageous due to growing interference from other teleosts and elasmobranchs in the exhibit.

The current group of broadnose sevengill sharks in the Aquarium of the Bay collection appears to have responded favorably to the operant conditioning in this training plan. However, by the end of the year-long study period, no sharks had been brought into the acclimation pool for the purposes of performing an animal health procedure or release. Several variables must also be considered when measuring the success of this program, and due to the low sample size in this study group any statistical analysis would result in low confidence. Although there was no observable difference in the response of males or females to the training, three of the sharks that had consistently high average behavioral scores and successful responses can be considered developing adolescents based on previous research on size-at-age (Van Dykhuizen and Mollet, 1992).

Studies have also shown sevengills sharks are intermittent feeders that have short periods of highly active feeding followed by longer periods of reduced predatory activity. This activity also appears to vary with body size due to decreasing growth rates and possible slower metabolic activity as the sharks grow larger (Braccini, 2008). Because the three previously mentioned sharks are in a period of increased growth and development, their feeding activity may not accurately reflect how mature individuals would respond to the training. The three sharks that received the lowest average behavioral scores and lowest number of successful responses over the study period were all mature adults, two males and one female. Additionally, another shark that received both lower behavioral scores and successful responses was a smaller juvenile male. Although this particular shark seemed to respond more consistently toward the end of the study, it was also easily disturbed by other tank mates of similar size during the training sessions. Variations in responsiveness to the training on days immediately following the scheduled diver feeds was also not analyzed.

Anecdotally, sharks that had only been in captivity for a few months seemed to respond better to the training than those that had been in the collection for years, but other factors may be influencing behavior. For example, adult male sevengills are believed to have increased activity levels prior to mating season (Lucifora et *al.*, 2005). The Offshore Tunnel is subject to seasonal water temperature-fluctuations, and reproductive behavior has been observed between adult sevengills in the exhibit. The increased response by two of the three mature males in the collection during the summer and fall months may be directly related to reproductive activity. Similarly, the largest sevengill in the collection, a mature female, has been periodically examined with an ultrasound since the spring of 2012 to monitor follicle development. This female was one of the first sharks to respond to the training, has maintained a relatively high average behavioral score, and has had a high number of successful responses. Although this shark is no longer devoting metabolic energy toward somatic growth, its increased appetite compared to other adults may be directly related to the extra metabolic demands of follicle growth.

The secondary goal of providing additional vitamin C to help mitigate or more quickly heal rostrum abrasions, a common problem when keeping this species in captivity, has yet to be validated or refuted. While some sharks have shown minor improvement with rostrum abrasions, others have shown no improvement or slightly worsening conditions. Providing the sharks with additional feeds during periods of heightened feeding activity appears to have lessened the number of incidental bite wounds and predatory activity on tank-mates. The effectiveness of this training will also be challenged as the water temperatures cool over the winter and spring months and the sevengills' appetite decreases. As new sevengills are collected and added to the Offshore Tunnel, they will be included in the operant conditioning process however they will be trained to immediately respond to stimuli at the acclimation pool doors and reinforced to voluntarily enter the pool.

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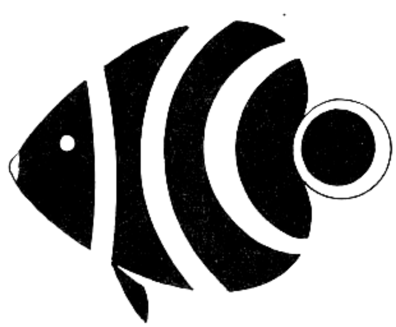

THE CREATION OF THE WORLD'S FIRST PEANUT BUTTER AND JELLYFISH

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"Man cannot live by bread alone, he must have peanut butter"
-Bill Cosby

Introduction:

The science and art of aquaculture has advanced tremendously in the past few decades. Nowhere is this more evident than in the production of the most delicate of invertebrates, now commonplace at zoos and aquaria worldwide. The aquarium maintenance and culture of zooplankton, especially gelatinous zooplankton, has always been notoriously challenging (Baker, 1963), but great strides have been made in the husbandry of these delicate animals in recent decades (see Caughlan, 1984; Norton, 1993; Gershwin-Nelson and Schaadt, 1995; Raskoff et. al. 2003; Widmer, 2008; and AZA, 2013).

The use of novel feed items in culturing aquatic or marine animals has always been desirable for a variety of reasons; primarily due to economic concerns and the convenience of having a stable, reliable food supply. An overview of alternative protein sources for aquaculture is presented in Lim et. al. (2008) and Gatlin et. al. (2007); but a huge body of literature exists on the topic and is beyond the scope of this paper. The general biology and physiology of invertebrates differs so radically from teleost fishes that novel approaches and "outside the box" thinking is often required for success closing the life cycle or rearing juveniles to adulthood. In one of the more interesting attempts at finding inexpensive protein sources for *Octopus* and *Sepia* culture Lee et. al. (1991) reported on the palatability of chicken, surimi, and even turkey hot dogs! The question of sourcing non-traditional protein sources for use in aquatic animal feeds reaches beyond economics and convenience, and in recent years has become a focal point to increase the overall sustainability of aquaculture. It has been increasingly evident in recent years that the use of many fish or shrimp-based protein sources in aquaculture is a wholly unsustainable practice (Naylor et. al., 2000, Naylor et. al., 2009) which begs the need for novel solutions.

That having been said, we would love to claim we conducted this trial with noble purpose, but the truth is that we just wanted to make peanut butter and jellyfish simply to see if it *could* be done. Whether or not it *should* be done is a question no doubt to be debated by philosophers for the ages (or at least by some aquarists over beers). We herein report on what we believe to be the first known unholy amalgamation of America's favorite lunchtime treat and live cnidarians. The success of our trial group of *Aurelia* on this experimental diet was surprising, and we hope this ridiculous experiment illustrates that unconventional approaches in husbandry are at the very least, worth trying once.

Methods:

A group of approximately 250 *Aurelia aurita* ephyrae were collected immediately after strobilation and placed in a 4L improvised plastic pseudokreisel (PET plastic goldfish bowl) on a water table with recirculating artificial seawater (18°C, 28-30‰). A stock solution of 45ml (as 3 tbsp.) creamy peanut butter (free of preservatives and corn syrup) was emulsified in 300ml seawater using a 16,000 RPM blender. This stock solution was kept refrigerated and used for several days before being refreshed. These young jellies were fed 2 ml of the emulsified peanut butter solution twice daily for a period of 5 weeks. They were initially offered some supplemental *Artemia salina* nauplii (48hr+ Instar II-IV) that were enriched (via gut-load) with the same peanut butter solution described above, but this was discontinued to evaluate the potential of peanut butter as a sole food source. Approximate measurements were determined from high resolution (15 megapixel) scaled photomicrographs of a haphazardly selected subset of jellies during the trial.

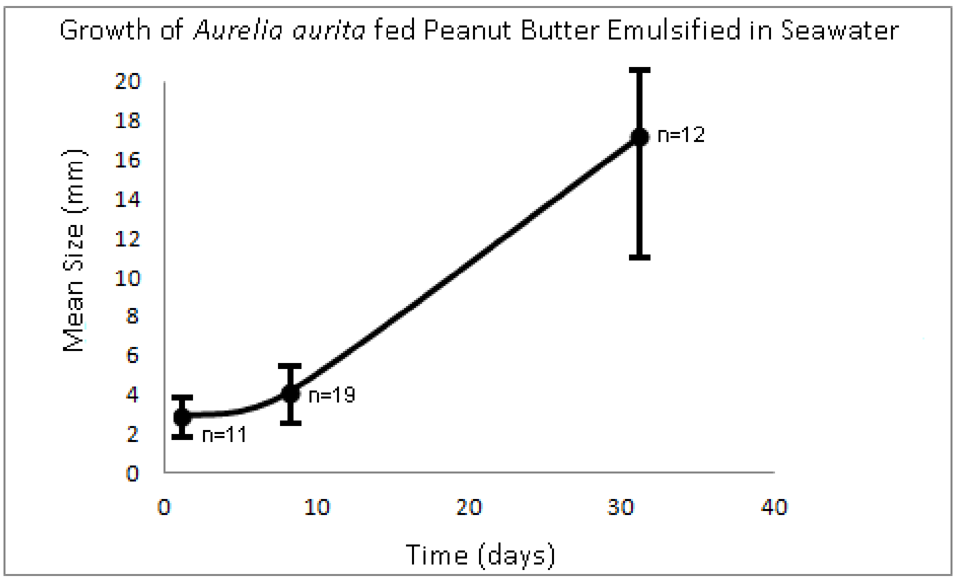

Figure 1: Growth of *Aurelia aurita* fed almost exclusively peanut butter emulsified in seawater over one month. Error bars indicate standard deviation, sample sizes were extremely limited, and are indicated next to each average size value given.

Results and Discussion:

Mean size was determined to be 2.86±0.55mm (n=11) just after strobilation. Mean size had increased to 4.17±1.06mm (n=19) after 8 days of peanutbutterification. By day 31 the jellies had attained a mean size of 17.17±7.44mm (n=12). Growth is plotted graphically in Figure 1, and representative photographs are shown in Figure 2(a-f). The measure sizes amount to an average growth of 542µm day^{-1} over the study period. Throughout this period it was noted that jellies that had recently fed displayed a distinct brownish hue owing to their high degree of peanutbutterocity (see fig 2b). Since this look at the peanutbutteriliciousness of *A. aurita* was done solely as a preliminary (i.e. half-hearted) attempt to evaluate the nutrition potential of peanut in cnidarians diet (i.e. just to see if something crazy would work), we did not properly control the experiment with a group fed a normal diet. The growth did appear, at least anecdotally, to be on-par with normal *A. aurita* outgrowth, though this was not intended to be a definitive examination.

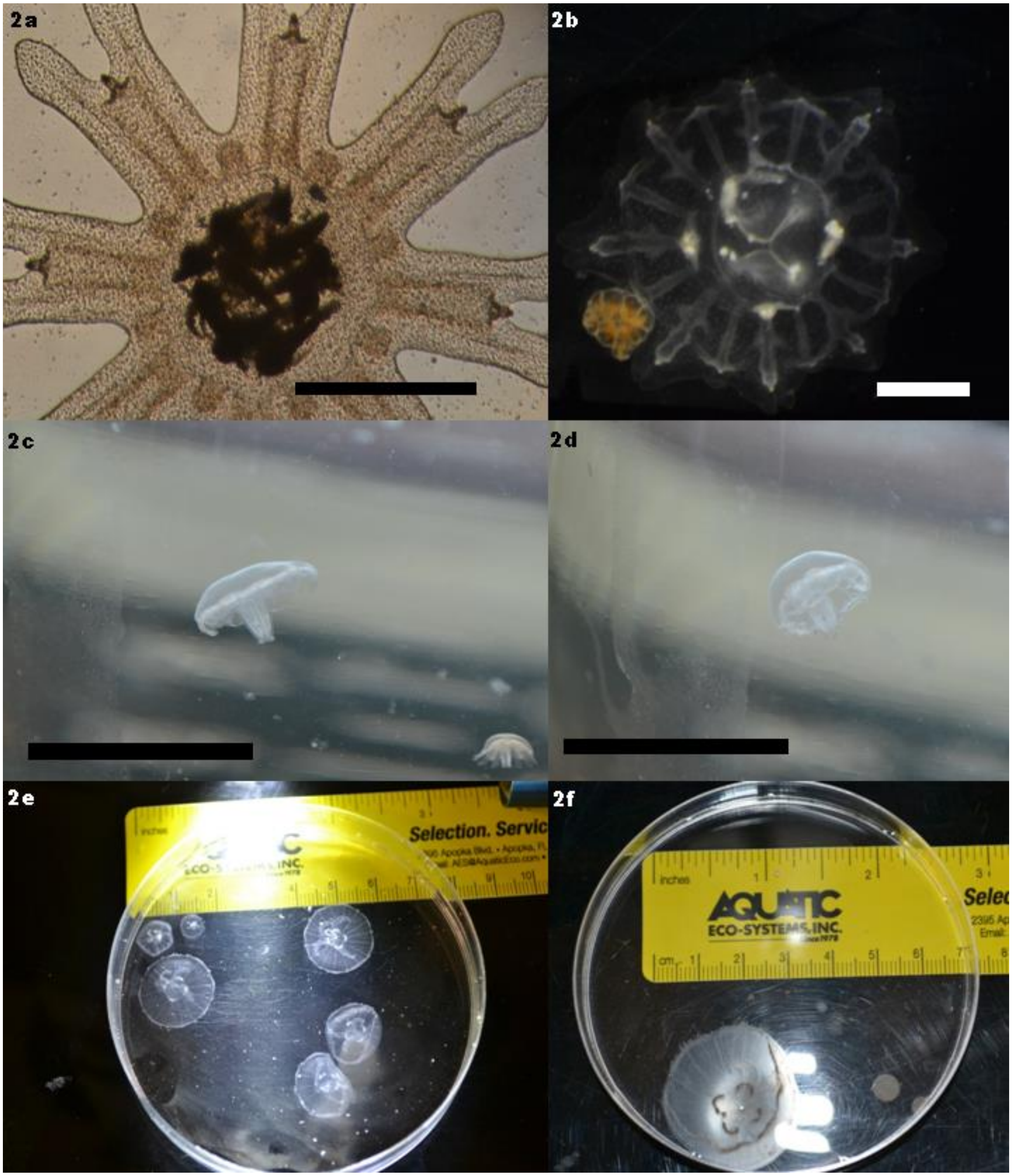

Figure 2. Progress in the outgrowth of *Aurelia aurita* on an emulsified peanut butter diet. 2a. *A. aurita* ephyra at beginning of experiment (day 1), just after strobilation. Scale bar is approx. 1mm, magnification 250x, note that the specimen had recently fed on peanut butter enriched *Artemia* nauplii- this was discontinued after the first several days of feeding in favor of a pure emulsified protein strategy. 2b. A pair of *A. aurita* specimens on day 8 of the trial, magnification 100x, scale bar is approx. 1mm. Note the color of the (contracted) specimen on the left, this specimen has recently fed and is full of creamy goodness. 2c & d. *A. aurita* specimens swimming on day 16, scale bar is approx. 1cm. 2e. A representative sample of peanut butter and jellyfish after one month of being fed the experimental diet. 2f. The largest specimen after one month of peanutbutterification

Peanut butter holds potential in invertebrate aquaculture, in this case the endeavor was undertaken for the sake of frivolity, though this whimsical exercise is not that far from serious aquacultural research. In recent years peanut meal has been evaluated as a potential additive to finfish diets (e.g. Allan et. al., 2000; Zhou et. al., 2004), and has been shown to be an effective surrogate for fish protein (12% dietary peanut meal in lieu of up to 20% animal protein) in the culture of decapods shrimps (Lim, 1997).

In closing, moon jellies have seen a storied past. They have delighted children at aquaria worldwide, captivated researchers with their elegant simplicity and functionality, and even traveled into space (Spangenberg, 1994); but we feel that becoming one with peanut butter helps them fulfill their ultimate destiny as a species – to become peanut butter and jellyfish!

Acknowledgements:
The authors wish to thank Julia Davis Chandler, who introduced America to the peanut butter and jelly sandwich in 1901. Thanks to Mr. Peanut are of course in order, as well as our undying gratitude to that dancing banana on the internet. Yes dancing banana, it is indeed peanut butter jelly time, peanut butter jelly time. Finally, we wish to thank Harold Burnett Reese, without whom the world would have been a darker, more inhospitable place.

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OVARIECTOMY OF SUB-ADULT SOUTHERN RAYS (*Dasyatis americana*) AS A TOOL FOR MANAGING AN EXHIBIT POPULATION

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The Southern ray, *Dasyatis americana*, is a large batoid that is commonly displayed in public aquariums. In both aquariums, and in the wild, female Southern rays are reproductively fecund and can produce 2 to 10 pups a year in one or two litters. Female Southern rays are seldom, if ever, not gravid[2] as they are impregnated within hours to days of parturition. Because Southern rays are constantly pregnant the uterus never involutes and villus trophonemata do not atrophy. As a result the trophonemata continue to produce thick mucoid histotroph and the uterus remains dilated. It appears that Southern rays are induced ovulators; therefore copulation must occur in order to stimulate hormonal signals for ovulation. If copulation does not occur, the mature follicles are not ovulated and remain on the ovary- irrespective of previous ovulations.

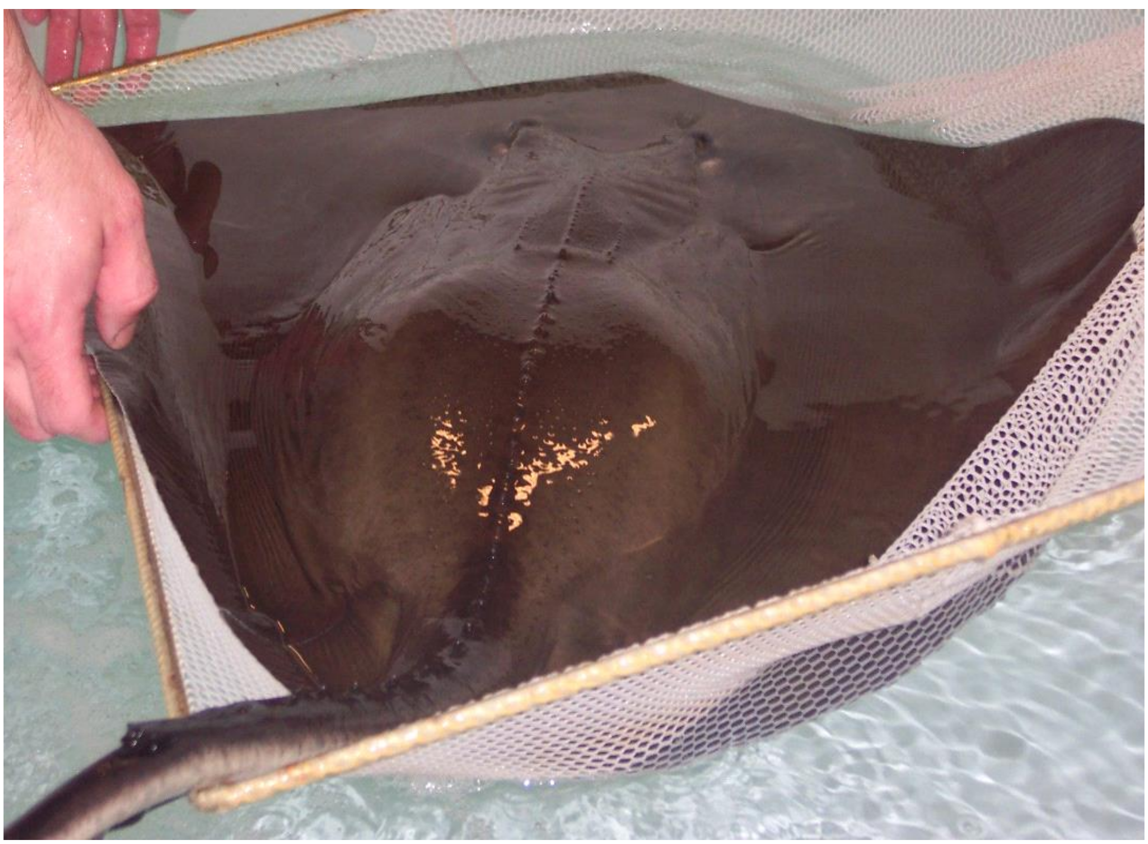

Figure 1: A female adult Southern ray displaying outward signs of mucometra with a distended abdomen area.

To decrease the overproduction of rays in captivity many aquaria have resorted to single sex exhibits that are inhabited by large adult female rays. When there are no males to induce ovulation and pregnancy in the female ray, her uterus continues to produce a large amount of histotroph[1]. This accumulation of histotroph leads to a marked mucometra. Female rays that develop this condition will appear distended; however, there are no pups present when the uterus is examined via ultrasound. Ultrasound often reveals a large hypo-echoic fluid filled uterus. Some gray proteinacious particles may be noted floating in the histotroph. When left untreated, this condition will continue to worsen as more histotroph accumulates. Since the ray may become distended ventrally as well as dorsally, they may develop pressure sores when resting on the exhibit floor. If the pressure sores become severe, there may be a rupture of the coelomic wall with salt water intrusion into the coelomic cavity and death. While the mucometra may be symptomatically treated by cannulating the cervix and evacuating the uterus, the fluid accumulation recurs in a few months.

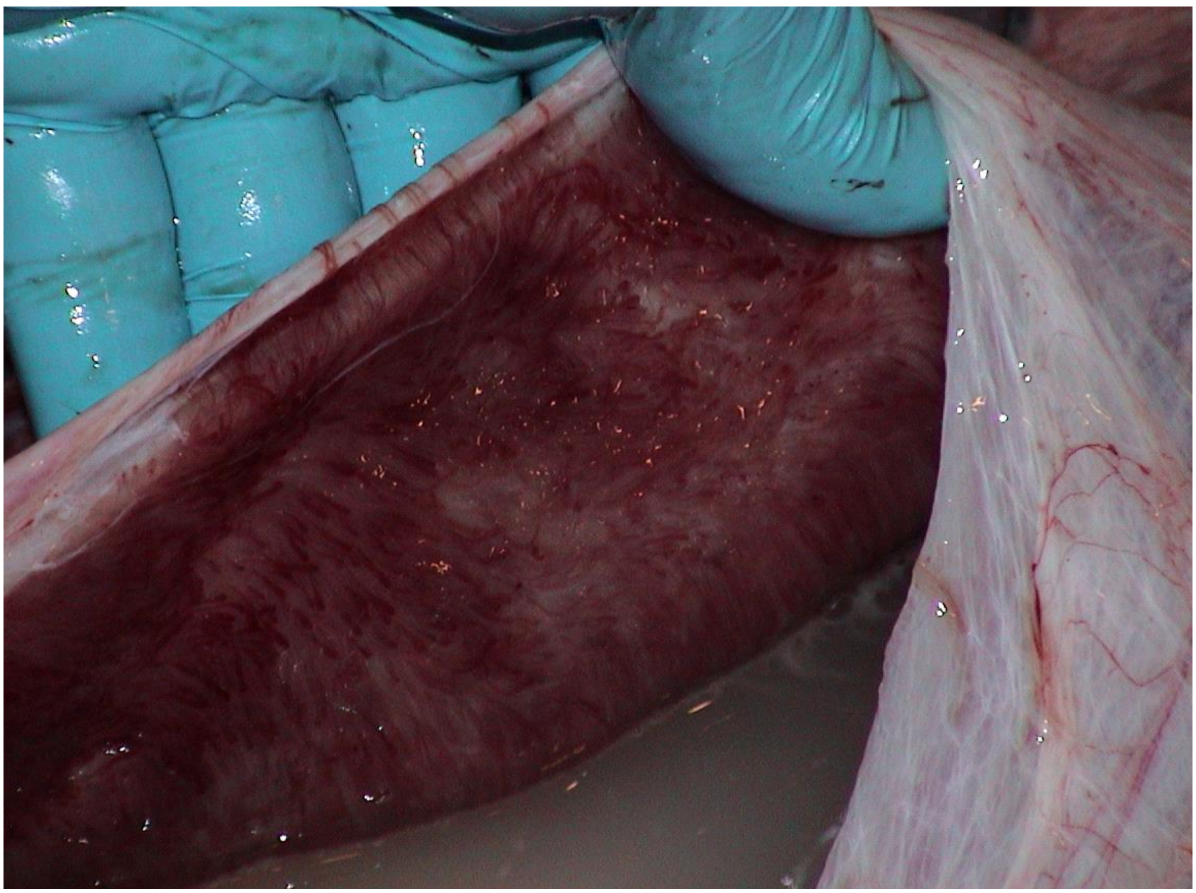

Figure 2: A female adult Southern ray uterus filled with histotroph

Similar to the uterus, the left ovary continues to produce follicles despite lack of pregnancy in the Southern ray. Continuous follicle production without induced ovulation will cause the ovary to accumulate a large number of mature follicles over a period of time. The ovary will secondarily become cystic and can achieve huge dimensions, weighing up to a kilogram. Ovarian tissues are thin and friable, so when ovaries attain such a huge size they are prone to tearing. This can lead to hemorrhage and death.

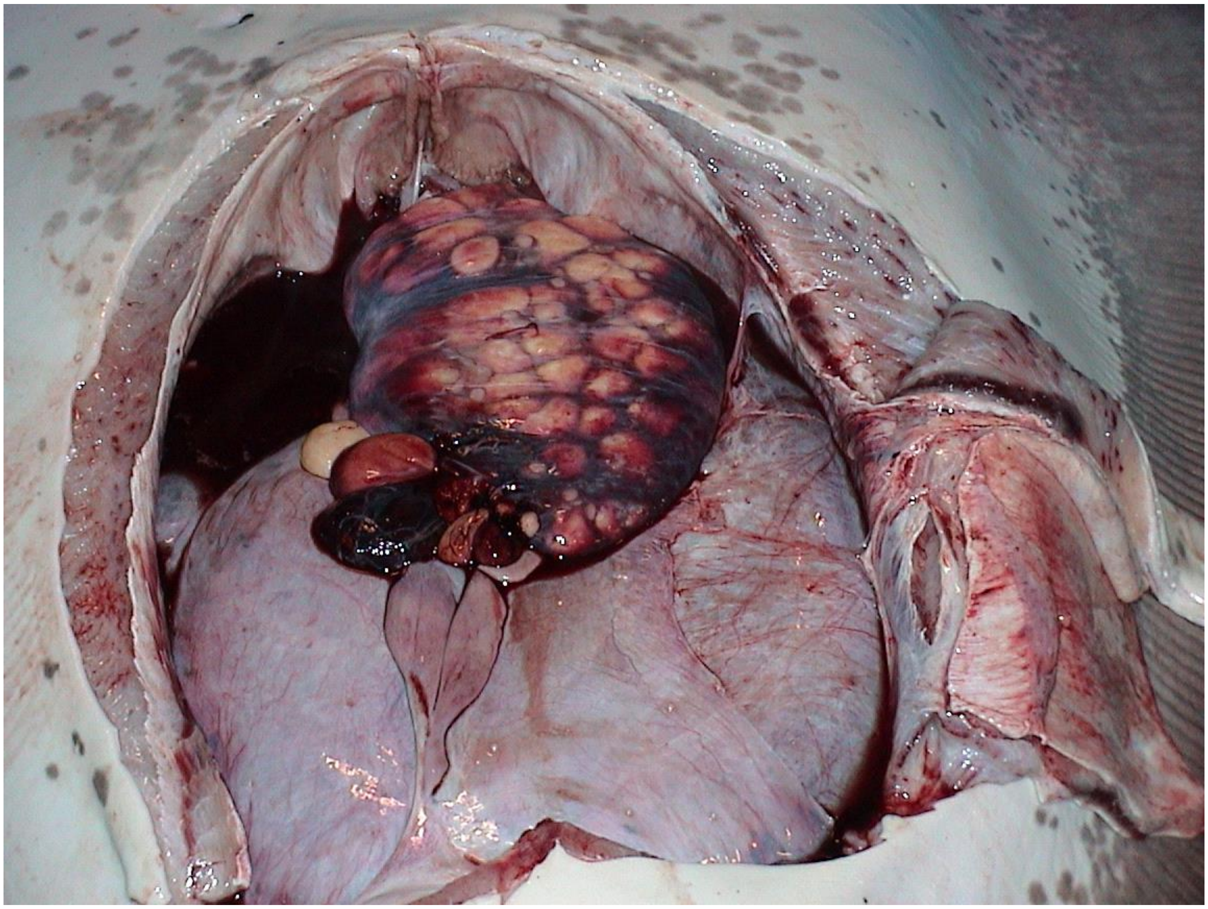

Figure 3: Internal organs of a female adult Southern ray displaying advanced reproductive complications. Note towards the top of the image the enlarged ovary with a buildup of mature follicles.

In an effort to prevent these reproductive complications in a single sex collection, ovariectomy in juvenile female rays was pursued. During the development of this procedure 6 rays underwent successful exploratory laparotomies but were not ovariectomized. A group of 4 additional rays had successful ovariectomies. Patient size was critical for the success of this procedure; an individual that was too large or too small made it challenging to safely complete the surgery. Ultimately, the ideal ray had a disc width of approximately 60 cm. The ovary was too large, vascular, and, as a result, difficult to remove in rays any larger than 70 cm. It was difficult to identify the ovarian tissue embedded in the anterior portion of the epigonal gland in rays less than 50 cm. Ovarian size can be evaluated pre-operatively with ultrasonography to choose the ideal candidate.

The rays were anesthetized and maintained with MS-222 at 75 ppm in a recirculating system. The left para-lumbar area was sanitized with an iodine surgical prep solution. A six to eight cm incision was made parallel to the spinal column and 2 cm lateral to the dorsal lumbar muscles. The skin incision was initiated just caudal to where the fin rays join with the epaxial muscles. After incising the muscle and the peritoneum, the cranial portion of the ovary and the enveloping oviduct were dissected free from the dorsal suspensory ligament, clamped and ligated with a single encircling ligature using 3-O PDS (Ethicon). The thick anterior pedicle appeared to supply the majority of the blood supply to the ovary. A thin suspensory ligament ran along the ray's dorsal mid-line between the ovary and the body wall. It was relatively avascular and was transected with scissors or blunt dissection. Mosquito forceps were applied for hemostasis as necessary. The caudal pole of the ovary was broadly attached to the cranial end of the epigonal gland. A clamp was placed across the epigonal gland caudal to the junction, an encircling suture is placed around the epigonal gland and the ovarian tissue was transected and removed. The peritoneum was sutured with 3-O PDS on a half round taper needle in a simple interrupted pattern. The muscular layers were not directly sutured as the tissues were too weak to hold sutures. The skin was sutured with 3-O PDS on a cutting needle. Care was taken to place the sutures relatively close together and tighter than would be done for mammals as the tissues do not swell. It was important to get good tissue apposition so there is no salt water intrusion that could lead to incisional dehiscence.

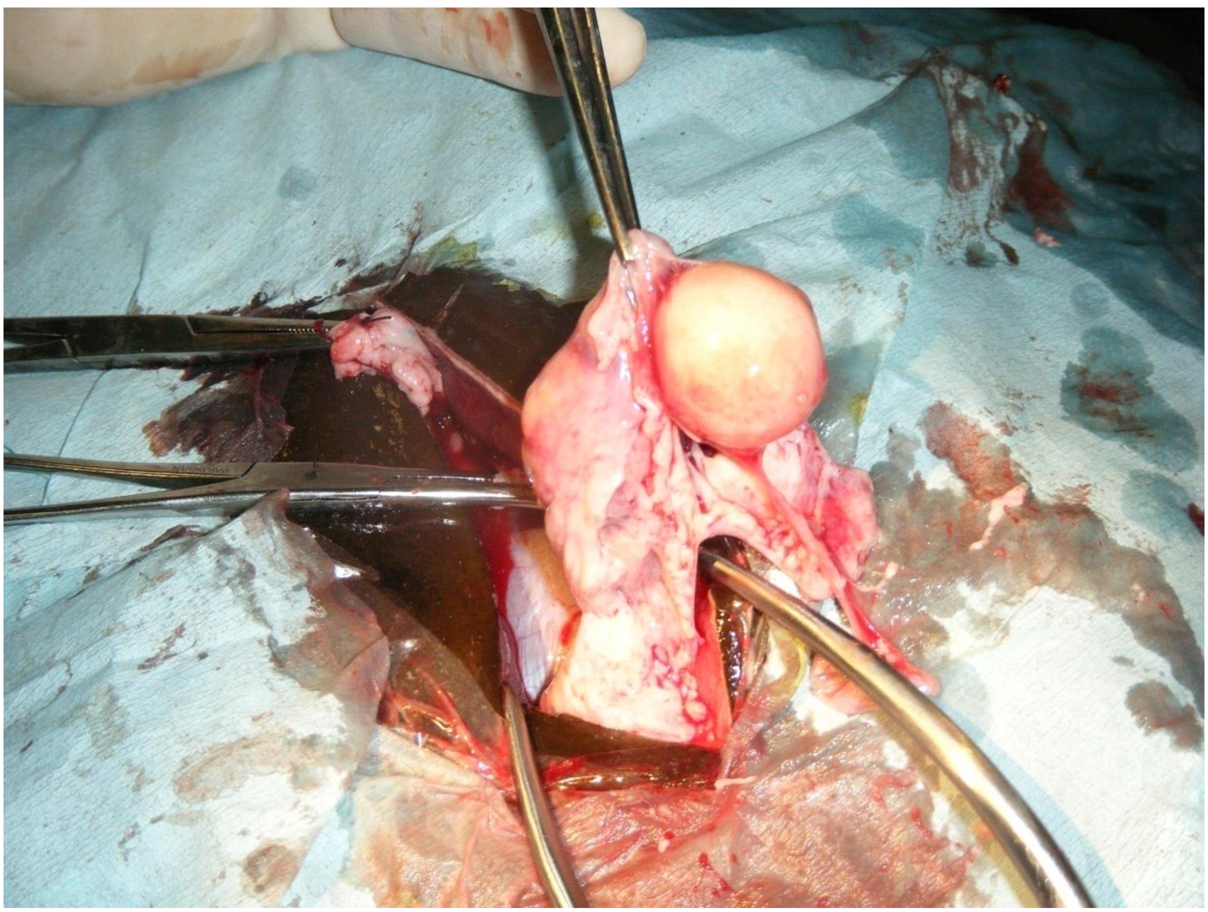

Figure 4: Removal of the ovary

After surgery, the rays were administered ceftiofur at 8 mg/kg (Excede, PFizer) and meloxicam at 0.3 mg/kg (Merial). The rays recovered from anesthesia rapidly and fed within 3 hours of the surgery. Sutures were removed one month after surgery. Other than mild bleeding, no additional complications were noted during and following surgery on any patients.

This technique for ovariectomy in Southern rays appears to be a safe procedure for controlling reproduction and population size in a display exhibit; however; it is too early to tell if removal of the ovary in sub-adult animals will cause any subsequent growth, metabolic, or reproductive problems in the future. Additional hormonal and long-term follow up studies should be performed in the future to determine the long-term safety and side effects in Southern rays.

Acknowledgements

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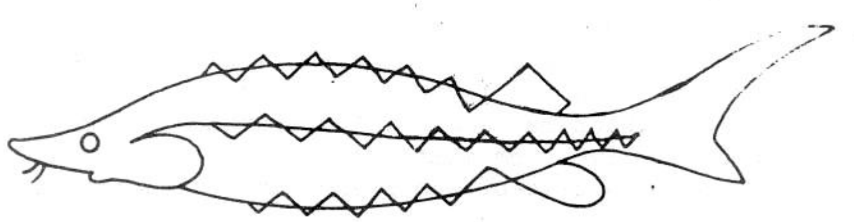

1 – 800 DIAGNOSE MY NAUTILUS

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Introduction

Significant progress has been made into several aspects of *Nautilus* biology including migration, behavior, buoyancy, genetics, and population structure. However, *Nautilus* husbandry and specifically their veterinary care are still misunderstood. Carlson (1987) provided a detailed report of the required methods of *Nautilus* care and it does not appear that these methods have changed significantly based upon current husbandry publications (Barord and Basil, 2014; Barord and Daw, In Prep). This leads to several questions regarding the captive care of nautiluses. *Is current nautilus husbandry perfect? Has progress actually been made but gone unreported? What aspects of nautilus husbandry need to be improved?* While changes in the general husbandry of nautiluses may be difficult to track, veterinary research into nautiluses has been presented in the literature and through anecdotal reports. Nonetheless, the dissemination of these new findings to the parties of interest has been difficult for various reasons. Here, I intend to present a step-by-step key on diagnosing and treating several well-known, and some lesser-known, health problems in captive nautiluses.

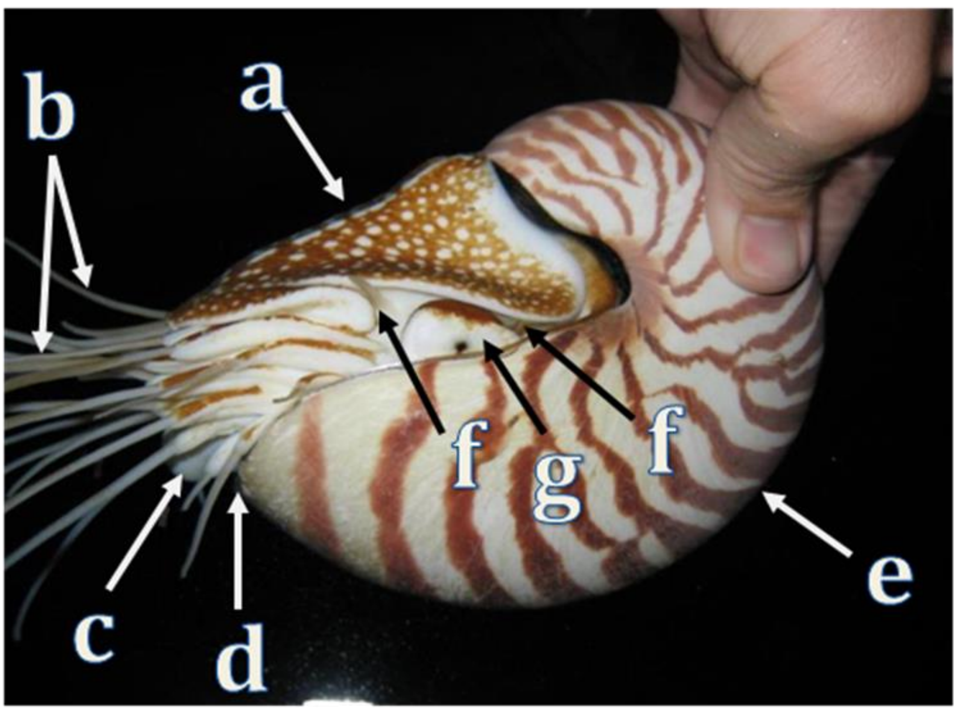

Figure 1: Photograph of captive *N. pompilius* illustrating key anatomical features; a – hood, b – tentacles, c – hyponome (funnel), d – shell aperture, e – shell, f – ocular tentacles, g – eye.

Nautilus Diagnostic Key: The following key addresses some of the major health problems that arise in captive nautiluses. To use the key, start at Step 1 and simply follow the directions at each subsequent step.

1a. Abnormal shell growth	....	Go to Option #1
1b. Normal shell growth	....	2
2a. Shell broken	....	Go to Option #2
2b. Shell intact	....	3
3a. Abnormal buoyancy	....	4
3b. Normal buoyancy	....	5
4a. Positive buoyancy	....	Go to Option #3
4b. Negative buoyancy	....	Go to Option #4
5a. Abnormal behavior	....	Go to Option #5
5b. Normal behavior	....	6
6a. Abnormal diet	....	Go to Option #6
6b. Normal diet	....	7
7a. Mucus production around hood, eyes, or tentacles	....	Go to Option #7
7b. No mucus production around hood, eyes, or tentacles	....	8
8a. Hood color abnormal	....	Go to Option #8
8b. Hood color normal	....	9
9a. Abnormal tissue growth (dysplasia)	....	Go to Option #9
9b. Normal tissue growth	....	10
10a. Bacteria present	....	Go to Option #10
10b. Bacteria absent	....	11
11a. Parasites present	....	Go to Option #11
11b. Parasites absent	....	12
12a. Fungal hyphae present	....	Go to Option #12
12b. Fungal hyphae absent	....	Go to Option #13

Option #1: Abnormal Shell Growth

The shell of captive nautiluses appears to have two distinct types of abnormal growth. The first, and perhaps most common, is the presence of black lines along the new shell growth, (Figure 2). This black material has been referred to as "black shell syndrome" and "black shell deterioration" in the literature. Sherill *et al*. (2002) describe a treatment method for black shell syndrome by scrubbing the shell lightly to physically remove the black appearance. However, the scrubbing did not eliminate the black shell from reoccurring and appears to be a superficial treatment, neglecting the underlying cause. Barord (2007) reported on a year-long alternative treatment of calcium supplementation in the diet of *Nautilus pompilius*. The treatment was based on the assumption that the black shell was a result of a calcium deficiency in the diet of captive nautiluses. However, the increased calcium did not reduce the occurrence of black shell. Currently, there are no known treatments of the black shell appearance though the condition does not appear to cause any negative effects on *Nautilus* health and welfare.

The second type of abnormal growth results in new shell growth that forms large lumps in the shell and is wider than normal shell growth (Figure 3). There have been a handful of anecdotal reports of this type of growth and one treatment option that appeared to have some success eliminating the abnormal growth (Pers. Comm.). In this case, the underlying cause of the abnormal shell growth was presumed to be a result of an improper diet that included too much protein. Subsequently, the protein in the diet was reduced and this appeared to have positive changes in the growth condition.

***Next Steps** – Although these two types of abnormal growth have not resulted in any observable negative effects, careful observation is critical to ensure that no other problems arise. Continue with Step 2 of the key to determine if any other issues exist.*

Figure 2: Black shell appearance on a captive *N. pompilius.*

Figure 3: Abnormal shell growth of captive *N. pompilius* illustrated by "bump" in shell.

Option #2: Broken Shell

Shell breaks are relatively uncommon in captive nautiluses but appear to be very common in wild nautiluses (Figure 4). As wild nautiluses must interact with a dynamic environment with various predators and interactions, major shell breaks occur frequently in the wild. Thus, most shell breaks in captive nautiluses are minor. Minor shell breaks may occur along the aperture of the shell as a result of jetting into hard structures within the aquarium. Another type of minor shell break may result in small, v-shaped breaks along the aperture of the shell which actually might be a result of mating behaviors. Nautiluses have the ability to heal a broken shell given time and the appropriate conditions. The nautilus will first begin laying down a thin, black layer at the edge of the break (as in Figure 4) and this will subsequently fill up the entire broken area. When the shell is completely healed, the black scarring will usually be reduced to a single line where the break occurred (Figure 5). The total healing time of broken shell will take at least 45 days (Meenakshi *et al*., 1974). Shell breaks that occur along chambered portion of the shell may have other side effects as the break may affect the buoyancy of the nautilus and may also lead to other infections. In these cases, while healing was taking place, additional shell material was grafted over the shell break to promote healing while also reducing the chance of any other health problems (Pers. Comm.).

***Next Steps** – Both minor and major shell breaks are usually no cause for concern in nautiluses. During the healing time, observation and pristine water quality conditions are critical to ensuring that no other issues arise. Continue with Step 3 of the key to determine if any other issues exist.*

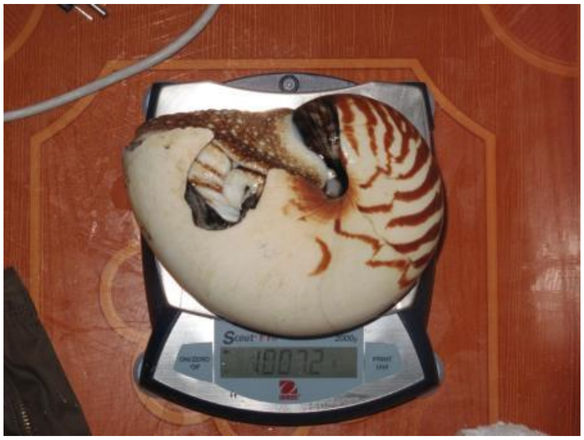

Figure 4: Example of shell break in *N. pompilius.*

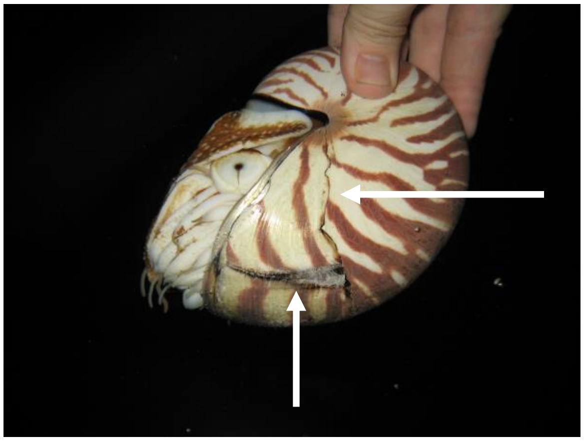

Figure 5: Evidence of shell break repair in *N. pompilius*. The arrow denotes the left over scar from the shell break.

Option #3: Positive Buoyancy

The majority of abnormal buoyancy cases in nautiluses are reported as positive buoyancy (Carlson 1987). Positive buoyancy appears to be most prevalent in newly acquired nautiluses although the causes of this condition are not clearly understood. The assumption is that the internal chambers are lacking cameral fluid in their chambers which is critical for attaining neutral buoyancy. In most cases, a "wait and see" approach is undertaken and given time, positively buoyant nautiluses seem to be able to regain neutral buoyancy within one to six months. The ability of nautiluses to regain neutral buoyancy without intervention suggests that this condition could be related to an internal stress response during shipping. Thus, the nautilus regains neutral buoyancy after being acclimated to the system and new conditions. However, specimens that have been in captivity for extended periods of time and then become positively buoyant may be experiencing something different. In these cases, positive buoyancy still might be from a stress response but the trigger would likely be different than newly acquired nautiluses.

Next Steps *– Although no literature is available to support the "wait and see" approach in positive buoyancy cases, the anecdotal evidence all appears to support this method in newly acquired nautiluses. A modified approach might be required in nautiluses when this arises after being in captivity. In prolonged cases, radiography may be an advisable course of action to determine the levels of cameral fluid in the chambers (Barord and Henderson, 2008). Continue with Step 5 of the key to determine if any other issues exist.*

Option #4: Negative Buoyancy

Negative buoyancy cases are far less common in the literature (Carlson 1987). Barord and Henderson (2008) reported on a single case of a *Nautilus* exhibiting negative buoyancy. In this case, the first step was again to "wait and see" while recording observations and any changes. After there were no changes in the condition, additional steps were taken to determine how to treat the condition.

Next steps *– Negative buoyancy is less common in nautiluses. In prolonged cases, radiography may be an advisable course of action to determine the levels of cameral fluid in the chambers (Barord and Henderson, 2008). The only reported case also found systemic bacterial infections and parasitic infestations. While the "wait and see" approach is important during the first stages of this condition, additional diagnostics may provide evidence of underlying problems which would require immediate treatment. Continue with Step 5 of the key to determine if any other issues exist.*

Option #5: Abnormal Behavior

The behavioral changes of captive nautiluses can be difficult to assess. The normal behavior of captive nautiluses is really a *lack* of behavior. The ability to observe behavioral changes also relies on being able to identify individual nautiluses in a system by their unique striped patterns or by simply marking the dorsal portion of their shell. Although the stereotypic view of *Nautilus* behavior is that they prefer to just stick themselves against the tank or other structures, individual nautiluses may behave different.

Next Steps - *Continue with Step 6 of the key to determine if any other issues exist.*

Option #6: Abnormal Diet

Dietary fluctuations of captive nautiluses may be a more effective means to track nautiluses. Nautiluses will readily accept many different types of food in captivity so tracking any changes in diet should be relatively efficient. Although a change in diet may signify an underlying problem, unhealthy nautiluses may also continue eating normally so it is critical to eliminate any other problems as well.

Next Steps *– Continue with Step 7 of the key to determine if any other issues exist.*

Option #7: Mucus Production

The presence of mucus may be an indication of a potentially fatal mucodegenerative disease. Visible mucus around the eyes or tentacles appears to be the first symptom of this disease and until recently, had resulted in mortality in the reported cases. Barord *et al*. (2012) presented a case study of a single *Nautilus pompilius* that started to produce mucus around one eye and the ocular tentacles (an example of mucus production can be seen in Figure 6). In this case, additional diagnostics were collected to pinpoint what else was going on and what the treatment options were.

Next Steps *– The presence of mucus in nautiluses is a significant condition that can quickly lead to mortality. Additional diagnostics should be performed to determine exactly what type of infection may be present. Methods of sample collection might include skin scrapings, mucus collection, and hemolymph (blood) withdrawal. Barord and Henderson (2008) report on a method to withdraw hemolymph. Continue with Step 8 to determine if any other issues exist before Steps 10-12 for analysis of the samples collected.*

Figure 6: Mucus production around the eye of *N. pompilius*.

Option #8: Hood Discoloration

The most prevalent discoloration of the *Nautilus* hood occurs begins as white spots. The white spots may appear small and transparent when they first appear. The spots may grow larger (Figure 7) and lesions may begin to form where the white discoloration is, resulting in depressions into the hood. There are no reports of this condition or a treatment option in the literature although it is fairly common in anecdotal reports. The one thing that appears certain is that the progression of the white lesions invariably leads to mortality in nautiluses. The lesions can double in size in less than 30 days so effective treatment is critical (Author's Observation).

Next Steps *– Initial topical treatments of 10% povidone appeared to have slowed the progression while additional diagnostic samples could be collected (Author's Obs). Methods of sample collection might include skin scrapings, mucus collection, and hemolymph (blood) withdrawal. Barord and Henderson (2008) report on a method to withdraw hemolymph. Continue with Step 9 to determine if any other issues exist before Steps 10-12 for analysis of the samples collected.*

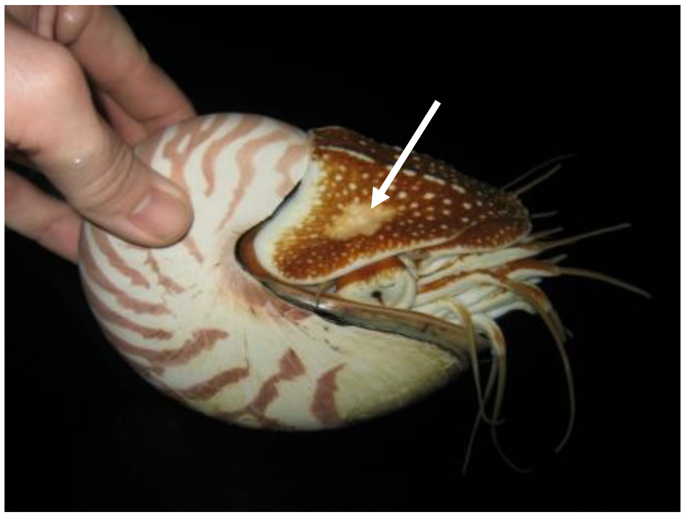

Figure 7: White lesion in *N. pompilius*.

Option #9: Dysplasia

Abnormal tissue growth (dysplasia) has not been reported in the literature for nautiluses. An example of an abnormal tissue growth is shown in Fig. 8. In this case, the growth continued to become larger over the course of observation and had a height of approximately 2cm (Author's Obs.). There were no other observed problems with the nautilus and the growth eventually fell off, leaving a scar of where it had originated.

Next Steps *– When dealing with similar growths, a tissue biopsy might be the best course of action to determine what type of growth it is. Methods of additional sample collection might include skin scrapings, mucus collection, and hemolymph (blood) withdrawal. Barord and Henderson (2008) report on a method to withdraw hemolymph. Continue with Step 10 for treatment options.*

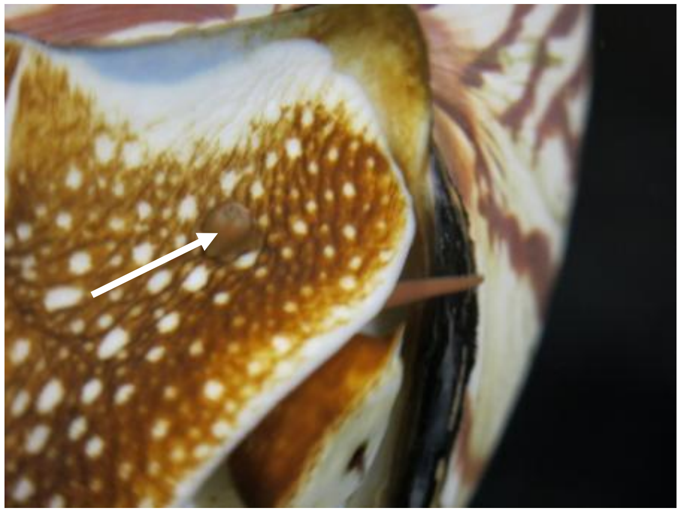

Figure 8: Abnormal tissue growth in *N. pompilius*.

Option #10: Bacteria

Bacterial infections can be present in several different types of diagnostic samples. The proper diagnosis of a bacterial infection will enable effective antibiotic treatment. Barord and Henderson (2008) found bacteria present in both skin scrapings and hemolymph samples. Subsequent antibiotic treatments with enrofloxacin (25mg/kg orally) and oxytetracycline (25mg/l in a bath) were conducted. The oral treatments were difficult to assess given how nautiluses feed so the oxytetracycline baths were conducted next. In both cases, the bacterial infection remained and it was determine that the infection had become systemic and was untreatable at this point. Bacterial infections have also been correlated to a mucodegenerative disease (Barord *et al.*, 2012). In this case, the condition was diagnosed quickly and subsequent treatment of 10% povidone and 25mg/l oxytetracycline baths eliminated the infection and the nautilus survived with no other occurrences. There are other reported treatments of bacterial infections and treatments in the literature but the results and methods are varied (Pers. Comm.).

Next Steps *– Proper diagnosis of bacterial infections should enable appropriate treatment options with specific antibiotics. However, there might also be parasitic infestations or fungal infections that coincide with the bacterial infections. Continue with Step 11 of the key to determine if any other issues exist.*

Option #11: Parasite

Copepods are common parasites of wild nautiluses (Willey, 1897c); Haven, 1972) but they do not appear to be problematic in captivity (Ho, 1980). Other parasites that have been observed in captive nautiluses include protozoans (Pers. Comm.) and nematodes (Barord *et al.*, 2012). It is difficult to determine whether the parasites were the initial problem or if they were secondary to additional infections.

Next Steps *– Although it is difficult to determine if parasites were the primary cause of the health issues, it is important to know whether or not they are present. The presence of the* *parasites may impact additional infections and decrease the likelihood of treatments for bacterial or fungal infections. There are some anecdotal reports of parasite treatments but the results are variable (Pers. Comm.). Continue with Step 12 of the key to determine if any other issues exist.*

Option #12: Fungal

There is one case of a fungal infection in a captive *N. pompilius* reported, however the diagnosis was made post mortem and no treatments were employed (Scimeca, 2006). A second fungal infection was also observed in a living *N. pompilius* and a treatment protocol was initiated (Barord *et al*., Unpub. Data). In this case, the fungal infection was preceded by a white lesion. Samples collected from the lesion site showed bacteria as well as fungus (*Fusarium* sp.). The treatment was adapted from a similar condition reported in the blacktip shark *Carcharhinus limbatus* (Christie and Henderson, 2008). Over a six month period, an antifungal/antibiotic cream was applied to the lesion twice daily. At the end of the treatment the lesion disappeared and no other occurrence was noted.

Next Steps *– Fungal infections may often be associated with other health issues. Definitive identification of a fungal infection is necessary to apply proper treatment. In the lone anecdotal report, the treatment was successful in eliminating the fungal infection.*

Option #13: What now?

If you are at this step then you are out of luck (just kidding of course). There are other health conditions reported in captive nautiluses that are rare with even less information on them. The first seldom seen case was an iridovirus in *Nautilus* (Gregory *et al*., 2006). In this case, the virus was identified post-mortem and no treatment protocols are available. Eye disorders resulting from trapped air in the eye have also been reported (Carlson 1987). This can result from taking the nautilus out of the water or from air stones in the system. These problems are evident by a white mucus-like material in the eye. Gentle pressure on the eye can remove the material. There were no known other side effects of this condition observed.

Conclusions

As you can see, there are some areas of *Nautilus* veterinary care that have improved over the years but there are also several other aspects that need further investigation. It is possible that many answers to these questions have already been studied but may have not been reported in the literature or to other institutions. At this point, negative results are just as important as positive results so that the field can move forward and will not have to reinvent the wheel each time. The importance of continuing to improve the captive care of nautiluses is even more important given the current conservation implications of nautiluses (Dunstan *et al*., 2010; DeAngelis, 2012; Barord *et al*., In Submission). The captive care of nautiluses has already directly impacted research into the population status of nautiluses and will most certainly continue.

Going forward, there are several aspects of *Nautilus* care that could be benefited by future collaborations and investigations. These areas include black shell deterioration, hemolymph analysis, causes of abnormal buoyancy, antibiotic use, as well as many more. If you or your institution is interested in assisting the efforts please send email inquiries to Gregory Barord at gjbarord@gmail.com.

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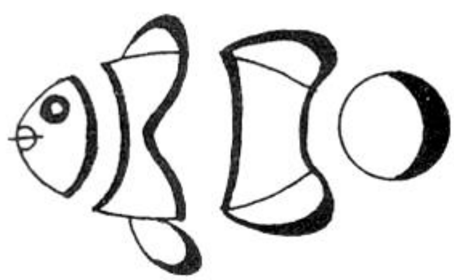

BEHAVIOR MODIFICATION TO ALLEVIATE STEREOTYPIC BEHAVIOR IN A WHALE SHARK (*Rhincodon typus*)

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Abstract

Georgia Aquarium houses four whale sharks (*Rhincodon typus*), two males and two females, in the Ocean Voyager Gallery. One of the males, known as Yushan, developed a stereotypic swim pattern in 2010 that prevented him from utilizing the entire exhibit. Stereotypic behaviors can develop in response to environmental stressors and often include unvarying, repetitive patterns with no obvious goal or function. Yushan's stereotypy has resulted in a virtually complete right-turning swim pattern, in circles of varying diameter, interrupted only to participate in the daily feeds. While the cause of Yushan's behavior is unknown, it became evident that the swim pattern could potentially become detrimental to his physical appearance and long-term health. Indicators such as asymmetrical swimming and dorsal fin curvature developed over a relatively short time period. The Aquarium team developed a training plan to counteract this behavior including additional feeds for enrichment and physical therapy. As a result of this training, Yushan now utilizes a greater portion of the Ocean Voyager exhibit, the diameter of his circles has increased, and his body condition appears improved. Additionally, and as a result of Yushan's underwater feeding sessions, we have obtained updated measurements and blood samples from him while under stimulus control. The Aquarium team continues to work with Yushan to address his current condition, and extinguish the stereotypy, with the goals of him achieving adult size and long-term residency on display.

Observations and Behavior Plan

The primary behavioral goals for whale sharks upon entering Georgia Aquarium included feeding horizontally at their individual stations while tracking a ladle placed in the water. Secondary goals included all four animals using the entire exhibit randomly and without repetition. Initially all animals were fed at the same time to prevent them interfering with each other. From the start Yushan did very well; he had a voracious appetite and once feeding was established would station immediately upon initiation of his session. Yushan also utilized the entire exhibit perimeter throughout the day, often swimming at a very fast speed compared to the other animals. Yushan did seem to have difficulty tracking the ladle to the left early on, but he never left the area and almost always consumed his entire diet. Up until June of 2008 Yushan was using the entire exhibit throughout the day. About that time the team began to see some changes in his behavior including reduced use of the exhibit that resulted in a clockwise circling pattern that was restricted to a small fraction of the 2.3 million liter exhibit. Yushan continued this pattern 24 hours a day, only breaking out of it to participate in the two regularly scheduled whale shark feeds. Although Yushan continued to station for feeds, his ladle tracking and horizontal feeding pattern had essentially disintegrated. It resulted in a vertical corkscrew swim pattern underneath his feeding platform. Yushan began to spin even in the middle of the straight passes although the target remained straight ahead. Consistent swimming in one circular direction with no gliding behavior to break the swimming cycle is a repetitive and unvarying behavior and as such resembles stereotypic behavior (Mason, Clubb, Latham, & Vickery, 2007). Following a large increase in diet Yushan stopped stationing altogether for several days in a row. Although the cause of this behavior is unknown, it became evident that this swim pattern was becoming detrimental to Yushan's physical appearance and potentially his long term health. Indicators such as asymmetrical swimming and dorsal fin curvature to the right developed over a relatively short time period.

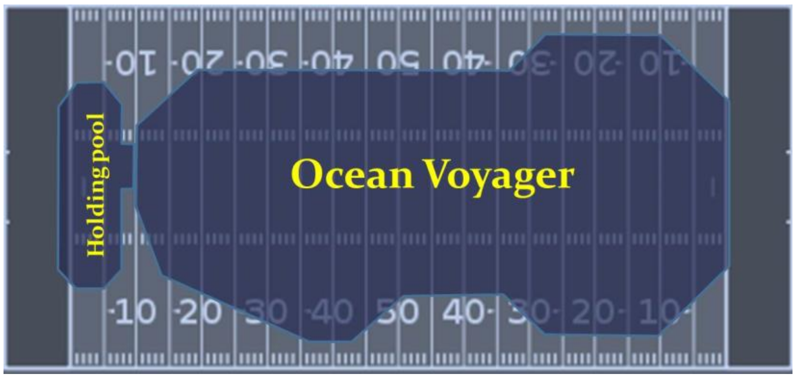

Figure 1: The Ocean Voyager exhibit is 269m long, between 27.4-36.6m wide, and between 6.1-9.1m deep.

It became imperative for the Aquarium team to develop a behavior modification plan to address this stereotypy and the resulting deformities. The goals of the revised training plan included less time spinning, improved body condition, improved horizontal feeding behavior to allow for a better range of motion for his tail, improved ladle tracking behavior, and a varied swim pattern that made use of the entire exhibit. The team decided to employ behavioral enrichment strategies with the goal being a reduction of stereotypical behavior. These enrichment strategies can include any combination of variation in the amount, type, timing and position of food offered (Gindrod & Cleaver, 2001). The plan addressed all of these areas and has continued to adapt based on his needs and responses. The behavior modification plan implemented with Yushan included altering the current feeding mode and including additional training sessions which consisted of boat feeds, perimeter walks and dive feeds. Yushan's diet consists of Superba krill (*Euphausia superba*), Pacifica krill (*Euphausia pacifica),* squid (***Loligo*** *vulgaris*), silversides (*Menidia menidia*), Mazuri omnivore gel diet, as well as Mazuri shark and ray vitamins. These items are split throughout the day to provide the maximum enrichment time.

The initial aspect the plan addressed was the twice daily fifteen minute whale shark feeds that occurred with all four sharks. Yushan originally fed from a small platform positioned over the middle of the exhibit in the deep end; the original goal was to have him move in a figure eight pattern under the platform. By mid-2008 his behavior had deteriorated enough that he would station for his feeding session but then would very quickly begin corkscrew swimming under his station. During most feeds, the team was unable to get him to travel in a straight line or to the left at all. By October of 2009 he was transferred off of the platform to a small raft that was set up in the water, as the platform restricted the ability of the feeder to move Yushan adequately in a straight line without running into another shark or exhausting the length of the ladle. The boats were set up in such a way as to give them a greater range of motion and the ability to travel further in a straight line. Through a phased approach, all four sharks became able to utilize the entire length of the exhibit while feeding.

Figure 2: Yushan with curved dorsal fin. Image: A. Collier

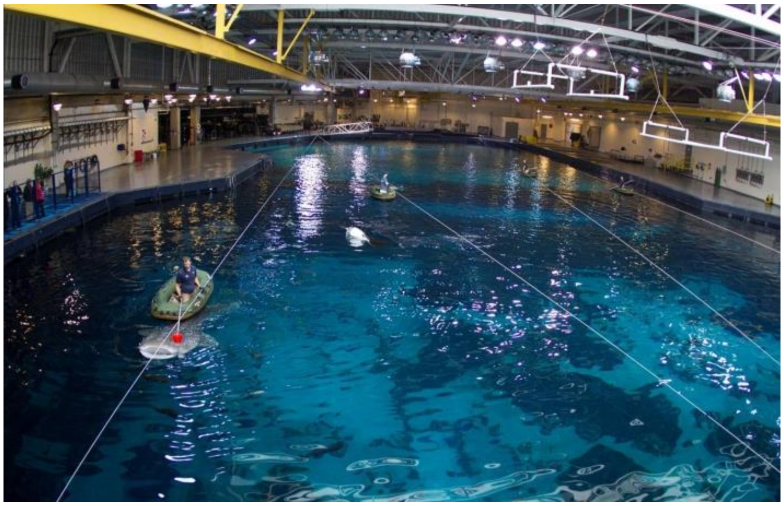

Figure 3: Whale shark feed with all 4 animals, Yushan is on the far left.
Image: A. Collier

Once the twice daily boat feeds became an established part of Yushan's day an auditory cue was added that would act to signal the start of the session and aid in our ability to provide additional sessions randomly throughout the day. Approximately a month after this transition to the boat, a third boat feed was added every day exclusively for Yushan in the early afternoon. The team used the overhead ceiling fans (designed to create water ripple) as Yushan's initial cue to come to the surface and later transitioned to the auditory cue previously mentioned. The purpose of this feed was to break up Yushan's day and provide him some relief from the constant spinning. Yushan took to the third feed quickly and easily and was consistent in showing up as soon as the cue was provided. This period was Yushan's worst to date as far as behavioral degradation is concerned, and at one point the team carried out as many as 5-9 boat feeds per day to provide further enrichment.

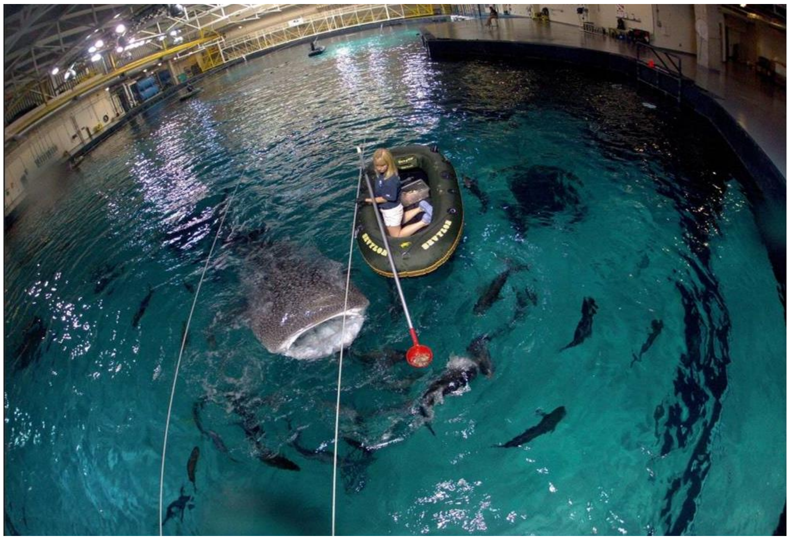

Figure 4: Yushan boat feed. Image: J. Drobny

In April 2010 the mid-day feed was modified from a boat feed into a counterclockwise walk around the perimeter of the exhibit, using the same red ladle and the auditory cue already established during the boat sessions. The aquarists initiated this session at Yushan's usual boat feeding station to ensure participation. The walk idea stemmed from a desire to extend the time spent on Yushan's sessions as well as the distance travelled in a straight line. The team hoped this would provide a gradual 90 degree left turn. Once this concept was established as a basic behavior it was the opinion of the team that we could use it to train Yushan further to utilize the remainder of the exhibit based. The auditory stimulus used as a start of session cue during the boat feeds was incorporated to initiate a pass back to a second feeder at the edge of the exhibit. The goal of this A→B transition was to encourage Yushan to travel into areas of the exhibit that he does not regularly use via stimulus control and food reward. The goal was developed from simply having Yushan move around the perimeter of the exhibit to travel thru the middle of the exhibit and to be able to pass him from any point in the exhibit to any other point in the exhibit using the ladle and auditory cue. By March 2013 Yushan met the goals set out for the walk and successfully transitioned back and forth across the length of the exhibit. Presently, after three years of training, as soon as the ladle is lifted providing the food, Yushan generally spins one time looking for it before he travels back to the location of the second ladle. Future goals of this particular feed are to be able to eliminate the extra spin, pass more frequently, and for the pass to be more consistently reliable. Another option to utilize this particular training method in the future could be stretcher training. The stretcher infrastructure is already in place on top of Ocean Voyager exhibit for our manta ray training program and could easily be applied to one of these walk sessions. If this plan were pursued it would require an incremental process of incorporating small stretcher panels next to and under Yushan before asking him to swim into a completed stretcher. If successful it would allow us to lead Yushan into the stretcher and handle him under stimulus control.

In May of 2011 the team began to consider using divers to alter Yushan's swim pattern during our daily morning dives. The plan started as an attempt to simply get in the animal's path and use divers, without touching him, to block his path and aid him in opening the circles a bit wider. The team used this process to some extent with one of our other whale sharks with some success. This method evolved from a passive method of simply getting in his way to a more active method of actually leading him by using several squeeze bottles filled with Pacifica krill (*Euphausia pacifica*). By June 2011, the team incorporated the auditory cue used during the walk sessions and boat feeds into the dive feeds and began a schedule of integrating these feeds 5 days a week in the early morning. A method was established in which Yushan successfully tracked a diver with food around the entire volume of the exhibit. The divers were able to turn him in all directions and to some extent control his swim speed and depth. Originally this was done using one diver and several squeeze bottles with a second diver in the water observing as a safety precaution.

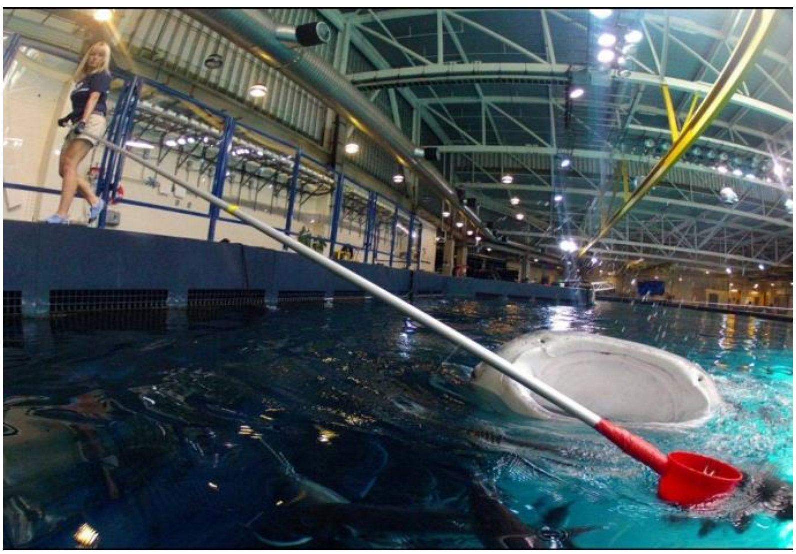

Figure 5: Yushan perimeter walk. Image: J. Drobny

In August 2011, this progressed further into two divers taking turns passing him back and forth between them. During the transitions, the feeding diver drops down out of Yushan's line of sight as the second diver within visual proximity signals the auditory cue. Yushan is expected to travel between the two divers directly. Divers are also able to alter the distance between them with the ultimate goal of utilizing the entire length of the exhibit to allow a straight path at a pace that divers do not impede. After establishing this protocol, the team was able to use this method to obtain a blood sample from Yushan's right pectoral fin on four separate occasions as well as a full set of updated measurements under stimulus control. The long-term goals for this particular feed include increasing the distance between divers during the transitional passes with the hope that eventually the team will be able to pass him the entire length of the exhibit regularly. This would allow Yushan to swim at greater speed than the divers are able. The problems encountered along the way were abundant and some quite unexpected, the spinning behavior being the most apparent and in need of immediate attention. The standard whale shark feeding mechanism includes ram filter feeding however they are able to use a suction type mechanism as well. This system allows them to remain stationary in the water and forcefully inhale water and their prey (Compagno 1984). Yushan has an exaggerated suction feeding behavior when he opens his mouth, and he tends to inadvertently ingest surrounding teleosts. The other three whale sharks spit when this occurred while Yushan clamps down on them. The majority of the time that Yushan ingests teleosts, the aquarists ended the feeding session because Yushan does not return to his station. Another problem encountered is that since the initiation of the dive feed, Yushan has had a tendency to generalize divers and will sometimes follow divers, especially in the morning, that are within visual proximity even without food present. This is problematic as we have a large volunteer diver base as well as a large number of guest divers in the form of the Dive Immersion Program, coming through the exhibit on a daily basis. This means that the majority of the people that Yushan encounters at those times are not proficient in behavioral training.

Figure 6: Yushan dive feed. Image: A.Collier

Conclusions

The aquarists observed a number of positive results from the training; Yushan has been observed utilizing the entire exhibit more frequently as seen by Georgia Aquarium staff as well as the Georgia State University observers that carry out behavioral observation several days a week. Yushan has been observed doing larger diameter circles and utilizing a full 1/2 of the exhibit on a regular basis. This improved use of space and increased size of Yushan's circles has lead to improved body condition, reduction in dorsal fin curvature, and improved horizontal and symmetrical swim position with bilateral tail movement. Another positive result has been that Yushan's behavior changes just prior to an anticipated feeding session, and he begins scanning the exhibit for food. Yushan's swimming speed increases, meaning improved use of the full length of his tail and he begins circling a much larger portion of the exhibit to include the areas where his feeds take place. In addition, Yushan has learned to pay attention to various stimuli around the exhibit that mean it is his feeding time. Examples include, a ladle dropping on the ground at the station, divers entering the exhibit in the early morning, splashing fish or people at the surface of the exhibit all send Yushan racing towards his feeding station.

Figure 7: Yushan dive feed with blood draw from the right pectoral fin. Image: J. Drobny

While Yushan's spinning behavior has not been eliminated completely, there have been some marked behavioral improvements and his overall body condition appears improved as well. Yushan is desensitized to divers very close to him, and because of that better quality blood samples are obtained than if he were put into a stretcher. These blood samples reflect the sharks actual health better due to less stress, which allows better baseline pH and lactic acid values. The team has also had the ability to obtain accurate body measurements under water during feeding sessions. Yushan has improved targeting on his ladle and improved response to his auditory cue which gives the team more control during feeds and more options for altering feeds and providing enrichment when necessary. All of these items combined give us the ability to accurately position the shark anywhere within the exhibit at any point throughout the day. The Aquarium team continues to work with Yushan to address his current condition and extinguish the stereotypy, with the goals of him achieving adult size and long-term residency on display.

Acknowledgments

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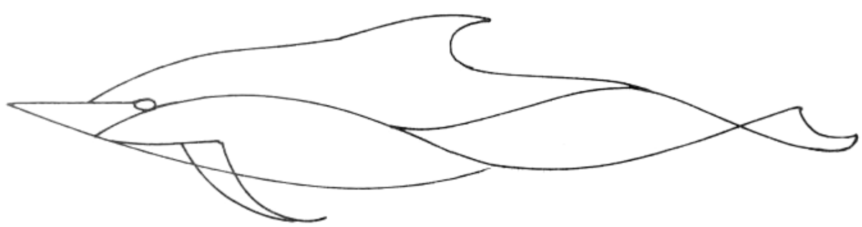

BUFF GUYS BUFFING: A GUIDE TO BUFFING ACRYLIC

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As acrylic becomes more common within public and private aquariums, so does the need to keep it clear of scratches and scuff marks. A clear view into an aquarium is not only important for animal health observations and maintenance for the caretaker, but for the guests who look through the acrylic to view a section of a world that is not easy for them to normally see. Acrylic comes in different sizes, shapes, thickness, and even hardness. It doesn't always take much to scratch acrylic. Tools such as scraper blades with a sharp edge, gravel caught between the scrub pad and the acrylic, internal décor being moved about within the aquarium, zippers from divers who clean, or animals that rub their teeth up against the acrylic can all cause unsightly scratches. Once this happens, how do you fix it? Well, one option is to hire a company to come and buff the scratches out for you, or a more cost effective but time consuming option is to do it yourself.

Since budget is on everyone's radar, doing it yourself seems like the best approach right? Well, there are several things to consider when tackling such a project. You should start by taking the time to determine the overall scope of the project. Below is a quick outline and each section will be broken down into more details.

An Outline for Determining the Scope of Your Project:

1. Size of project
    a. Small 150 gallon system with 1 side needing work vs. 3000 gallon double sided system?
    b. Curved surface or flat?
    c. Wet or dry?
2. Timeline to achieve goal
    a. Project done while open to guests or after hours?
3. Tools needed
    a. Compressors and air lines if wet buffing
        i. Size of compressor (for underwater buffing)
            1. Size of disks depends upon the compressor used (Ex. 5" disc easier to spin than 6" disc)
        ii. Compressor to run how many sanders?
        iii. Sanders for underwater
            1. Brands that work
                a. Dynabrade vs. Chicago for underwater
        iv. Sanders for Dry work
            1. Rigid, etc.

    b. Pads of various grit
        i. List different companies and types of pads to be used
            1. 3M, Micro-Surface,
            2. Micron vs. Micromesh
            3. Hook and Loop vs. Sticky
            4. Grits to start at or finish
        ii. Size of pads (5" seems most common)
    c. Buffing polisher and compound
    d. Miscellaneous Equipment
        i. Spray bottles
        ii. Rags (Type that will not scratch the acrylic)
        iii. Wax pencils/window crayons
        iv. Squeegees
        v. PPE
            1. Eyes, ears, etc.
            2. Surrounding area
    e. Hand polishing kit (MicroMesh MA1)
4. Cost to complete project – Can be expensive to purchase all the above
5. Method
    a. Grids
    b. Speeds
    c. Moisture if dry
    d. Patterns to avoid burns
    e. Blending
    f. Curved surfaces?
6. Maintenance and care of equipment in between jobs
    a. Pneumatic sander and hoses
    b. Air compressor
    c. Miscellaneous equipment

Project Details and Methods:

1. <u>Size of Project</u>:

It is very important to ask yourself some very direct questions related to the project in mind. Is this a small 150 gallon tank or is this a 3000 gallon system? Does it need buffing on just the inside or does the outside need to be touched up as well? Is the surface you are planning to work on curved or flat and do you plan to do this work with the system drained dry or while wet with animals still in there? How bad are the scratches? Are they all over the panel? Are the scratches deep? These are all very important questions to answer at the start of a project because it will help you plan accordingly for the longevity of the project. Smaller, single sided projects tend to take very little time but as soon as you add another side to the scope, your time will usually double. It is also easier to blend a smaller panel/system (4ft long x 2ft high) as opposed to a large panel/system (10ft long x 6ft high) as there is much more room on the larger panel/system to see mistakes. The grid system will help avoid this and is discussed more in the "methods" section. Knowing if you are working on a flat or curved surface is critical as it requires different equipment. Flat surfaces are the easiest to work on it requires even pressure throughout your procedure where a curved surface could cause problems such as burning or divots if not taken into consideration. More on how to work on curved surfaces is discussed in the "methods" section. Lastly, you need to decide if you have the ability to empty the system and work on the panel dry or if you have to leave it as is, and work with the water still in place. Working dry offers you more flexibility and is much easier from a resource perspective, however; not every system offers that ability. Some large systems require it to be done as is or wet which will require special tools to facilitate this situation and is discussed more in the "tools" section.

2. Timeline:

With every project, it is important to put a plan together including equipment cost and estimated work time associated with the project. If this is your first time doing such a project, it will be difficult to gauge the length of time that it will take, but there are several things to take into account to be able to plan accordingly. Things like the size of the project (150 gallon one side vs. 3000 gallon double sided) and length of time to acquire the materials. How many helpers do you have? How many and how severe the scratches are? How much time can you free up to work on the project and still complete daily work load? Will your project interfere with the public or daily operations of your facility? Those are all very important questions to consider when developing a timeline. Once you decide on a set amount of time that it will take (ex. 500 gallon single side panel will take me 40 hours), add 10-15% more time as protection (ex. 500 gallon single side panel will take me 46 hours) for a worst case scenario.

3. Tools:

There are a variety of tools that will be needed for a buffing project and they will vary depending on if it is a wet or a dry project. For a wet project you will need to consider the compressor, airlines, disc size, sander type, safety equipment, spray bottles, rags, ladders or scaffolding, wax pencils/window crayons, dive gear, squeegees, buffing compound and buffing polisher. The same will be needed for a dry buffing project although you will not have to worry about the compressor and airlines as the pneumatic method will not be used (although they can be used for dry side); rather individual electric sanders/buffers can be used. If you choose to go the electric sander route, you should use a GFI since water will be used during the sanding process.

Compressors are one of the most important pieces of equipment when buffing underwater (Figure 1). You will need a reliable machine that will run consistently and provide enough power to run the number of sanders you wish to operate at the speed necessary to buff. This can be achieved by calling a compressor company and having a conversation with what your needs are. Example: Need to run 4 sanders at X RPM's (varies on your sander type and disc size). Since your underwater/pneumatic sanders require air, another important component to consider is the length of your air lines from the compressor to the sanders. There is nothing worse than getting all the necessary equipment organized, suited up to dive, gear tested and then you fall 3 ft short of the longest span of which you need to buff. It is also important to take into account the fittings. Most are standard fittings but there have been occasions where the quick disconnect fittings were not available or were not chosen and the airlines are threaded in. On the quick disconnects it is recommended to get male ends with a double nipple vs. a single nipple. It is important to know what fittings you have/need to accommodate for any repairs that might need to happen mid project.

Figure 1: Sample compressor.

Sanders are an important component whether you are buffing dry or wet. It is important to select sanders that are comfortable for you to use as you'll be using them A LOT. Brands that seemed to have held up underwater the best are Dynabrade and Chicago while many brands such as Rigid, Dewalt, Milwaukee, Makita, or Bosch work well on the dry side (Figures 2, 3 and 4). The 5 inch disc size seems to work the best for a couple of reasons. First, a 5" disc is easier to spin and control than the larger disc sizes. Second, the 5" size a more common size when purchasing your sander and discs. This helps keep costs down and makes it easier to find what you need in the event of an emergency. There are differences in padding on the sanding discs and should be chosen according to your needs (Figure 5).

Figures 2-4: Examples of suitable sanders

Figure 5: Sanding discs showing variable padding thicknesses.

Buffing pads come in different sizes, colors, attachment methods, and grit sizes and are produced by a variety of companies. Companies such as 3M and Micro-Surface (www.micro-surface.com) are common places to find the buffing pads needed for such a project. It is important to note the surface of your sander and the surface of your pads. Both the sander and the pads can come with a flat surface which accommodates the sticky pads or a Velcro or hook and loop system (commonly referred as HOOK-IT Pads). The hook and loop system is preferred as it provides the pads to stick more securely where the sticky option often allows the discs to spin off when minimal friction is created. If the buffing disc spins off, it can create a burn (melted plastic) in your acrylic which would make the situation much worse (Figure 6).

With any buffing project, traditionally you would start at a lower grit and work your way up to the finer grit. For example, there is a very deep scratch and you decide to start with 1500 grit. After buffing for a period of time, you notice that the 1500 grit did not remove the scratch. You can go down to 1200 to see if that does the job or even down to 800 to remove the scratch. The 800 grit removed the scratch and now you will have to begin going up in grit to keep removing the scratches the previous grit made until you reach the polish stage. You should continue sanding with whichever grit size you are using until the scratches look uniform. Keeping with this example, if you start with the 800 grit and that worked to remove your worst scratch, you would need to do the entire panel in 800 grit to maintain a consistent look. Next, you would go to 1000 grit to remove the 800 scratches. After 1000 grit is complete, you might move to 1200 or 1500 grit. Upon noticing that 1500 removed the 1000 grit scratches, next could be 1800 and so on until you reach the buffing stage. Keep in mind that the lower grit (or more course) you go, the longer your project will be because you will have to work your way back up to the finer grits. It is important to completely remove the scratch before moving to the next grit size. Failure to do so will result in the remaining scratch being visible in your end product. Note, that about 75% of your polish time is to remove the initial scratch or scratches from the acrylic.

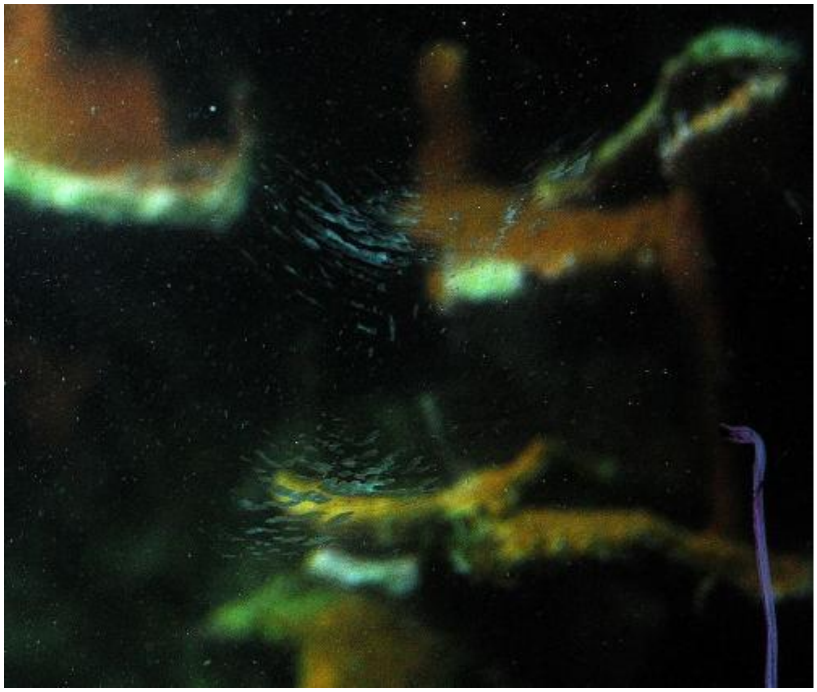


Figure 6: Burn marks are caused by overheating such as from detached sanding discs.

Wet polishing will be the same technique as above with your finest grit size. Micro scratches will be filled in by the water and not noticeable unless the water is drained away from the glass.

Once all the buffing is complete, the next step is to polish the acrylic. This requires the acrylic polishing compound, an orbital polisher, spray bottle with R/O water, and a non-abrasive polishing material. Lamb's wool has been a common and successful material for this and can be found online or at most automotive stores. It is very important to clear any debris. The smallest abrasive debris will re-scratch your polished area. There are lots of other pieces of equipment that are needed but will be labeled as miscellaneous. These are things like spray bottles, rags, wax pencils/window crayons, squeegees, personal protective equipment (PPE), benches, etc.

Supply List for Larger Polishing Projects:

- Spray bottles / Pressurized spray bottle: Spray bottles are needed to spray a mist of water over the surface you are buffing. This is not needed in the wet/underwater process but essential during the dry process to prevent friction and acrylic burn. Water fed sanders are available, but expensive. For example a Viking Air Tools pneumatic sander retail at $580.
- Microfiber rags: It is important to use a type of rag that will not scratch the acrylic as you wipe the buffing mess away to check on progress. Microfiber rags work great for this but it is important to know that they can attract other debris so make sure they are debris free before wiping the acrylic.

- Wax pencils/window crayons: These work the best to mark out your grid system and not scratch the acrylic in the process. The grid system helps you keep track of the area you are polishing and allows you a pattern to work with for overlap and consistency standards.
- Squeegees: Squeegees can be used in place of the rags as they are used for the same purpose but the same guidelines apply. These can be a bit more challenging as imperfections in the squeegee as well as the acrylic dust you are removing could re-scratch the polished area.
- Personal Protective Equipment (PPE): PPE is necessary for any project. It is just as critical here as the equipment being used can be quite loud and create a large mess. Eye protection and ear protection is a recommended minimum but wearing old clothing or a smock are useful to help protect your work or personal clothing from potentially becoming stained. It is also important to protect the area surrounding the work area. The sanders tend to spin at a fast rate and will often spin white material in all directions. It is worth the time to set up and protect the area around the work area to minimize the mess and clean up time in the end.
- Miscellaneous Equipment: Depending on the project other pieces of equipment that are useful are ladders or scaffolding, radio or cell phone with ear buds, fan or heater depending on your location or time of year. When diving suction cups are useful to keep in place. A underwater slate or dry erase board for communication between spotter and diver.

For smaller projects a more economical option is to use a hand polishing kit. Micro-Surface Finishing Products Inc. has several kit options to fit your needs. For example, the MA-1 Kit is recommended for polishing up to 100sq. feet of acrylic and retails for around $100. The kit also includes an instruction booklet with a trouble shooting section to determine how to proceed with your scratch removal.

Procedure for Using Polishing Kits:

- The first step is to determine the severity of the scratch or scratches. Deeper scratches will take additional sanding steps since you will have to start your sanding with courser sandpaper. For example for a deep scratch you should begin with 220grit wet/dry sandpaper to uniformly remove the initial scratch then proceed with the course 1500 MICRO-MESH included in your kit. If your scratches are visible but not deep you could start with 400 wet/dry sandpaper or 1500 MICRO-MESH. Hairline scratches and slight hazing, it may be possible to start your sanding with the 4000 or 6000 MICRO-MESH. Whichever your situation is, it is important to completely remove the scratch with your first sanding. Failure to remove the scratch on your first sanding will result in the remnant of the original scratch visible after your final polish.
- To start the process, determine what grit size to start with to remove the scratch. Wrap the sanding sheet around the provided foam block. To help keep the sheet around block I find it helpful to use a rubber band. Start the sanding by pressing the block firmly against the acrylic and it moving up and down or side to side, depending on the orientation of your scratch. You want to sand perpendicular to direction of the scratch. Doing so will allow you to see the contrast of the scratch you want to remove and the uniform scratches you are creating. As you are sanding continuously spray water to flush away the sanded

material from the sand paper as well as the acrylic (It is possible to dry sand but debris tends to build up on the sand paper and shortens the life of the sheet). Work the area until you have completely removed the scratch. To check your progress, rinse the debris away and wipe the area with a soft clean towel or micro-fiber rag. It is important to dry the area, as any water will fill in the scratch and may mask the scratch from being visible. Once you have completely removed the scratch you can continue onto the next step.

- You now have a uniformly scratched surface to continue the process. Change out the sanding sheet to the next grit size. For example, if you started with the 1800 sheet for your initial scratch removal, you will change out the 1800 sheet for the 2400 sheet. You will now sand in the direction perpendicular to your first sanding (if your first sanding was up and down your second sanding will be side to side). As with your first sanding you will continue this step until you have completely removed any remnants of your first sanding. It is important to increase your sanding area (approximately 2") with each subsequent sanding. Failure to increase your sanding area can result in visual distortion in your end product as well as cause an unsightly divot in your acrylic.
- The third sanding and all subsequent sanding will follow the same method as above (sheet 1 up and down, sheet 2 side to side, sheet 3 up and down, sheet 4 side to side), working your way through the Micro-Mesh sheets up to and including the finest grit of 12000.
- If you are polishing the inside of a filled aquarium your final step would be sanding with the 12000 grit paper. If you are polishing the outside of the aquarium or you are able to drain the inside of the aquarium there is one additional step. You will need to polish the entire area with the Anti-Static Cream provided in the kit. Add a few drops of the cream to the surface and spread it over the acrylic with the cotton cloth provided or micro-fiber cloth. You want to make sure you completely remove any residual cream to finish your polish. Any remaining polish will harden and become difficult to remove.

4. <u>Costs to Complete the Project</u>:

As you start to add up the costs of buffing pads, sanders, compressors, hoses, squeegees, PPE, and people's time, the costs can get quite high. Don't let this scare you away from doing the project. Although the upfront cost seems high, it is usually still less expensive than hiring a company to come in and do it plus you will have the bulk of the equipment for future jobs. Starting off with practice acrylic is a great way to hone your skill and not use your practice time on something that could go horribly wrong for all of the public to see. This also allows you to acquire equipment as needed which will usually help break down the costs over time. Costs will obviously vary depending on the size of the project and the equipment you chose to use. I do not recommend cutting corners on less expensive equipment because more often than not, the equipment will fail at a critical time which could cause a considerable error on the project (ex. acrylic burn or personal injury).

5. <u>Buffing Methodology</u>:

To help keep consistency and avoid confusion, it is best to draw a grid on the acrylic with our wax pencil or window crayon (Figure A). Each grid box should be roughly 2ft x 2ft on larger acrylic projects or just divided into equal sections for smaller projects. It is best to do this on the "dry" side of the acrylic or where the guests would be viewing from for two reasons. 1) If you are working on the inside of the tank, you can see your grid and not have to redraw it in between grits and 2) if you are work on the "dry" side of the tank, it is difficult to get in and draw your grid on the "wet" side so you will have to redraw your grid in between each grit that is buffed as the buffing should remove the lines as well.

Figure A: Grid pattern on window.

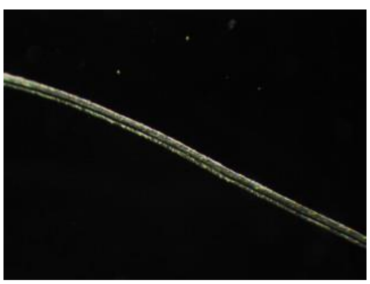
The speed or rotation of your sander is very important to monitor. You want to operate at a fast RPM in order to accomplish your task; however, too fast can make it difficult for some to control the sander and too slow will not accomplish the task. The variable speed sanders really come in handy here as you can adjust them to a desired speed and then just turn them off when you are done and when you come back, the speed will still be the same for a consistent finish.

The moisture of the acrylic while buffing is something that is very important to monitor. This isn't something that you need to worry about while buffing underwater but can cause major issues if it is not monitored closely on the "dry" side. It is recommended that while buffing dry acrylic, you spray water over your work area (Fig. B & C) to keep a squirt bottle with you so that as you work through your pattern (discussed below) on the grid, you can keep it moist to help minimize friction (which leads to overheating and acrylic burn, Figure 6) as well as to help rinse away the acrylic debris as it is sanded off. This will be in the form of a white liquid that will run down your acrylic.

There are a few patterns/methods that can be used while buffing your acrylic. 1) Up and down **THEN** side to side or 2) Up and down **AND** side to side. The first method is performed by alternating your pattern. As you work through the various grit sizes, you will also vary your sanding pattern/method. For example, with grit #1 you will go in an up and down motion with equal pressure on the sander until the scratch is removed and the acrylic is uniformly scratched


Figures B and C: Spray water on dry-side work areas while buffing.

from the up down motion. Grit #2 will be side to side motion until the scratches are uniformly going side to side. This alternating method should be done until you have reached your final polish. The second method is performed by alternating the up and down, side to side patternwithin each grid square for a period of time. For example, your grid is sectioned off into 16 2ft x 2ft squares. You would start at the upper left corner and work on that grid in an up and down, side to side motion within that one square for five minutes. Wipe the debris away and see if your scratches are removed. If they are not, buff for a bit longer (Fig. E, F & G). If they are, move over to the next square and repeat that process.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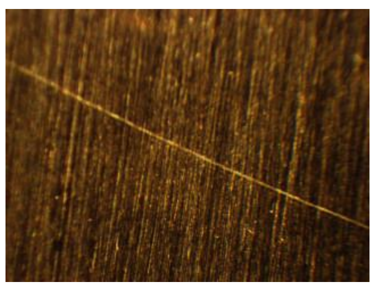
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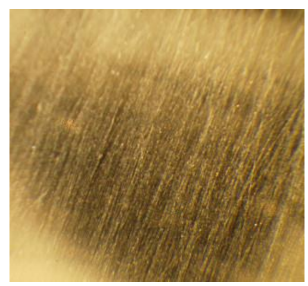
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Figures E-G: Progressive removal of a large scratch by sanding.

Blending is important to the finished product. Whichever pattern/method you chose, it is important to overlap a bit from square to square to help blend your work. This will help create consistency across the whole acrylic and hopefully eliminate any "warbles" or flaw that was created during the buffing process (Figure H).

Buffing a curved surface can be very tricky. If you are going to be working on a curved surface, it is important to layer several of the sponge buff pads to act as a cushion between the sander and the buffing pad. Without this sponge buffer, it is possible to apply unequal pressure across your sander, creating gouges that would need to be re-sanded to remove. The sponges might have to be doubled up depending on the degree of the curve and which side you are working on but they will help create a more even contact on the surface while sanding.

6. Maintenance and Care of Equipment in Between Jobs:

Now that you have invested in this wonderful buffing equipment, it is important to make sure it is properly maintained in between jobs.

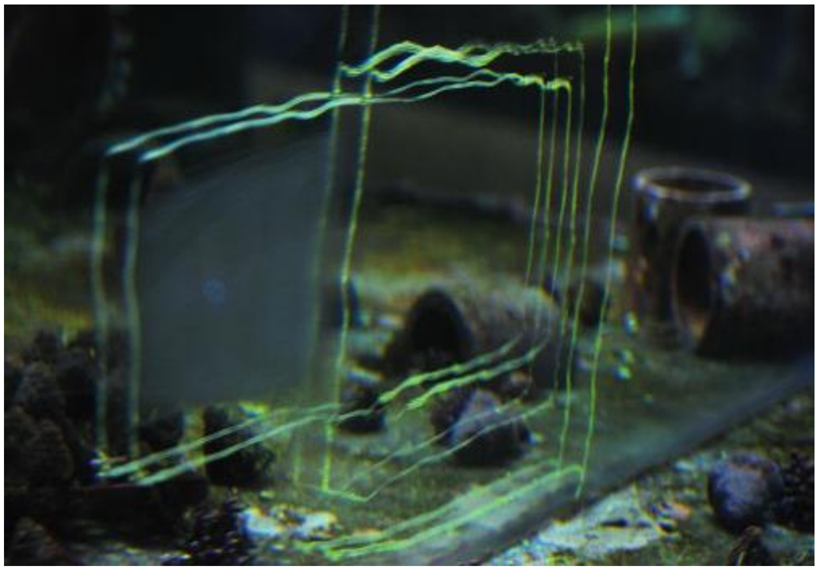


Figure H: Overlapping squares allow blending of work.

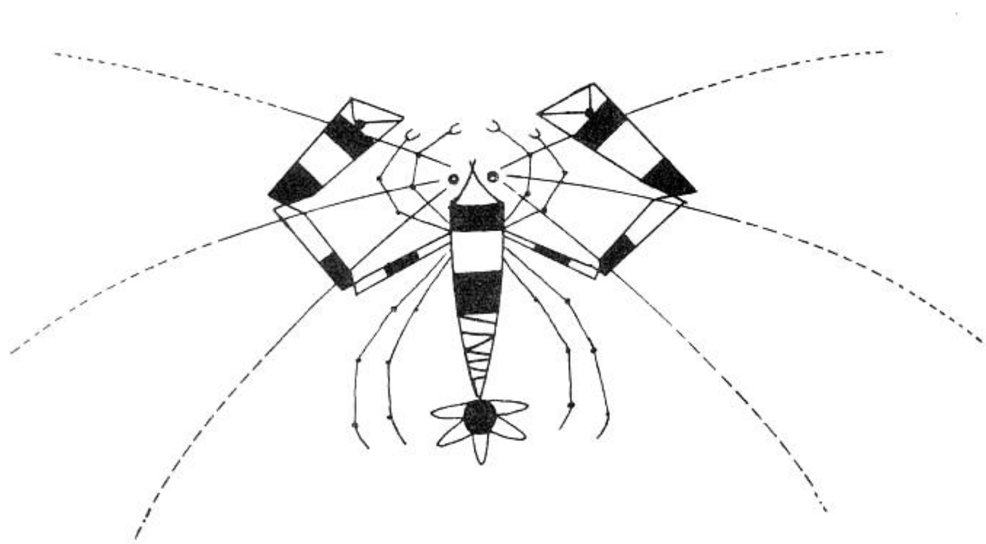
The first step to ensure the life of your sander and hoses is to leave the sander connected to the compressor and flush out any salt or fresh water. Next, disconnect the sander and run fresh water through it (necessary if used in saltwater). Third, reconnect the sander to compressor and blow out the rinse water. Fourth, disconnect the sander and fill it with oil (Vegetable or food grade machine oil). You should manually work the oil through the sander or reconnect it to the compressor and run it for a few seconds. It is important to remove any water and lubricate the inner gears of the sander to avoid rusting. Finally, for short and long term storage, you should completely fill the sander with either of the above recommended oil and store in a cool dry area. The hoses and quick disconnects should be rinsed with freshwater. The quick disconnects should be dried and then lubricated with oil. It is important once again to manually work the oil around the disconnect ball bearings to avoid rusting.

A regular maintenance schedule should be followed for your air compressor according to manufactures recommendations. For long term storage a scheduled monthly start up of the engine should be done and or drainage and removal of gasoline to avoid fouling of the engine.

Any equipment containing metal should be rinsed, dried and lubricated. All other equipment should be cleaned according to your company's standards and procedures.

Summary:

Every Facility that has acrylic displays will at some point need to consider how they will recondition their acrylic to look like new. The pros and cons of hiring a professional company or invest in equipment and training staff should be considered. Both choices have considerable monetary investments, but the second choice can give your facility more return on its investment. You will have staff with a unique skill set and having trained staff can offer more flexibility with a project's timeline.



INSTITUTIONAL COLLECTION PLANNING FOR AQUARIUMS

Jay Hemdal, Curator of Fishes and Invertebrates

The Toledo Zoo

An Institutional Collection Plan (ICP) is simply a list of species that are desired for inclusion into a collection of animals. However, developing such a list for aquariums has proven challenging; with over two million species of marine animals, (Mora, et al., 2011) how does one select one over another? The collection plan process becomes a tool to manage this immense task. The selection method is vital; it must be comprehensive, yet streamlined in application. It should be objective, without losing the ability to subjectively evaluate some species. The results must be technically accurate, but still useful when reviewed by non-technical persons.

Having a comprehensive, well-conceived collection plan aids public aquarium curators in maximizing the effect their animal collection has on inspiring their visitors, enhancing conservation efforts, and minimizing surplus animals. Resource allocation and communication are also improved when a high-quality collection plan is in place. The Association of Zoos and Aquariums requires that all accredited facilities have an approved institutional collection plan.

From the AZA web site: http://www.aza.org/institutional-collection-plans/

> *"All institutions must have an Institutional Collection Plan (ICP) that is re-evaluated and updated at minimum of every five years. The ICP should include a statement of justification for all species and individuals in the institution's planned collection."*

Collection planning processes run the gamut from a curator simply typing out a list off the top of their head, to results from a committee using specialized keys that use weighted values to rank various criteria against one another. A simple list may be too subjective, while running thousands of potential species through a lengthy key can be too laborious. Weighted scoring systems are particularily difficult to implement. Take two common criteria used in collection planning; conservation need of the species and acquisition cost. Most aquarists would agree that conservation need trumps cost (within reason of course). In order to reflect this difference, they may assign weighted scores to each of the criteria, say a 3x multiplier for conservation and a 1x for cost. There is then a strong urge to add up the scores and make a blanket determination such as, "any fish rating higher than 5.6 will be included in the ICP". Avoid this temptation! The curator must drive the process, and not be dictated to by the process itself. "The model made me do it" is not a valid response to problems with an ICP (Willis, 2000).

Simplified Collection Planning Process:

The Toledo Zoo Aquarium has utilized different planning methods over the past twenty years, some were very complicated (Hemdal, 2013). A simplified process was recently developed for use in the planning of a temporary exhibit, and it may have wider application to ICPs in general;

The process starts off simply enough; first, refering to the taxa's AZA Regional Collection Plan, a candidate species is selected. Each of the following four questions must then be answered with an unequivocal "Yes" in order for that species to be included in a collection plan. The curator would then need to subjectively weigh the strength of the affirmitive answers in order to select one species above another.

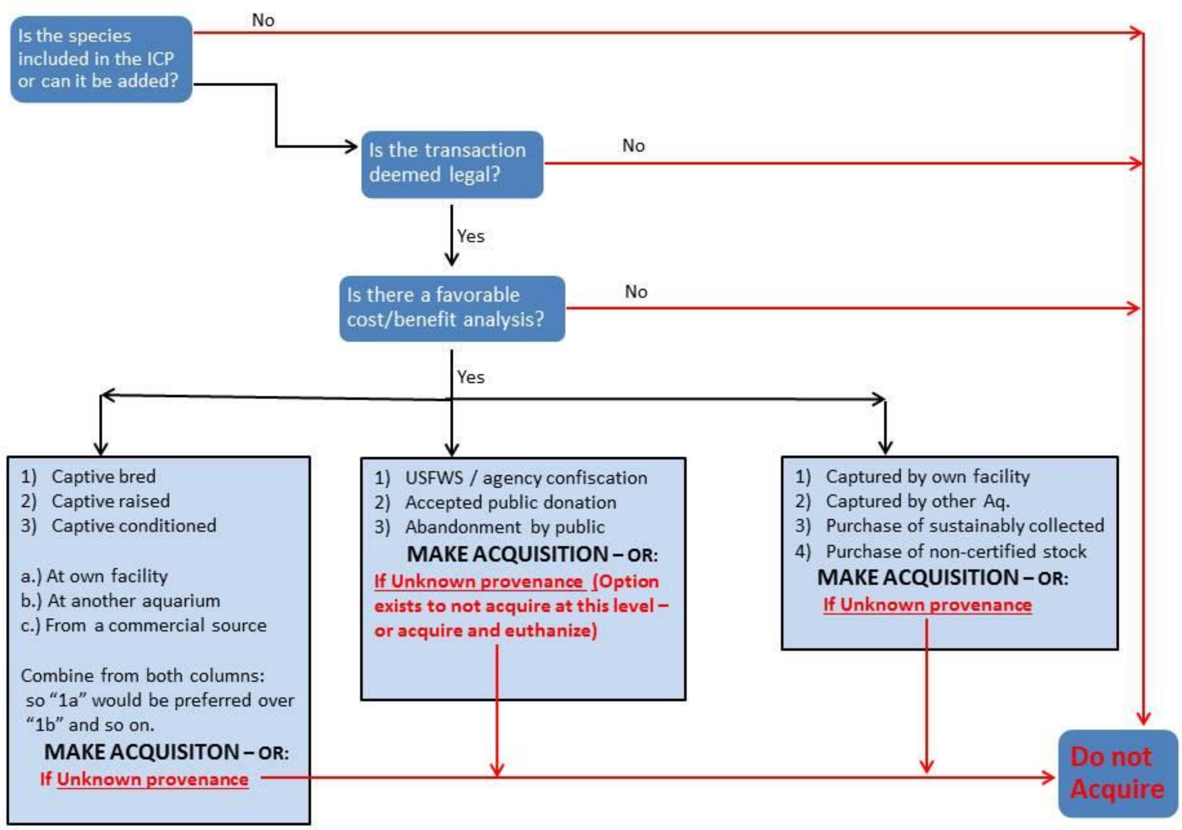
1) Are they a notable species from the exhibit's region or do they support the theme well?

2) Will they inspire our visitors? Color, form, unusual habits all build interest OR Do they have an important educational/conservation message to convey?

3) Can their husbandry requirements be fully met (including compatibility & quarantine)?

4) Are they legally available at the right time and cost, from ethical vendors?

Selecting a species for inclusion in an ICP is just the first step of the process. To be effective, the ICP must then be implemented. This of course means actually acquring the animals. As a continuation of the ICP process, the following animal sourcing flow chart outlines various animal sources and compares their relative viability.



Aquarium Animal Sourcing Flow Chart

Once the potential animal sources have been identified, it is helpful to analyze the different sources to determine their relative costs versus benefits. The first step is to determine the true cost of the animal. This is the sum of the purchase price of the animal multiplied by the number of animals plus the transportation costs. This figure is then divided by the number of animals from the group still alive after quarantine. The true cost is only part of the equation. There are some intangible costs that need to be factored in. For example, staff time needed to manage the animal may vary from one source to another. There may be a negative public impression seen when acquiring animals from a particular source that is not seen if another source is used. There is also a negative environmental cost associated with unsustainable collections.

Once all of the associated acquisition costs have been tabulated, they are then compared to the benefits. These include; lower true cost, value of the specimen as a public exhibit, P.R. value of making sustainable acquisitions, as well as staff development from collecting trips, captive propagation, or learning new husbandry techniques.

Conclusion

Collection plans are vital for the proper operation of a modern aquarium. Curatorial staff should make this process a key component of their work flow. The plan must also be a living document, reviewed and updated as-needed. Remember:

"*Everybody has a plan until they get punched in the face*."
― Mike Tyson

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THE BIOLOGICAL CHARACTERISTICS, LIFE CYCLE, AND SYSTEM DESIGN FOR THE FLAMBOYANT AND PAINTPOT CUTTLEFISH, *Metasepia sp.*, CULTURED THROUGH MULTIPLE GENERATIONS

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Background

Metasepia is a genus of small cuttlefish found in the Pacific Ocean. They are characterized by several definitive traits. The cuttlebones of this genus are small and diamond or rhomboidal shaped. Cuttlebones are also typically much shorter than the mantle and located in the anterior half to two-thirds of the mantle. It is believed that the small cuttlebone size results in negative buoyancy, allowing a unique method of locomotion in which they spend most of their time walking on the sea floor. The mantle itself is also unique, as the dorsal anterior edge lacks the traditional tongue-like projections of most *Sepiidae* genera. (Jereb and Roper, 2005)

There are two species in the genus *Metasepia*. *Metasepia pfefferi*, the flamboyant cuttlefish, is found in the Indo-pacific waters around Australia, New Guinea, the Philippines and Indonesia. *Metasepia tullbergi*, the paintpot cuttlefish, is found from Hong Kong to southern Japan. The two species can be quite challenging to differentiate based on morphology, making genetic analysis an important tool in species identification. Both species of *Metasepia* are neritic demersal species generally found in sand and mud substrates at shallow depths from 3-100m. *Metasepia* breeding is seasonal with mature individuals migrating into shallow water to spawn in the spring, and juveniles migrating to deeper depths in the late summer. (Reid et al., 2005) *Metasepia* lay individual eggs in crevices, on coral rubble, or under overhangs. Adults will live an average of 6 and 8 months, and throughout their life span they feed on various crustaceans and fish.

Commercially, *Metasepia* have been collected for the public and private aquarium trade. Unfortunately, success in captivity has been limited until recently. Ultimately, shipping has made it quite challenging to get healthy individuals from source to destination. These animals traditionally have shipped very poorly, which generally results in significant mortality occurring in transit. Furthermore, in the past, successful rearing of newborn hatchlings has been highly problematic.

Biology

For both species of *Metasepia*, gender can be determined starting at 90 days. At this point in time, there are some behavioral and physical distinctions indicating gender. The males' bodies will typically elongate and turn almost completely white in color during courtship. Males will retain some pink color at their arm tips and a yellow/brown coloration on their posterior mantle. During typical courtship display, males will approach females face to face and bob their heads back and forth on a horizontal axis (Image 1).

Image 1**:** Male depicted on left displaying courtship behavior to female on right. Females have occasionally been observed demonstrating this behavior in territory disputes but it is uncommon.

Another predictable method for determining gender is the size differential of males and females at maturity. At around 90 days of age, the growth rate of each individual changes depending on its sex. This results in a drastic sexual dimorphism in adults whereby females are larger than males. It has been noted that with some cephalopods it is necessary to create two growth curves: one exponential for the immature individuals and one logarithmic for the adults, creating a sigmoid pattern (Domingues et al. 2006). This is the case for *Metasepia*, and the following curves demonstrate the increase of dorsal mantle length and mass (Figs 1, 2) with respect to age. The immature individuals follow a typical exponential growth pattern, which slows upon maturity for males, but remains exponential. Females, however, transition to logarithmic growth upon maturity. The curves fit to these data are significant, with most R^2 values above .8. The rate of growth of a mature female is greatest upon maturation, and decreases with age. And so females grow faster and reach larger sizes, slowing down as they approach appropriate reproductive size. Additionally, the ratio of mass to mantle length is much higher in females, demonstrating that the females invest more energy into increasing their body mass (i.e. gonad development) than they do to their length (Fig. 3)

Reproductively mature females began laying eggs at four months of age. The youngest female to record a clutch was 108 days old. The oldest female to record a clutch was 213 days old. The median age at which females deposited egg clutches was 151 days. Female *Metasepia* can lay eggs through senescence. On average there were seven clutches per female through their lifespan. Females typically laid larger clutch sizes as they aged through their reproductive period. Figure 4 depicts the reproductive trend for one of our mature females. This trend is typical for most of our female broodstock. For this particular female, the first two clutches were small and non-viable and the clutch size increased exponentially as she aged with a minimum of 11 eggs and a maximum of 106 eggs.

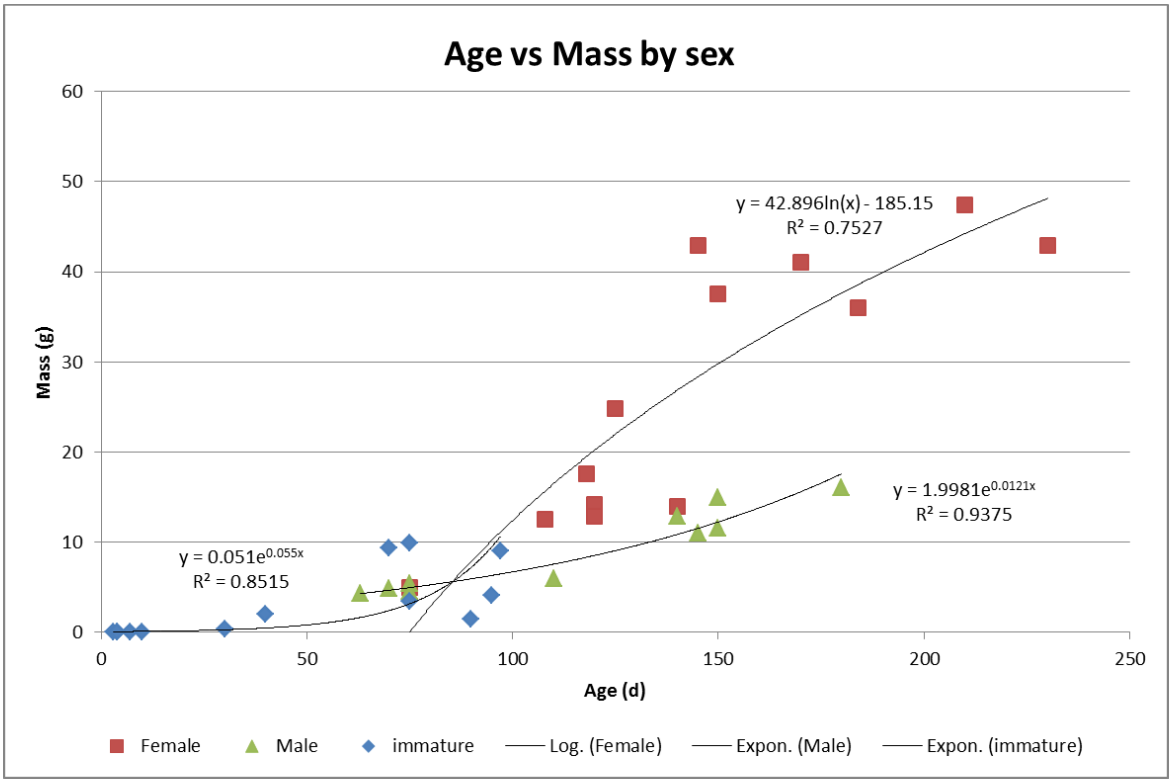


Figure 1: Mantle Length (cm) growth curves in developing *Metasepia* at MBA.

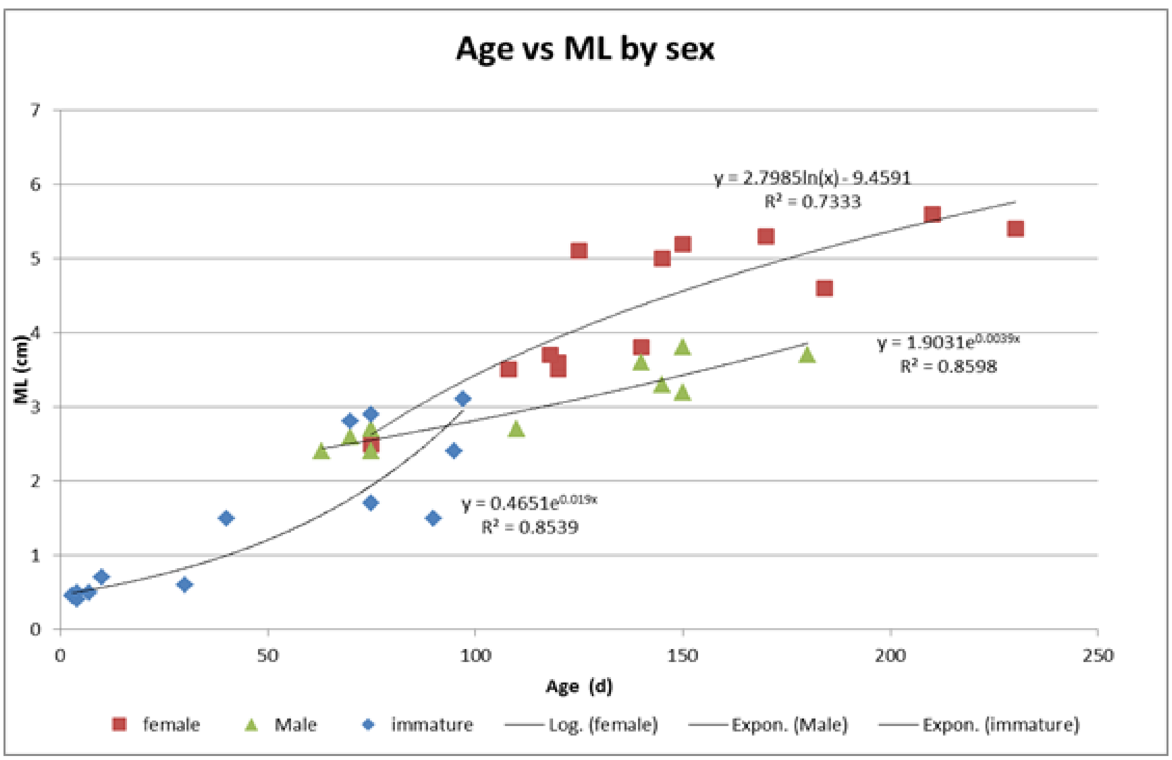


Figure 2: Mass (g) growth curves in developing *Metasepia* at MBA.

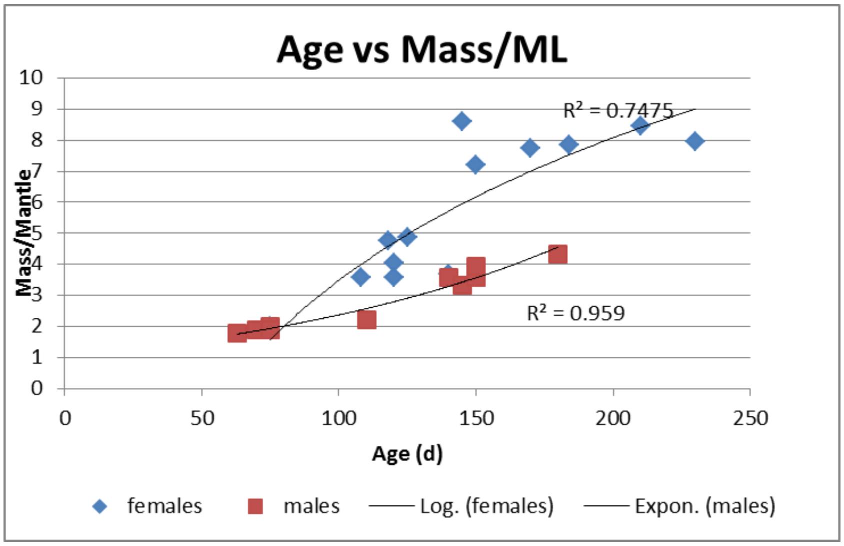


Figure 3: Mass differentials for each gender post maturity at MBA.

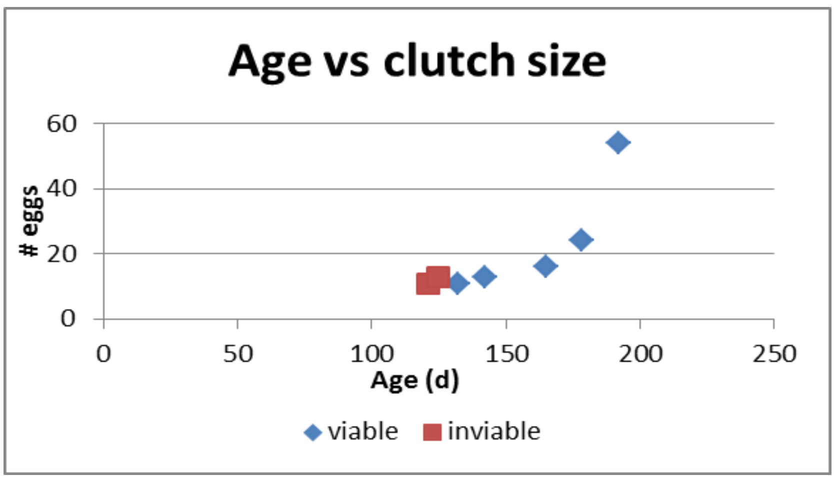


Figure 4: Clutch size in females during laying period and embryo viability trends.

In captivity, *Metasepia* will lay their eggs in live rock crevices, empty coconut shells, or in any other well protected artificial décor arrangement. Eggs are opaque and ovular when they are first laid (~1cm in diameter). As cell division proceeds and the embryo develops, the eggs become transparent and increase in size. Gestation period was a minimum of 18 days to a maximum of 32 days. Average gestation was between 23-29 days. After hatching, juvenile growth was exponential until reaching sexual maturity at around 90 days (Fig. 5; Fig. 6)

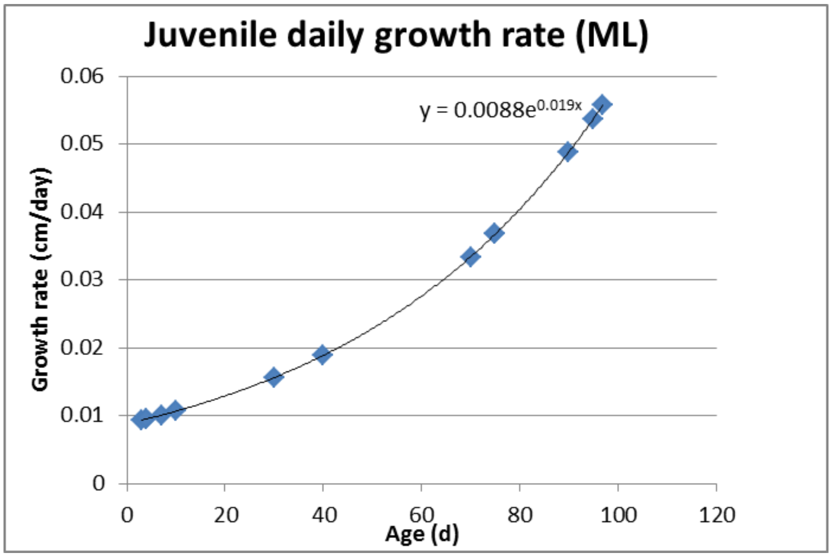


Figure 5: Juvenile exponential growth rate trend in mantle length (cm).

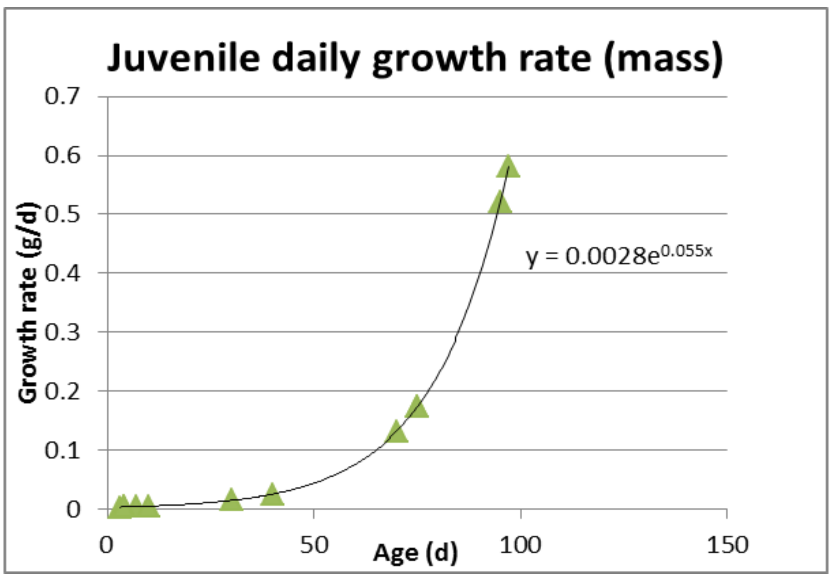


Figure 6: Juvenile exponential growth rate trend in mass (g).

Husbandry

System Design

The most commonly used holding at MBA for our sub-adult/adult system is a 230 L fiberglass tank with dimensions 60cm X 90cm X 50cm. Our holding tanks have three opaque sides that aid in prey detection and one clear acrylic window for observations.

The Monterey Bay Aquarium utilizes a semi-closed, flow through system which greatly assists with maintaining optimal water quality. Protein skimmers or other mechanical filtration can be used depending on system specifics and the bio-load of the *Metasepia* culture. These extra filtration methods will assist during inking events, a relatively uncommon event in our experience with *Metasepia* cultures when compared to other sepioid species.

Like many cephalopods, the skin of *Metasepia* possesses innumerous microscopic epidermal projections, a microvillus epidermis, which drastically increases the total surface area of the skin. This increases susceptibility to elevated ammonia and nitrogenous compounds in the water column. Due to this sensitivity, water quality needs to be carefully monitored and managed.

A water temperature of 77° F (25° C) has resulted in consistent and reproducible culture results, however, this temperature may be raised or lowered by (+/- 2° F; 1° C) as needed depending upon institutional culture goals. Higher temperatures result in more rapid growth, but a shorter lifespan. Conversely, lower temperatures provide for a longer lifespan, but a reduction in growth rate (Domingues et. al. 2002).

Substrate is important to incorporate on the holding tank's bottom so individuals don't rub their sensitive ventral mantle on the tank's fiberglass surface. The substrate also helps *Metasepia* with their crawling locomotion behavior. Crushed coral or sand works well. For sub-adults and adults, it is important to add vertical relief in their holding. Live rock, faux sea grass, or empty coconut shells work well. The additional décor helps maturing *Metasepia* by providing environmental complexity and visual security in the form of refugia and/or opportunities for escape from conspecifics.

Air diffusers should not be utilized because the tiny bubbles they create can occasionally get trapped inside the mantle cavity. Trapped air will negatively impact buoyancy control and increase stress. Traditional routine maintenance used in aquaria tends to increase stress levels in *Metasepia* and may hamper development of this culture. Siphoning/gravel washing should not be performed more than once daily. Similarly, window maintenance should only be conducted as needed. This maintenance will only add stress to the captive population and hinder culture progression. Using live food and low light levels to decrease the necessity of stressful "routine maintenance" is recommended for *Metasepia.*

Feeding

Metasepia sp. larger than 2cm mantle length (~2 months old) should be on a diet of glass shrimp, *Palaemonetes sp.*, or other similar live feeder shrimp (see the "Hatchling Care" section for feeding instructions for younger individuals). These *Palaemonetes* are a small (1-3cm) brackish water species of shrimp. These can be obtained from a number of venders and retailers, MBA orders through Northeast Brine Shrimp in Oak Hill, FL. Regardless of life stage, *Metasepia* prefer shrimp to crabs and fish. The mono-specific shrimp diet resulted in no observable untoward effects on growth or reproductive output of *Metasepia*. Glass shrimp should be size-selected based on the size of the cuttlefish cohort being fed. Prey items should be smaller than 3/4 the length of the mantle. Larger prey items may damage *Metasepia's* delicate feeding tentacles. Prey items of inadequate/small size will not provide an adequate nutrition for healthy growth and reproduction.

Adult *Metasepia* female using feeding tentacles to catch glass shrimp *Palaemonetes sp*.

Feeding sessions should be carried out three times daily. The *Metasepia* should be fed to satiation at each feeding session. Most individuals will accept 2-4 dietary items during each session (depending on the size of the food offered). Attempts to entice *Metasepia* to consume frozen food have been moderately successful at best. The use of a clear feeding stick to offer thawed, frozen food (shrimp, prawn, fish, etc) has been associated with the most successful non-live feedings. When one considers the time investment in offering frozen diets versus the minimal expense of live glass shrimp, little to no advantage to frozen diets has been demonstrated.

Excessive stress-related behavioral displays, such as uncontrolled buoyancy or intense/abnormal color flashing, may be an early indication of nutritional deficiency. Live food should be added immediately. If there is obvious prey present and the stress-related behavior persists, do not add more food and look for other sources of stress (i.e. aggression, water quality, etc).

Reproductive considerations

When selecting broodstock, the male to female ratio in each holding tank warrants consideration. If there are too many males, excessive competition for available females will occur and stress-related behaviors will likely ensue. Signs of stress-related behavior include uncontrolled buoyancy, excessive color flashing, inking, and jettisoning into the holding tank walls. Additionally, males may flush out a competing male's spermatophores with their siphon (Hanlon et. al. 1999), thus hindering reproductive efficiency. If there is a heavy female to male ratio, reproductive females will be forced to compete for food resources and laying grounds. This concern is dependent upon the size of the enclosure. A male to female ratio of 1:2 seems to be a reproductively efficient pairing for typical 75-200 liter holding spaces. Maintaining a small reproductive cohort also facilitates more accurate tracking of genetic lineage in multi-generational cultures.

Sustainable cultures benefit from fresh genetic input every second or third generation. In *Sepia officinalis* and *Sepia pharaonis,* inbreeding directly affects morphological appearance/condition and causes a reduction in size through progressing generations (Lee et. al. 1994). This does not appear to be the case with *Metasepia.* The Monterey Bay Aquarium has reared five generations of *Metasepia pfefferi* and three generations of *Metasepia tullbergi* with limited morphological variance through each generation. Decreasing female fecundity, mating compatibility, and overall reproductive success were the primary negative outcomes observed in multiple generations of *Metasepia* cultures.

<u>Egg care</u>

There are advantages and disadvantages for either natural maternal or artificial incubation of cuttlefish eggs. Maternal incubation provides consistent and reliable incubation for all eggs in the clutch. There is also less work for the attending biologist. Maternal incubation is a consistently reliable option if the goal is to simply have reproductive success. In order to maximize the reproductive efficiency of broodstock, artificial incubation should be considered. One significant negative aspect of maternal incubation is the fact that the female must allocate a large portion of her energy towards egg incubation rather than maximizing her reproductive productivity. If the eggs are removed for artificial incubation, the laying female quickly abandons brooding behavior and returns to mating and subsequent oviposition. Eggs can be removed with medical forceps. Artificial incubation permits biologists to record gestation period(s), egg hatchability statistics, and note exact hatch date of progeny. Artificial incubation also allows for an extensive knowledge of the development and progress of nearly every embryo produced. This greatly reduces the chance of an unmonitored hatching where the sensitive progeny may be in danger of cannibalism, starvation, or being flushed to discharge. Harvesting eggs for artificial incubation also allows alternate males to be introduced to broodstock females, genetically diversifying the female's reproductive output.

If eggs are artificially incubated, they should be agitated vigorously for the first two or three weeks. This initial vigorous agitation/circulation is critical, as it keeps the embryos oxygenated and prevents the settling of debris and bio-fouling organisms (hydroids, bacteria, and fungal recruitments). If the agitation is too intense, damage can occur to the egg tunic. It is important to find the correct balance.

This is one example of a vigorous incubation model that was successful at the Monterey Bay Aquarium (Image 2).

This design was constructed using two 12 fl oz empty plastic bottles as the incubator shell. A screen mesh partition was adhered to the inside of one of the bottles using non-reactive bonding compound (i.e. Dow Corning silicone 999). The two bottles are then combined using the same bonding compound. The upward facing cap should be removed to allow a discharge point for air bubbles and exiting sea water. The bottom facing cap face is removed and replaced with screen mesh, and adhered to the cap rim for sea water supply. The exiting air bubbles from the air stone drive fresh sea water in through the bottom and incubate the embryos. The diameter of the supply/discharge cap openings dictates flow mechanics within the incubator.

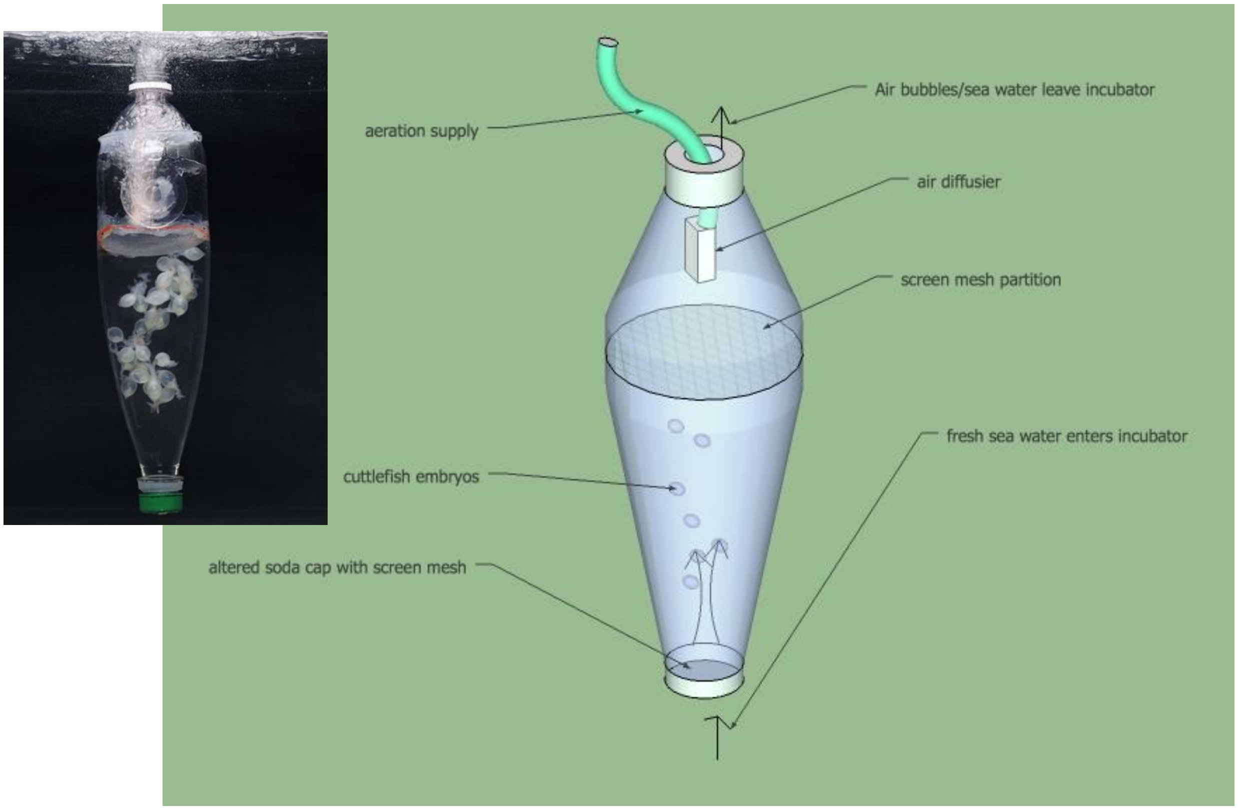
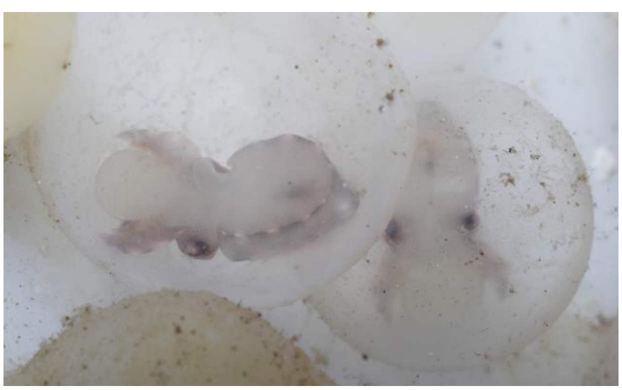


Image 2: Embryo incubation device used at MBA (Diagram with photo inset).



Developing *Metasepia* in eggs.

Metasepia eggs laid underneath clam shell.

Embryonic development can be observed with *Metasepia* eggs as the tunic/albumen is transparent. Days before the yolk is completely absorbed by the developing embryo, the egg should be transferred to a more passive incubation system. Cephalopod eggs incubated at the upper end of their species-specific temperature requirements develop more quickly than at lower temperatures (Domingues et. al. 2002). Therefore temperature will have direct influence on how long the embryos should be in each style of incubator. For our culture purposes we found this passive incubation model to be effective (Image 3). This design utilizes ¾ inch PVC as the primary structure. Sea water supply ports are drilled to guide fresh sea water over the plastic mesh basket. Sea water supply ports should be drilled so that there is equal flow over the entire mesh basket. As the hatchlings emerge, they can drift safely out of the flow and come to rest on the culture tank bottom.

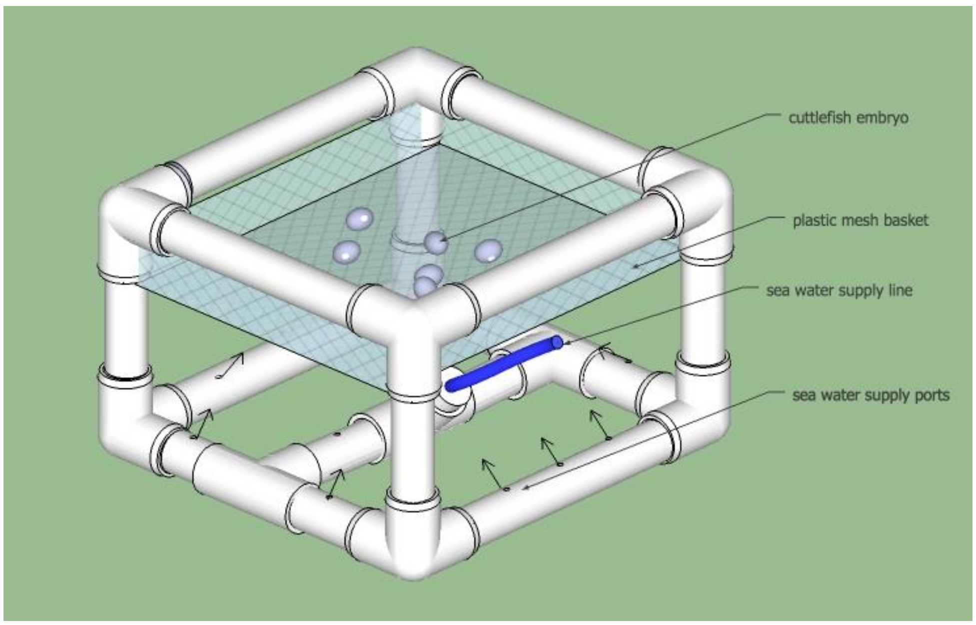


Image 3: Passive incubation device used at MBA.

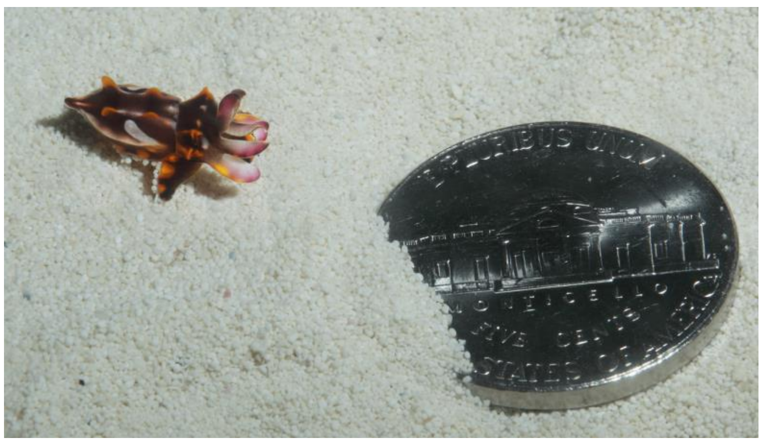
If excessive bio-fouling occurs on the egg tunic, the permeability of the embryo can be reduced. This can cause asphyxiation in developing embryos and eventually lead to embryonic mortality. Similarly, bio-fouling organisms can degrade and weaken the tunic, leading to pre-mature birth. To remedy this, an immersion bath of 1ml of iodine/Betadine to 1 L of seawater for 10 minute treatments every other day as needed can be applied (Lee et. al. 1994). This method has resulted in no harmful effects on embryos and has been successful in limiting the biological growth on the eggs' external tunic.

Hatchling Care

As with many aquatic invertebrates, the hatchling stage is when *Metasepia* is the most sensitive. Positioning the holding system away from high traffic areas or areas with excessive noise/vibrations is recommended.

Metasepia hatchlings should be kept in smaller holding tanks ranging from 15 to 200 liters depending on the hatchling population density. Tanks should be more horizontal than vertical, since hatchlings spend the majority of their time on or near the bottom. If the tank is too vertical, live food items may go un-noticed near the surface. Tank walls should not be transparent to assist in the visual detection of prey, as tiny prey items are difficult for hatchlings to locate with transparent walls. This may be achieved by adhering an opaque, non-permeable, non-reactive material inside of the holding walls (i.e. kydex). Seams where this material meets are also sealed with silicone to avoid hatchlings getting wedged in a corner. Tank discharge areas need to be screened (<3mm) such that hatchlings are not inadvertently swept out.

Smaller substrate (<1mm) is preferred to aid in locating hatchlings, facilitating population counts, and making behavioral observations. Smaller substrate also allows hatchlings to locate food with greater efficiency. Large decor is not necessary for the first two months of *Metasepia sp.* life span as it obstructs visual observations of the cohort and hinders prey detection by the hatchlings.



One day old *Metasepia* hatchling next to nickel.

Flow rates should be low (< 2L/min) during this sensitive developmental period to decrease stress. Low flow will also allow dietary items to remain in the tank for longer periods of time, thus extending feeding opportunities.

Captive light cycles should mimic natural light cycles with 12 hours of daylight and 12 hours of darkness. Intense light is not recommended as it tends to increase bio-fouling. Moderate florescent or LED lighting is preferred. Blue-tinted "moonlight" during the night period is necessary as it decreases captive stress and allows for crepuscular feeding.

Hatchling stocking density should never be greater than one individual for every 7.5 cm^2. Hatchlings exhibit uncontrolled buoyancy and stressed behavior if densities are too high. There is also greater competition for food resources and habitat.

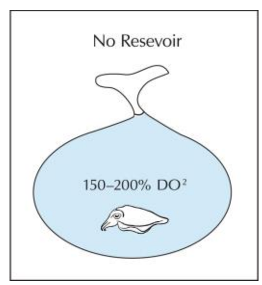
For the first two months of life, captive *Metasepia sp.* should be fed exclusively live mysid shrimp, *Americamysis bahia*. Adult *Artemia salina* are too nutrient deficient to nourish a growing cuttlefish. A culture utilizing *Americamysis bahia* nourished with a healthy *Artemia* nauplii (small *Artemia* hatchlings) diet has proven successful. Mysids should be offered three times a day at a rate of 3-5 mysids per individual. Excessive prey in the holding tank may be stressful for the hatchlings and may suppress their natural predatory behavior. Before each feed, be certain to search for any remaining mysids from the previous feed to prevent overfeeding. If there are still two or more mysids per hatchling, do not add more.

Offering the correct mysid size is paramount to hatchling survivorship. *Metasepia* less than three weeks of age should not be fed adult mysid shrimp. In the initial weeks of *Metasepia's* life span, they are too weak to capture adult mysids, therefore younger shrimp (1-8 days of age) is recommended. Although *Metasepia* are diurnal, some feeding occurs at night. It is important to leave two or three extra mysids per hatchling after the final feed of the day.

Tank maintenance, such as siphoning or gravel washing should only be conducted once a week. The live feeding regime and the small size of the *Metasepia* help preserve adequate water quality for a longer time period. Additionally, gravel washing is an added stressor that is important to avoid during early development.

Shipping Considerations

Hatchlings should be at least four weeks old (~1.5 cm ML) before shipping. Use 1-10 liters of sea water per individual, depending on the size of the *Metasepia*. The shipping water needs to be highly oxygenated prior to shipping (150%-200% dissolved oxygen). The preferred shipping method does not utilize an air/oxygen air space sealed inside the shipping bag. Removing any empty air space greatly reduces agitation inside the shipping bad and decreases stress during transit. Empty air space can also lead to air becoming entrapped in the cuttlefish's mantle cavity and subsequent buoyancy issues and stress. Shipping with no empty air space decreases shipping stress but reduces available oxygen and has other water quality implications. This concern may be mitigated by shipping *Metasepia* with an adequate volume of highly oxygenated sea water. *Metasepia* should be packed in individual bags. Smaller individuals are not only less expensive to ship, but are also associated with a higher survival rate.



Temperature considerations are necessary depending on the geographic location the cuttlefish will be shipped to. Heat/cooling packs will need to be implemented accordingly. Shipping animals on the cold side of their biological requirements will lower the metabolic rate of the animals and hold more oxygen.

Metasepia less than six weeks of age should not be fasted prior to shipping. Fasting hatchlings at this age is dangerous due to the high metabolic requirements at this early developmental stage (Foresthye et. al. 1994). The impact of malnutrition over a 24 hour shipping period is actually of greater concern than deteriorating water quality.

Consistent and efficient shipping methods are important in facilitating successful *Metasepia* culture collaboration between institutions. This collaboration will prove to support genetic diversification between various cohorts of the species The ability to maintain and share diverse genetic populations not only enhances the sustainability of the species in zoos and aquariums, but also significantly reduces potential collection pressures of wild populations.

Conclusions

The flamboyant and paintpot cuttlefish (*Metasepia*) are highly charismatic marine invertebrates with significant potential for public display. Until recently, however, the transport and captive culture of these species have proven to be significantly challenging and largely unsuccessful. Advancements in *Metasepia* propagation and transport techniques at the Monterey Bay Aquarium have proven that these species can be successfully maintained in captivity over several generations. Cultured offspring have subsequently been provided to several other AZA institutions. MBA developed husbandry techniques have allowed some of those institutions to begin successful propagation efforts of their own.

These techniques will serve to relieve wild collection pressure on what has historically been a challenging genus to handle and bring to public display. Ultimately, we hope that what we have learned about *Metasepia* biology and captive life cycle will help to inform future understanding and conservation of these species in the wild.

Acknowledgments

I would like to acknowledge the Monterey Bay Aquarium staff, volunteers and interns for their diligent, detail oriented work with throughout this culture. In particular, Ellen Umeda (now at SLP Toronto) was a major contributor to this project during her internship at MBA. I would also like to thank Alicia Bitando for assisting with data analysis and graph creation. Thanks to Marcus Zevalkink, Paul Clarkson, and Dr. Mike Murray for assisting with the writing and editing process. Thanks to Randy Wilder for taking most of these beautiful photographs. A final thanks to the MBA husbandry management team for their continued support on this project.

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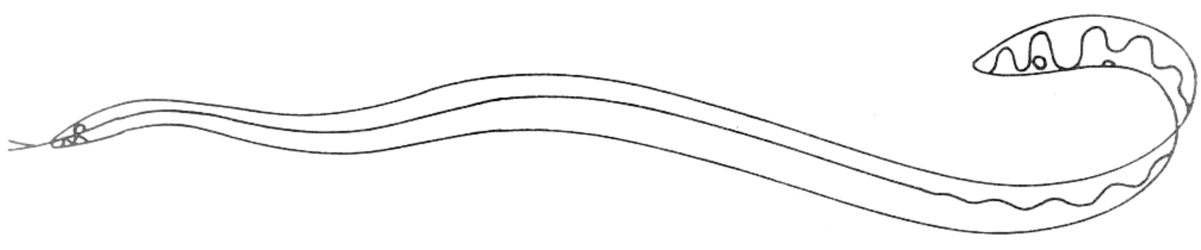

RAW
NORTH CAROLINA AQUARIUMS
2014

General Information for RAW 2014

Host Institution: North Carolina Aquariums; Wilmington, NC, USA
Conference Hotel: Hilton Wilmington Riverside
301 N. Water Street, Wilmington, NC USA 28401
Telephone: +1-901-763-5900
Conveniently, book your RAW 2014 lodging **here**
Rate: $139 +$18.07 (tax) per night for a double, $149 triple, $159 quad
When: April 21–25, 2014 (TAG meetings on Monday April 21st.)

Tentative Schedule for the Week:

Megalodon Tooth/Shark Dive	April 20 and 26
Pine Knoll Shores/Sea Turtle Hospital	April 20 and 26
TAG Meetings	April 21
General Sessions	April 22-25
Ice Breaker; Cape Fear River Shores	April 22
Aquarist Olympics and Aquarium Evening Event	April 23
Hospitality Venues	April 21 and 24

Abstract Deadline: **Feb. 7th. See website for more information**

www.rawconference.org

Registration: **Before March 28th, 2014** (fee per person)

$75 Public Aquarium or Non-profit affiliate
$125 Commercial Affiliate

Late Registration Commitment Form; Form can be found on the RAW website www.rawconference.org

Your facility will commit to send you to RAW for a non-refundable $25 (fee per person).
The $25 commitment fee is an addition to the normal registration fee bringing the total registration fees to:
$100 Public Aquarium or Non-profit affiliate
$150 Commercial affiliate

Airport & Other Transportation Options:

Wilmington is located on the southeastern coast of North Carolina. The city can be accessed directly by Wilmington International Airport (ILM) and several major interstates and highways.

Currently US Airways and Delta fly into ILM. Below you will find sample roundtrip fares from major metropolitan areas. These fares are not guaranteed and are offered only as a budgetary guide.

Additionally, Raleigh-Durham International Airport (RDU) and Myrtle Beach Airport (MYR) are within a two-hour drive of Wilmington.

Transportation Between Hotel and ILM; 15 minute travel time

Type	Typical Minimum Charge
Courtesy Bus To and From ILM 5am-11pm	$0.00
Taxi	$20.00 USD

RAW 2013 ABSTRACTS

Regional Aquatics Workshop, April 23 - 26
Georgia Aquarium, Atlanta, GA, USA

Monday, April 22
Pre-RAW AZA Conservation Group Working Meetings

Coral Reef CAP
Aquatic Invertebrate TAG
Freshwater Fishes TAG
Marine Fishes TAG
Aquatic Interest Group (AQIG)

Tuesday, April 23
Conservation Part 1, Session 1

Animal Professionals

Animalprofesional.com has partnered with RAW this year to document presentations online. Attendees will have access for one year.

Keynote Address
Vice Adm. Dr. Conrad Lautenbacher
Ocean Health and Conservation

Sponsor Presentation – Piscine Energetics

Monterey Bay Aquarium's Project White Shark at 10 Years Old
(or How to Shoot Yourself in the Foot)

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Paul Clarkson
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Monterey Bay Aquarium

In 2002, the Monterey Bay Aquarium (MBA) initiated Project White Shark. From its inception, several goals were established for this program: to better understand the biology and movements of these ocean predators through scientific research, to systematically determine whether it would be possible to display a young white shark successfully, and to communicate with our visitors and the public in ways that would lead to continued protection of white sharks and to highlight the need for global conservation of shark species.

As the program enters its eleventh year, we have documented compelling impacts upon our guests and visitorship, education programs and research initiatives, and conservation-based advocacy efforts. MBA-supported field research has redefined what we know of this species' biology and movement patterns in the Eastern Pacific Ocean. Initially, significant aquarium attendance impacts were realized; however, in the following years those impacts declined, raising some interesting questions. Survey data support the assumption that the presence of a white shark on exhibit has indeed raised public awareness of this species. Our advocacy efforts related to the protection of sharks have had quantifiable public policy results. 2013 now sees the beginning of an evaluation period to consider the listing of white sharks under the federal and California Endangered Species Act. If this listing is formally adopted it will have significant implications on the future of the program in its current form. While the husbandry advances achieved through this program have been considerable, Project White Shark exemplifies the kind of broad-scope impact that public aquarium programs can and do achieve.

An Update on Sawfish in Aquaria, the Global Conservation Strategy

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Dallas World Aquarium

This presentation will include information originally presented at the London Zoo in May 2012 during the IUCN Shark Specialist Group's Global Sawfish Conservation Strategy Workshop. The presentation synopsizes the work being done in public aquarium facilities in terms of cooperative breeding plans and public education, research, etc. The outcomes of the meeting, including the opportunities for future collaborative programs for sawfish conservation, will also be addressed.

New Marine Zoning in the Florida Keys National Marine Sanctuary and Other Regulatory Issues Affecting the Supply of Marinelife to Public Aquariums and Zoos

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Dynasty Marine Associates Inc.

As caretakers and sustainable suppliers of marine life and as ardent supporters of environmental sustainability, most of us stand behind the concept of Marine Protected Area's (MPA's) and the listing of threatened and endangered species as protected.

In fact, one could also say that most, if not all of us, consider ourselves to be conservationists, environmentalists, or environmentally conscious individuals. But what do MPA's and protected species ultimately mean to the supply of display animals to public aquariums and zoos that are responsible for promoting education and conservation to the hundreds of millions of individuals who visit their facilities each year?

As the operations manager of a company whose primary responsibility is the supply of many different marine species, as display animals, to zoos, aquariums and educational facilities; it is paramount that we are acutely aware of the many changes that are occurring across a wide variety of fisheries and fisheries management regimes on a continual and ongoing basis.

Operating primarily in the Florida Keys and within the FKNMS creates a unique set of circumstances. We are currently under the management jurisdiction of six different fisheries agencies on a State and Federal level and are surrounded by more than a dozen different state and federal parks, MPA's, wildlife refuges, and other restricted use zones within the FKNMS. All of these regulations affect what is, or is not allowed to occur, in and out of these areas and all the time bearing in mind that many of these regulations are changing on an annual basis.

This presentation will focus on the different agencies, rules and regulations, how those rules and regulations are evolving, and how that is affecting the collection of display animals.

Sponsor Presentation – Quality Marine

Whale Shark Aggregations in Yucatan Mexico

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Georgia Aquarium

There has been a veritable explosion of research interest in whale sharks over the last 10-15 years. Among this body of work has been the discovery that whale sharks are not always solitary open ocean nomads. Rather, under the right conditions they can and do form large groups, seasonally and sometimes quite close to shore. Two such aggregations occur in Yucatan Mexico: one near Isla Holbox and one offshore from Isla Contoy. The former is a turbid water aggregation that feeds on zooplankton, whereas the latter is a blue water aggregation that feeds on fish eggs and is the largest whale shark aggregation known to science by a large margin. Tools like aerial surveys, satellite-linked tags and photo identification by spot pattern matching are beginning to unravel the biology of these extraordinary aggregation events and tell us more about whale sharks in general. This is important because they are ambassadors for a threatened and much maligned class of vertebrates and, indeed, for the entire pelagic zone. Understanding this species and the aggregation events are key components of enlightened science-based conservation strategies for the oceans.

Project Piaba: 2013 and Beyond

Scott Dowd
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New England Aquarium & Project Piaba

Project Piaba is an entity well known by the RAW community. This presentation will give a very brief overview of the background of the project, critical issues, goals, and challenges. Much has happened with Project Piaba since the presentation at RAW in 2012. The role of the project within the AZA is coming into focus. New partnerships with key members of the aquarium fish industry have been established. Project Piaba's goals of fostering sound fishery management with benefits to local communities appears to be generating harmonized enthusiasm from mainstream conservation organizations as well as leaders in the commercial trade of live fishes.

Comprehensive Blood Analysis of Free-Ranging Sandtiger Sharks *(Carcharias taurus)* In Delaware Bay

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Georgia Aquarium

The sandtiger shark (Carcharias taurus) is a popular exhibit specimen among public aquaria. While ranges for hematologic, biochemistry, blood gas, nutritional and hormone parameters have been reported for sandtigers maintained under human care, ranges for these parameters have not been established for wild populations. The objectives of this project were to measure baseline health and nutritional indices of free-ranging sandtiger sharks in Delaware Bay. These data will not only contribute to the management of sandtigers under human care, but may also contribute to the development of a conservation plan for this species in the wild. During the summers of 2011 and 2012, 162 sandtiger sharks were captured via longline using non-barbed, circle hooks. Animals were positioned in dorsal recumbency, morphometric data was collected, and tooth, skin and blood samples were obtained. All sharks received a dart tag and adequately sized animals also received acoustic and/or satellite transponders. Blood was collected from the caudal tail vein, and blood gas values were immediately measured using a point of care analyzer (iSTAT, Abbott Labs, Princeton, NJ 08540). Blood samples were analyzed for additional indices including complete blood counts, blood cultures, comprehensive chemistries, trace minerals, heavy metals, vitamins, fatty acid profiles, sex and stress hormone levels and serum protein fractions via electrophoresis. Preliminary results suggest some variability amongst many of the individual parameters, and numerous analytes varied between those reported for managed sandtigers and this wild population.

500 Million Years on Earth and We Still Don't Know Jack: The Life of Nautiluses

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Humans and nautiluses have coexisted on Earth for 200,000 years but have experienced very little interactions, until recently. In the late 19th century, formal research into nautiluses began with Arthur Willey's expeditions throughout the Indo-Pacific. In the late 20th century, humans began to view nautiluses not just as a research endeavor, but as a marketable commodity. Nautilus fisheries were created throughout the Indo-Pacific, primarily in the Philippines, to supply a new, world-wide demand for their ornamental shell. Since these fisheries formed, anecdotal reports of nautilus population decline have become common throughout their habitat. However, without scientific data to report these claims, there have been no conservation efforts or regulation measures in place to protect nautiluses. In 2011, a team of researchers began a project to determine the state of nautilus populations throughout the Indo-Pacific to quantify the effect of fisheries. Four separate locations were selected in the Indo-Pacific: Philippines, Australia, Fiji, and American Samoa. The Philippines population represents a fished population whereas the other three locations represent un-fished populations. The data collected confirms the earlier anecdotal reports of population decline in fished areas, like the Philippines. Further, the population levels of nautiluses in un-fished areas appear to be relatively low in its natural state, suggesting that nautiluses are highly susceptible to unregulated fisheries. Based upon these studies, nautiluses are highly sensitive to overexploitation. Conservation initiatives and management practices should be in place to ensure healthy populations of nautiluses, but also sustainable fisheries. Ensuring the survival of nautiluses will enable researchers to investigate other questions and mysteries of an animal that has lived on Earth for 500 million years, unlocking even more secrets of the Earth and its history.

The Most Important Corals You Have Probably Never Heard of

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The corals of Alaska's Aleutian Archipelago may be some of the most important corals you have probably never heard of. From an ecological standpoint, they are believed to be the most biologically diverse collection of cold water corals in the world. These corals are also extremely important economically. Eighty-five percent of commercially fished species in Alaskan waters are, at some point in their life cycle, associated with corals and other deep water, structure forming invertebrates. With the commercial fisheries of Alaska valued at 2.4 billion dollars annually, the argument could be made that the health of the corals in Alaskan waters is a 2 billion dollar a year issue. Threatening these animals are a suite of issues ranging from mineral exploration and bottom contact fishing operations, to water chemistry shift due to climate change. These threats are further amplified by the slow growth rate and recruitment limitations of many corals, which leads to extremely slow recovery rates after a disturbance. Considering the value of the coral community of the Aleutians, the threats they are facing, and their long recovery times, there exists a very clear and present need for greater understanding of cold water coral communities. Studying these communities, as well as their connectivity to each other and surrounding habitats, is vital to developing proper management practices. While there have been previous studies that have looked at the coral community structure of the Aleutians, new genetic techniques are emerging which may allow us to get a picture of these communities at a resolution never before possible. This study utilizes 3 mitochondrial gene markers from nearly 1000 deep water corals off the Alaskan coast to attempt to determine if genetic methods provide a substantially different picture of community structure than the traditional species designation based studies.

Collection and Successful Long Distance Transport of Atlantic Tunas

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A collection, husbandry and transport method has been successfully developed to catch and transport juvenile individuals of Atlantic blackfin tuna, *Thunnus atlanticus*.

Challenges that were discovered and solved will be discussed along with the discussion of results of a 45 hour duration trans-Pacific air transport. The authors feel a compelling case can be made for the ability to catch and transport other additional species of Atlantic tunas and other pelagic species.

Tuesday, April 23
Animal Management & Training

Sponsor Presentation – CaribSea

Behavior Modification to Alleviate Stereotypic Behavior in a Whale Shark *(Rhincodon typus)*

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Georgia Aquarium houses four whale sharks (*Rhincodon typus*), two males and two females, in the Ocean Voyager exhibit. In 2010, one of the males known as Yushan, developed a stereotypic swim pattern that prevented him from utilizing the entire exhibit. Stereotypic behaviors, such as Yushan's, can develop in response to environmental stressors and often include unvarying, repetitive patterns with no obvious goal or function. Yushan's stereotypy has resulted in a virtually complete right-turning swim pattern, in circles of varying diameter, interrupted only to participate in regularly scheduled daily feedings. While the cause of Yushan's change in behavior is unknown, it became evident that the swim pattern was detrimental to his physical appearance and potentially his long-term health. Indicators such as muscle atrophy and dorsal fin curvature developed over a relatively short time period. The Aquarium team developed a training plan to address this undesirable behavior including changes in feeding approach, additional feedings for enrichment, and physical therapy. As a result of this training, Yushan now utilizes a greater portion of the Ocean Voyager exhibit, the diameter of his circles has increased, and his body condition appears improved. Additionally, and as a result of Yushan's underwater feeding sessions, we have obtained updated measurements and blood samples while under stimulus control. The Aquarium team continues to work with Yushan to address his current condition, with the goal of extinguishing the stereotypy and encourage a swimming behavior that will promote achieving adult size and long-term display residency.

"Brushing" Up on Octopus Enrichment

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Harrison Baker, McKenzie Reeves, Nadia Stegemen, Aimee Reed, Mark Farley, Tim Miller-Morgan
Oregon Sea Grant

In 2012, husbandry staff at the Hatfield Marine Science Center (HMSC) Visitor Center in Newport, Oregon identified a need for a new enrichment protocol for a Giant Pacific Octopus (*Enteroctopus dofleini*). This coincided with a need for improved visitor engagement with the display octopus. Husbandry staff decided to venture away from conventional methods of enrichment. Instead, they constructed a PVC frame device to hold a canvas with brushes above water attached to a stylus-toy device in the water allowing the octopus to "paint" by manipulating and retrieving food from a Mr. Potato Head toy.

From an animal enrichment perspective, the project was a success. The octopus remained engaged with the stylus-toy device for prolonged periods compared to similar enrichment sessions without the painting apparatus. The use of an elastic pulley system to smooth the movement of the stylus also provided passive resistance, affording some degree of physical exercise and triggering the octopus's prey drive.

From a visitor engagement standpoint, the project was also a success. However, husbandry staff eventually elected to hold the painting sessions during closed hours due to the difficulty of managing crowds around the tank and the tendency of the octopus to become distracted. Artistic collaborations with HMSC researchers/artists added depth and definition to the abstract paintings, and provided a link for the public to the broader research community at HMSC. The "painting octopus" generated industry recognition, sparked public interest in the animal's behavior and intelligence, and provided a signature outreach product for HMSC's visitor center.

Acquisition and Operant Conditioning of the Lesser Devil Ray

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Transportation and husbandry of *Mobula sp*., has shown to be a difficult endeavor. Georgia Aquarium has had success with a particularly resilient Lesser devil ray (*Mobula hypostoma*) acquired in May of 2009. One of the challenges faced was the establishment of feeding behavior. The aquarist team attempted various combinations of food items, delivery devices and start-of-session cues. After feeding behavior was observed, operant conditioning techniques were developed and utilized to provide the foundation for the ray's addition to the Aquarium's Ocean Voyager exhibit. Environmental changes included exhibit size and shape, and tank-mate composition. These variations caused a fluctuation in feeding behavior, health and body condition. Medical treatments were necessary at times, so it was important to establish a plan that would minimize the ray's stress while allowing for tube feedings, supplement administration, wound care, and general examinations. Building upon success and experiences with this particular animal led to acquisition of six additional *Mobula sp*. in August of 2012.

Management of *Arapaima gigas* with Operant Conditioning

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Arapaima gigas is the longest freshwater fish species in the world. This fact makes them popular exhibit animals in zoos and aquariums. But the power, large size and flighty nature of this species makes them difficult to handle.

The Pittsburgh Zoo's PPG Aquarium began training with our large arapaima in 2010 as a means of ensuring that it received the proper diet. Since then the training program has expanded to include more behaviors and loftier goals. The large arapaima has been desensitized to tactile, target-trained and trained to transfer. We have highlighted the training in regularly-scheduled demonstrations for our visitors during the busy season. And we are working toward stretcher training with this flighty species. We have also added two younger specimens to the collection and have achieved progress in their training over the past year. This presentation will discuss the challenges and solutions for training. Videos and photos will show the various behaviors we have trained with our *arapaimas*.

Wednesday, April 24
Animal Health, Session 1

Sponsor Presentation – Pentair

Lazy Nettle Syndrome: A Brief Update from the Tennessee Aquarium

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In 2009, the *Chrysaora fuscescens* at the Tennessee Aquarium began to suffer from a total loss of pulsing ability, but they continued to collect some food and did not appear to die. The condition persisted through several groups of sea nettles at our facility and was also observed at other institutions. At RAW 2010, this distinct group of symptoms was affectionately dubbed "Lazy Nettle Syndrome" (LNS). The chronic and repeated nature of the syndrome made it clear that it was not an anomaly and required investigation. Subsequently several theories about cause(s) were put forward including source water quality, group origins (i.e. wild vs. captive raised), and nutritional deficiency. Following a colleague's suggestion, our jellyfish diets were fortified with the omega-3 fatty acid, DHA. Within a short period following this diet change, several specimens did display improvement but recovery was not complete or consistent throughout our collection. Most recently, a group of sea nettles reared at the Tennessee Aquarium on the enriched diet have been on exhibit since June 2012. This entire group remains healthy with no signs of LNS. Although unsuccessful at completely reversing the symptoms, the addition of supplemental DHA seems to be the key factor in the prevention of LNS.

Techniques for Diagnosis and Treatment of *Spironucleus vortens* in Discus *(Symphysodon aequifasciatus)*

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Spironucleus vortens infection in Discus (*Symphysodon aequifasciatus*) is a persistent problem both in the public aquarium industry and in the ornamental fish trade. Further, many have linked this pathogen to cases of the poorly understood and insidious condition called Head and Lateral Line Erosion (HLLE). Consequently, hobbyists and aquarium professionals alike have tried, with varying results, to formulate techniques for diagnosis and treatment of *Spironucleus*. Diagnostics have often been limited to skin scrapes and/or fin clips. Treatments traditionally involve metronidazole - either added to food or administered via prolonged immersion. These approaches can work to a degree, but well-informed assessment of parasite load, before and after treatment, and accurate formulation of drug doses have always been problematic. Attempts to address these issues have led to techniques some may have avoided in the past due to Discus' relative fragility. Findings suggest that the preferred diagnostic tool, instead of a skin scrape, is a distal gut-contents analysis. Gut samples are collected by flushing the cloaca with sterile saline. Checking parasite load in the gut is critical since the infection is often not detectable in epidermal tissue. To solve the dosing issue, medicated gruel is administered, via catheter tube, directly into the stomach of the infected fish. Despite the relatively invasive nature of these procedures - which involve anesthesia, handling, and esophageal and cloacal tube insertion, vastly improved results were achieved with no negative effects on treated specimens.

Wet Preps 101 – An essential Part of Diagnosis Making

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The Aquarium Vet

Wet Preps 101 – Good wet preparations from either live or dead fish form the basis of making many diagnoses that involve external and internal parasites, bacterial infections and in some cases even viral infections. What is a wet preparation and how to make them. We will look at gill preps, skin preps and fin clips, as well as the internal wet preparations (liver, spleen, kidney and gut) that should be conducted at every post-mortem. I will show a variety of examples of how useful they are as well as demonstrating what is normal and what is abnormal. (Live Webinar).

Wednesday, April 24
Animal Health, Session 2

Sponsor Presentation – New Era Aquaculture

Treatment of a black spot shell disease in California Spiny Lobster (*Panulirus interruptus*)

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A common problem in crustacean health is black spot shell disease and it seems there have been few successful cases of treating it in the commercial and aquarium industry. Last year while responsible for a large California Spiny Lobster, I observed a black spot in the center of its carapace. It was removed from exhibit and placed into an isolation tank for further observation.

The diagnosis was an extensive erosion of the end of its tail progressing forward as an open infection sight. It also had black areas on its legs and carapace. Microbiology results were inconclusive. Research found that malachite green is the best treatment previously used in the industry in similar situations, it is unobtainable in California, which led us to try something new. The lesions were treated with Doxycycline Hydrochloride bath. The lobster was removed from the tank every morning in conjunction with the antibiotic treatment and I topically applied a chemical called MinnFinn to the affected areas. The active ingredient in MinnFinn is Hydrogen Peroxide and has been traditionally used to treat Koi for bacterial and parasitic problems. The erosion on the tail resolved and it was then my goal to get the lobster to molt and get rid of any remnants of the disease. It was fed a special high Calcium diet and within 2 months, it molted successfully into a spot free shell. The tail area was still slightly deformed, but it was completely healed of diseased tissue. It has continued to get a high Calcium diet and has since molted another time with an even better tail region. It is going to be put back on exhibit in the next month and as far as we can tell, the situation has completely resolved.

Ovariectomy of Sub-Adult Southern Rays *(Dasyatis americana)* to Prevent Reproduction and Future Reproductive Problems

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The Southern Stingray (*Dasyatis americana*) is reproductively prolific in captivity. In an effort to curb the over production of rays many aquariums maintain single sex populations in their exhibits. Mature female Southern rays that are unable to reproduce in a natural manner while on exhibit often develop a suite of pathological problems. To avoid reproductive pathologies as well as limit the production of young rays the husbandry and veterinary staff at Ripley's Aquarium teamed up to perform laparotomies on five female rays of varying ages and sizes. The rays varied in size from 55cm disc width to 83.5cmdisc width. The female southern rays were anesthetized using 70 ppm Tricaine Methanesulfonate. Before surgery, the ovary of each ray was located using digital ultrasonography. Each surgery lasted approximately 35 minutes, the rays recovered quickly from anesthesia and were returned to their respective exhibit or holding tank. Only two individuals were of the correct size and follicular development to allow for successful removal of the ovary. As a result, it was determined that the prime candidate for this surgical procedure must be in the 60-85cm disc width range. We believe that ultrasound can be used to stage animals in this size range to determine if the candidate has an ovary of appropriate size prior for surgical removal and that ovariectomy is a practical procedure for limiting the reproductive potential of Southern rays.

Coelomic Distention and Anemia in a Manta Ray *(Manta alfredi)*

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A reef manta ray, (*Manta alfredi*) at the Georgia Aquarium sustained impact to her dorsum during breaching in the exhibit, a normal activity for manta rays. An abnormal swim pattern and different posture was occasionally noted soon after, but food intake and behavior remained normal. Examination at the time was relatively unremarkable other than a possible abnormality along the vertebral column visualized via ultrasound. Over the next few months, her body condition declined despite normal food intake and coelomic distention became evident. Ultrasound revealed free fluid in the coelomic cavity and sand in the spiral colon, but no vertebral abnormalities were noted. Gastroscopy revealed large amounts of sand in the stomach, the result of frequent substrate sifting, a normal behavior in some mantas and one noted consistently in this animal since the time of acquisition. Stomach ulcers of varying degrees of severity were also visualized via endoscopy. Suction removal of the sand was only marginally successful. Blood work revealed a hypoproteinemia and anemia. Initial treatment consisted of a gel diet containing psyllium and mineral oil to help bind and remove the sand, sucralfate to coat the stomach ulcers and oral amikacin to treat possible infection in the gastrointestinal tract. The manta started refusing feeds within 5 days of the gel/psyllium/mineral oil addition to her diet. Normal feeding behavior returned when the gel was removed from the diet. Follow up exams revealed persistent sand and ulcerations in the gastrointestinal tract as well as persistently declining hematocrit and total protein. Sulfadimethoxine/ormetoprim was added to her treatment protocol to combat infection and as prophylaxis against *Eimeria southwelli*, previously diagnosed in other batoids in the same exhibit. Yunnan paiyo, iron dextran, and vitamin B complex were added to the treatment protocol because of the persistent anemia. After careful planning, permanent physical barriers were strategically placed on the bottom of the exhibit to prevent further sifting of substrate. With medication, physical removal of sand from her gastrointestinal tract and prevention of sand sifting, the manta gradually improved. The etiology of the manta's decline is unclear and the progression of her clinical signs not well understood, but the collaborative efforts of animal care and veterinary staff resulted in clinical resolution.

Wednesday, April 24
Natural Disaster Preparation & Recovery

Sponsor Presentation – Living Color

Surviving Superstorm Sandy

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Our presentation will include an intro of Jenkinson's Aquarium, brief description of Sandy, how our team responded, strategies to assess damage, strategies that provided initial support to the animal collection, progress mileposts and where we are today.

Disaster Prep and Recovery at New York Aquarium

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On the night of October 29, 2012 the New York Aquarium experienced severe flooding from the unprecedented storm surge associated with Hurricane Sandy. In a matter of minutes all of the Aquarium's animal life support systems and electrical distribution equipment, located below ground, were flooded. Rushing storm water quickly engulfed the first floors of most of the Aquarium's exhibit and support buildings. Eighteen staff members, who remained on site throughout the harrowing storm and for the next week, were able to stabilize life support systems and save the collection of marine mammals, fish and invertebrates with very little loss. Disaster recovery efforts were immediately put into place to mitigate flood damage and restore critical infrastructure. In this presentation the disaster proportions, recovery efforts and the future of the New York Aquarium will be discussed, including the storm's effect on the staffs morale and wellbeing.

Disaster Challenges in Aquatic Collection Management

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Contingency planning is a prudent exercise for any business, but has become a necessity for zoological facilities in the wake of recent natural disasters, changing AZA accreditation standards and emerging USDA APHIS requirements. Aquatic collection managers face unique challenges in disaster planning with the complexities that living water systems introduce. Modern life support systems rely on technologically advanced components to maintain water quality parameters with more precision than those simple systems of the past. Under disaster conditions we must understand the basic biological requirements of our specimens and the living components of our lss to successfully bridge the gap until full electrical and communications support is restored. The incorporation of basic lss components within aquatic systems that don't require significant power consumption or automation, and can serve as the sole source of support under adverse conditions is gaining.

Managing staff and resources before, during and after disasters strike is vital to the survival of collection specimens and the short term viability of a facility's recovery. Living aquatic collections pose challenges that are more complex than most terrestrial collections. Providing for aquatic animals safety while continuing to maintain their captive ecosystem under adverse conditions requires knowledgeable staff with the tools to adjust operations immediately. Disaster ride-out and recovery staff must possess unique skills including diverse aquatic husbandry, plumbing, electrical, use of S.C.U.B.A., chemical water treatment, capture and restraint of aquatic specimens, and aquatic specimen transport.

Aquatic exhibit design plays a role in collection managers' ability to respond to short term and longer term crisis situations. Using information from previous challenges allows us to be better prepared for future emergency operations. Infrastructure details including electrical supply grid protection, redundancy or flexibility in emergency power supplies, low energy emergency lss strategies, and provision of adequate access to lss and exhibit areas are all considerations that have come to light following real world experiences.

The long term recovery of aquatic facilities hinge on decisions that are made during and immediately after disasters strike. Providing for the short term survival of living specimens is generally considered a primary goal, but equally important are the choices that are made to protect equipment, resources and infrastructure that will be necessary to the organizations successful recovery. Aquatic system operations can be dramatically affected by how remediation measures are performed and understanding not only the biological implications, but also the mechanical and material nuances is important.

This presentation brings together several distinct aspects of contingency planning, preparation, ride-out and recovery with a focus on those challenges that are unique to aquatic collection management.

Thursday, April 25
Exhibitry, Part 1

Sponsor Presentation – EcoXotic

Two New Species of Octopus for Display: Coconut and Larger Pacific Striped Octopus

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Octopuses are one of the most captivating and crowd pleasing exhibit animals in public aquariums. The California Academy of Sciences is proud to be the first public aquarium to display two unique species of octopus: the Coconut Octopus (*Amphioctopus marginatus*), and a currently undescribed Central American species, the Larger Pacific Striped Octopus (LPSO). This presentation will discuss collection, display, husbandry and research of these engaging cephalopods.

The Sea Lasso: How to Catch a Kelp Paddy in One Swoop

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It is a common occurrence to come across drifting kelp (*Macrocystis pyrifera*) off the coast in southern California. If this drifting kelp does not wash ashore, it may drift at the ocean surface for approximately 100 days before decomposing. Also known as “Kelp Rafts” or “Kelp Paddies”, these mats of kelp have the potential to house many species of both pelagic and benthic fishes as well as invertebrates.

In 2003 the Cabrillo Marine Aquarium opened up a 1500 gallon “crawl under” plexiglas display tank which demonstrates what it's like to be under a blue water kelp paddy. This tank displays common fauna that is found under a wild kelp paddy, however collecting enough life to fill this display proved to be a tedious task. Over time we evolved to use a freediver driven-purse seine net to collect thousands of specimens in a single netting. The type of fauna vary from season to season and we have somewhat pinpointed the best times of the year to collect targeted specimens.

The Temperate Reef Tank

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Cabrillo Marine Aquarium

The surprisingly colorful temperate reef tank and the husbandry challenges accompanying it. A cold water temperate reef tank can be both diverse and surprisingly colorful, rivaling its warm water counterpart. We have been assessing the growth and changes of many invertebrate species in a 5-year long study in trial and error of the Plantlike Animals Tank at Cabrillo Marine Aquarium. Besides the obvious temperature difference, what most sets it apart from its tropical counterpart is the need for much more food and much less light, presenting its own set of maintenance and display challenges. Water flow requirements for different temperate cnidarians run the full range, from raging to very still. Even so, some species are tolerant of a wide range of conditions and others seem to be absolutely unsuitable for captivity. And like tropical cnidarians, many are territorial and will battle or overrun other species. A fascinating tank to have on display, one can see changes in the resident species evolve month to month and year to year.

A Living Coral Reef in Golden Gate Park: A Year 5 Update

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Begun with ambitious goals, one of the largest and deepest living coral reef exhibits in the world, The Steinhart Aquarium's Philippine Coral Reef exhibit will mark its 5th anniversary of operation in the spring of 2013. With over 1500 fish of some 120 species, and more than 800 living plants, anemones, giant clams, corals and other invertebrates this 212,000 gallon (803 m3) mixed taxa exhibit has not been without its challenges.

This presentation will cover some of the lessons learned over the last 5 years of operation, and will look at the challenges faced in water chemistry, feeding, water motion, lighting and collection building, and some of the ways in which each are being addressed.

Wednesday, April 25
Propagation

Sponsor Presentation – Dynasty Marine Associates, Inc.

How to Raise Your Dragon: Captive Propagation of the Weedy Sea Dragon, *Phyllopteryx taeniolatus*

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The weedy sea dragon, *Phyllopteryx taeniolatus*, is a popular exhibit animal in public aquariums around the world. The mysterious breeding habits of this species have resulted in only a few successful captive reproductive events. On December 24th, 2011, a successful egg transfer took place in a quarantine system at Georgia Aquarium.

During the 45 day brooding period, a variety of preparations were made in advance of egg hatch. Difficult decisions were made on fry collection strategy, assist feeding intervention and whether to separate the gravid male to ensure appropriate diet and caloric intake. Despite these challenges, 120 sea dragons hatched in early February, 2012.

This reproductive event was the largest single brood of sea dragons hatched in a U.S. public aquarium. With a survivorship of 56%, specimens from this group were shipped to six other U. S. institutions. A long-term strategy for repetitive successful breeding is currently under development.

Breeding Beluga Whale Food: Captive spawning and Rearing Arctic Cod, *Boreogadus saida*

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In 2009, the Vancouver Aquarium renovated its Canada's Arctic gallery and resumed displaying Arctic fishes and invertebrates again after a 15-20 year hiatus. As part of this redevelopment a series of collecting trips to the Canadian high Arctic were conducted and a variety of Arctic marine species were successfully transported to the aquarium for display. The Arctic cod, *Boreogadus saida*, was one of those species and is considered to be a keystone species in the Arctic food web. Due to the importance of this species for display, interpretation, and potential research, as well as the high acquisition costs to obtain them, captive reproduction was a major goal. In September 2011, viable eggs and sperm were successfully stripped from two adult Arctic cod. Fertilized pelagic eggs were incubated for close to one month at ~3^{0}C before hatching. Yolk sac larvae developed functional eyes and a mouth over a two week period following hatching. Following yolk sac resorption, larvae initiated feeding on enriched live rotifers. Harpacticoid copepods that had incidentally recruited into the rearing tank also may have provided an additional live food source. Larvae were transitioned from rotifers to enriched *Artemia* nauplii. Transitioning larvae from *Artemia* nauplii to a larger sized food type was challenging and a wide variety of items were offered including Cyclopeeze, finely chopped *Euphausia pacifica* and Otohime micropellets. Mortalities increased during this period until consistent feeding was finally established on the Otohime pellets. By spring/summer of 2012, ~500 *Boreogadus saida* juveniles had been reared successfully and were being displayed, dispositioned to another aquarium, and were being utilized outside researchers.

Reproduction of *Heterodontus portusjacksoni* at the Oceanário de Lisboa

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Ensuring the best maintenance and reproduction conditions for the species exhibited at Oceanário de Lisboa is a part of the institution`s mission. Portjackson sharks (*Heterodontus portusjacksoni*) are kept at Oceanário since 1998. This species was maintained successfully over the years, however reproductive behaviors were not observed and the females would lay only non-fertile eggs. Recently, in order to understand whether there is a direct effect of temperature in the reproduction, an annual variation in water temperature was performed. The natural temperature cycle for this species was followed. As a result reproductive behaviors were observed and viable eggs were produced. Embryo development is being followed by ultrasound and endoscopic observations. After more than 400 days of development the embryos are growing well and almost ready to hatch.

A Simple, Low-Cost, Low-Maintenance Design for In-House Live Brine Shrimp Culture

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The common brine shrimp *Artemia salina* is an important component of dietary requirements for public aquariums. Many aquariums spend a significant percentage of their food budget on importing live *Artemia* for feeding newly acquired and/or wild-caught specimens, or for supporting high maintenance species such as syngnathids. The North Carolina Aquarium at Fort Fisher (NCAFF) has successfully incorporated several methodologies for live brine shrimp culture into a low-maintenance design that utilizes a simple system including a hatch out bucket, 6 individual grow-out tubes and two “harvest barrels” for HUFA enrichment. Since 2006, this in-house setup has provided an average of 1 lb (0.5 kg) of enriched adult *Artemia* per week, yet requires less than 2 hours/week to maintain. The level of production is easily adapted to individual aquarium needs, and the design is especially advantageous for facilities struggling with limited space. The estimated total cost of in-house production is approximately 35% that of importing adult live brine from standard suppliers. Most importantly, the ready availability of multiple stages of live *Artemia* is critical to the successful propagation of seahorses and other species at the NCAFF.

Thursday, April 25
Live Collection Management

Sponsor Presentation – Emperor Aquatics

Use of Tablets for Data Entry and Record Keeping

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The field of aquatic husbandry is in a state of constant evolution. New techniques, findings, information, and technology continue to move our field forward.

At SEA LIFE Kansas City we are utilizing tablets and cloud technology to keep records, store data, and exchange information. There are many advantages to this, including having access to information and contacts almost anywhere, real-time updating of data, and the ability to take photos and videos for records. One of the bigger advantages is also the savings in terms of cost of labor, supplies, and waste. As we move from more paper oriented systems to more digitized ones, these savings become more important. While many institutions are using computerized systems such as ZIMS, ISIS, and TRACKS, the use of tablets allows aquarists to have all of their data and information with them at all times. This also reduces the time they need to spend compiling and entering the data, as they have their computer right there with them.

This is a system that has not been without challenges, and it is a work in progress, but it is working relatively well for SEA LIFE Kansas City. I would like to demonstrate the benefits and address the challenges, as this could very well be something that can be modified and tailored to other institutions' needs.

A New Kind of Fish Tattoo: Better Medical Management through Individual Identification of an Aquatic Collection

Megan Olhasso
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California Science Center*

As advancements are made in aquatic medicine, it is becoming crucial to maintain accurate, thorough records of individual animal treatments, movements and daily behaviors. Individual identification is the backbone that makes trending medical history possible; allowing for better decision making and interpreting success of treatment and of management choices. When fish are not individually identified, they become lost to follow up as soon as they leave the hospital system. It becomes impossible to determine if the next similar case is a reoccurrence or a new case.

At the California Science Center, we have implemented four ways of individualizing fish: visible implant elastomer for 'fish tattoos', notching, microchips and photo identification. The most broadly functional technique is visible implant elastomer (VIE). By grouping similar-looking species of fish into a spreadsheet, we are able to ensure that two fish cannot be mistaken for one another. It allows our divers to give accurate and detailed information about feeding behaviors, which then enables us to track medical treatments and easily communicate observations. VIE does not work on all dermal types. The use of notching and/or microchips on elasmobranches has proven to be an excellent way of maintaining current, accurate information and we are seeing similar results using photo identification as descriptors in marine invertebrate medical care – as we continue to explore new options in identification. Being proactive about individually identifying our collection has mainstreamed the flow of communication about individual animals rather than groups of animals. We are able to follow cases and determine if our treatment and management plans are successful. It allows us to recognize medical trends, identify problems, and create a data base of information that makes medical records and daily husbandry logs effective resources.

Why Cephalopods Need a National Set of Best Practices or Standards of Care

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Recently, the EU passed amendments to directive 2010/63/EU to include higher invertebrates in animal welfare considerations. This follows a similar ethical movement in Canada and Australia. As a university research lab in the U.S. studying cephalopods, while we are certainly concerned about animal care as it relates to research production, we do not have IACUC (Institutional Animal Care and Use Committee) guidelines or protocols to follow. Articles regarding cephalopod care are all over the map, making citation of one source over another problematic. Who has the best practices? Several EU-published journals now require that a statement or proof of animal care certification for cephalopods accompany a journal submission. Without a governing body knowledgeable regarding cephalopod health, behavior, reproduction, medicine, etc., providing such a certification is impossible. This poster reviews some existing publications regarding animal care and proposes suggestions for a national standard of care.

Thursday, April 25
Professional Development & Aquarist Training

Sponsor Presentation – Cairns Marine

Academics for Applied Science: A Brief Overview of the Aquarium Science Program; Past, Present and Future

Chris Spaulding
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Oregon Coast Community College

The Aquarium Science Program at the Oregon Coast Community College began in the Fall of 2003. Since that time, the program has formally educated and trained numerous students for a career in the aquatic animal care profession. The presentation will discuss this one-of-a-kind program along with its unique courses and practical hands-on learning opportunities. The history of the program, including the trends of its graduates and its contribution to the industry will be reviewed, along with the plans and strategies for offering practical learning to present and future aquarists entering a progressive field in an ever-changing academic environment.

Central Campus Revisited....What Have We Done?

Kirk Embree
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Central Campus Aquarium Science

A new 14,000 gallon Marine Science lab was built 3 years ago at Central Campus in Des Moines, Iowa. The 25 year old academic Marine Biology course was expanded at that time to also include a brand new career and tech course entitled Aquarium Science. The goal of the Marine Science program is to interest students in the world of Marine Biology and to provide them with a hands on experience to better help them understand what careers in this field might be like. A presentation of the new facility was given at the 2010 RAW conference in Omaha with promises of greatness, but included mostly just photos of shiny new empty tanks that had not proven themselves yet. This presentation will explore what worked... and what didn't, and the reality of operating a Marine Science lab run solely by high school aged students. The good, the bad, and the ugly.

Training the Next Generation; Developing an Intern Program

Rebecca Duchild
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Minnesota Zoo

We've all been there; the intern. Working for free, paying tuition to do all the dirty work of an aquarist, working the worst hours, etc. How do I know this will pay off, will I even get a job after this!? Now on the flip side, how should I train my intern? What do they need to learn and what should they know how to do to maximize their potential for getting a job in a market that has so very few openings, and how do I maximize their efficiency during their internship. As an aquarist for the last 13 years, intern coordinator for half of that, and with a dual degree in biology and secondary education, I have found myself asking these questions. I would like to share what I (and my coworkers) have gathered and created in that time period to answer these questions and help set our interns on a path to successful careers.

Making Science Work for You

João P. Correia
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Flying Sharks

In 2008 Flying Sharks began suggesting its clients the addition of a small fee to all invoices, which would be channeled to support research and conservation efforts. The vast majority of clients endorsed what then became the Flying Sharks Research Fund. Five years (and nearly 50.000 USD) later, multiple research programs have been made possible through this Fund.

This presentation provides a quick overview of conservation efforts that have been subsidized by the Flying Sharks Research Fund, such as Ximena Velez Zuazo's work with juvenile Hammerhead sharks in northern Peru, or the establishment of the IUCN's Shark Specialist Group Worldwide Sawfish Strategy, or Ilena Zanella's setup of "Misión Tiburón", a conservation organization focusing on shark species in Costa Rica, or Valentina Di Santo's PhD work on the effect of climate change in elasmobranch survival, or even Ismet Saygu's Master's thesis on elasmobranch commercial landings in Turkey, to name but a very few.

This presentation, however, mostly focuses on husbandry related results from research projects funded by the Flying Sharks Research Fund, such as the collection at depth of *Anthias anthias* and how its ascension rate should not exceed increments in 35% of pressure reduction for maximum survivorship, amongst other interesting findings. Or multiple sealed bags simulated transports with the objective of optimizing transport conditions – while ensuring 100% survivorship – of *Lipophrys pholis*, *Lepadogaster lepadogaster*, *Gobius paganellus* and *Diplodus sargus*, which yielded optimal bioloads, for up to 48 hours, of 20, 40, 4 and 10 g/L, respectively. Or even a biological filtering media currently being devised, which is focused on tackling nitrogenous waste during short and long-term transports.

These, amongst many others, are but a few examples of multiple research endeavors that have been awarded funds from the aquarium industry, which now benefit from some very significant results made possible from that research.

Friday, April 26
Exhibitry, Part 2

Sponsor Presentation – Fritz Pro Aquatics

Buff Guys Buffing!- An Acrylic Buffing Story

Ramon Villaverde
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Columbus Zoo and Aquarium
Ryan Czaja
Mystic Aquarium
Joe Yaiullo
Long Island Aquarium and Exhibition Center
Nick Zarlinga
Cleveland Metroparks Zoo
Steve Bitter
The Florida Aquarium

Buffing scratches out of acrylic windows is a subject that has come up repeatedly on the listserves and in conversations in recent years. Every institution that displays acrylic windows is likely to see scratches, and it is important to remove them properly. However, many aquariums still seem unsure of the best way to approach the task. This talk aims to discuss the most up-to-date products available and the techniques to use them effectively.

Information will include the following:

- Overview of tools and process
- Pros and Cons of different sanders
- Pros and Cons of different sanding discs and grit varieties
- Underwater buffing versus dry side buffing
- Window preparation and material organization
- How to estimate time requirements
- Photographic instruction and details of materials

Destroyers of Reefs or Docile Algae Grazers: Parrotfish in Your Reef Tank

Joe Yaiullo
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Long Island Aquarium

We have all grown up with the images of parrotfish crushing corals, and swimming away depositing a trail of coral sand in their wake. While this is true of some parrotfish species, as with many things in nature, it is not true of all. This talk will cover the inspiration of trying parrotfish in a reef tank and the successes and failures encountered along the way.

RiverGiants: The Message is Important Yet Nothing, if Not Heard

Thom Demas
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Tennessee Aquarium

River Giants. It's a biological purist's nightmare: an exhibit with fish from all over the world. It's a husbandry nightmare: finding ideal tank conditions for rearing large, predatory fish with attitudes. But it's our guests' dream: an exhibit they love and remember...with a message they take home. This departure from a traditional biogeographical exhibit format has paid big dividends at the Tennessee Aquarium by mixing important conservation messages with an engaging exhibit.

Friday, April 26
Conservation Part 2

Twenty Years of Imperiled Fish Surveys in Portions of the Conasauga and Coosa River Drainages with Watersheds on Cherokee and Chattahoochee National Forest Lands, Including Observations of Impacts of 2004 Floods Produced by Tropical Storm Ivan.

Patrick Rakes, J.R. Shute, C.L. Ruble, and M.A. Petty
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Conservation Fisheries, Inc.

Conservation Fisheries, Inc. has conducted snorkel surveys to monitor the status of imperiled fish populations in the Conasauga River for the Cherokee National Forest annually since 1993. Similar surveys were also conducted for the Chattahoochee National Forest in the Conasauga River and Coosa River tributary sites in 1995 and 2003; these include main stem Conasauga River sites from the upper Alaculsy Valley down to Minnewauga Creek, 3 Holly Creek sites, 2 Sumac Creek watershed sites, 2 Mill Creek sites, and 2 Etowah River sites. Surveys are visual estimates intended to gather baseline data and determine any trends in state or federally listed, Forest Service sensitive and Management Indicator Species (MIS)-- information gathering dictated by the Chattahoochee-Oconee and Cherokee National Forest Plans. These include the federally listed blue shiner, Conasauga logperch and Etowah darter; the state listed “bridled darter”, Forest Service sensitive species: the holiday darter, trispot darter, lined chub, frecklebelly madtom, freckled darter; and MIS species: coosa darter and redeye bass. Target species were counted. Relative abundance of all associated fish species were recorded as well as microhabitat preferences.

On September 17-18, 2004, the tropical storm remnant of Hurricane Ivan deluged many areas of the upper Conasauga and Coosa River watersheds with over 10 inches of rain, causing significant flooding in many streams. The Conasauga River rose 17 feet at the Eton, GA USGS gauge, increasing in flow from around 100 to nearly 20,000 cubic feet per second. Floodwaters inflicted substantial damage on floodplains and riparian zones, and significantly altered stream channel morphology and substrates. CFI conducted post-flood snorkel surveys at all sites routinely monitored for the Cherokee NF as well as sites established in the 2003 Chattahoochee NF effort to assess rare fish population and habitat impacts. Monitoring has continued through 2012, although effort and sites surveyed has varied annually.

Monitoring results after the flood revealed fish abundance declines in the most severely affected areas, followed by varying subsequent recoveries. The degree of decline and rate of recovery varied from site to site, correlated with the degree of habitat alteration. Affected abundances and recovery rates also varied from species to species, inviting speculation about underlying reasons such as habitat preferences and needs, life history attributes, and reproductive biology. The complex net result is the seemingly stochastic nature of stream fish community dynamics.

Posters

Nutritional Comparison of Enriched Diet Items Fed to Syngnathids

Juliet Eckert
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Georgia Aquarium

The nutritional requirements of Syngnathids are not well understood. However, studies suggest that a greater concentration of n-3 highly unsaturated fatty acids may be beneficial for optimal growth and survival. Vitamin E and selenium are also important for proper muscle growth and function, and deficiencies may be responsible for myopathies in some seahorse species. For these reasons, fatty acid composition, vitamin E, and selenium levels were measured in enriched live foods typically fed to Syngnathids. Results suggest that few of the enrichment strategies tested provided the recommended fatty acid ratios and that a combination of enrichment products may be needed to achieve proper highly unsaturated fatty acid ratios. Further testing is needed with other enrichment products in order to determine the effects they have on the growth, survival, and reproduction in Syngnathids.

The Perfect Specimen: Ziva's Journey

Amy Hupp, Michelle Kaylor, Rachel Thomas
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The Georgia Sea Turtle Center

Hundreds of thousands of guests visit animals in aquariums throughout the world every year. While visitors may remember a particular fish because it had pretty coloration, a sand tiger shark because it had jagged teeth or an octopus because it had eight legs, think how memorable a specimen with a deformity from a boat strike would be?

Ziva, a green sea turtle, was rehabbed at the Georgia Sea Turtle Center and became an icon for Sea Life Aquarium, in Tempe Arizona. Through collaborations with Sea Life Arizona, an injured and non-releasable patient has been able to educate guests about conservation and bring together two institutions to support future rehabilitation challenges. Sometimes it is the imperfect specimen that makes the perfect display for creating awareness and ultimately changing minds.

A Cautionary Tale: Two Florida Native Seahorse Species *(Hippocampus erectus* and *Hippocampus reidi)* Produce Hybrid

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Seahorses are a popular commodity in the aquarium trade because their unusual appearance and reproductive methods make them a charismatic icon for public aquariums. Aquariums commonly feature a syngnathid exhibit or system, where seahorses and pipefishes are co-exhibited in a multi-species system. Two prevalent seahorse species housed in public aquaria are the lined seahorse (*Hippocampus erectus*) and the longsnout seahorse (*H. reidi*), which are known to be co-exhibited. At our research facility, we co-housed *H. erectus* and H. *reidi*; interspecies courtship behaviors and copulations were observed between a female *H. reidi* and male *H. erectus*, despite the presence of a reproductively mature male *H. reidi*. The newly released hybrid offspring were viable and exhibited characteristics that aligned with the maternal species, *H. reidi*. Morphological features of the interspecific hybrid offspring through ontogeny were evaluated in contrast to the parent species. Photos of the hybrid seahorses were taken at 0, 5, 10, 15, 20, 30, 40, 60, 80, and 100 days old to track morphological ontogeny through sexual maturity. Photos will also be taken of *H. erectus* and *H. reidi* at the same ages. Unbeknownst hybridization from co-housing multiple species can jeopardize captive lines and work against conservation goals. This presentation will highlight how these photos can be used to assist aquarists and hobbyists in identifying seahorses produced in their aquariums that co-house *H. erectus* and *H. reidi*. In addition to the interest for aquarists, the recorded hybridization has appealing aspects for seahorse biology and provides avenues for further research into mate recognition, ontogeny, and behavior. This is particularly appealing in light of the overlap in habitat and distribution of these two species in the Western Atlantic, providing a natural vector for potential natural hybridization.

The Georgia Sea Turtle Center: Rehabilitation, Education, and Research

Michelle Kaylor, Rachel Thomas, Amy Hupp
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Georgia Sea Turtle Center

The Georgia Sea Turtle Center (GSTC), a department of the Jekyll Island Authority, is a non-profit facility located on Jekyll Island in southeast Georgia. Since opening in 2007, the GSTC has used sea turtle rehabilitation, research and education programs to increase awareness of habitat and wildlife conservation challenges, promote responsibility for ecosystem health and empower individuals to act locally, regionally, and globally to protect the environment. With the help of a dedicated staff, AmeriCorps members, and passionate volunteers, the GSTC has been able to carry out its mission and continues to make a difference for sea turtles and the environment.

Densitization and Training Techniques Employed in Safe Handling of a Queensland Grouper *(Epinephelus lanceolatus)* for Transport Out of a Multispecies Exhibit

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Pittsburgh Zoo & PPG Aquarium

Two Queensland groupers reside in the Ocean Exhibit at the Pittsburgh Zoo & PPG Aquarium. In the late summer of 2011, our largest of the two Queensland groupers displayed aggressive behavior towards the other, resulting in lacerations from bites and chaffing on rockwork. Aquarists were unable to catch and remove the aggressor in order to observe and manage the health of the wounded grouper. In time, the behavior subsided and the two groupers were left together in the exhibit. However, the same behavior was observed a year later, still without any methods to mitigate the scenario.

A training plan was initiated: 1) To increase the success of catching them; 2) to safety move a large teleost (200 lbs/100kg) specimen off exhibit; 3) to reduce the chance of injury to staff and tank mates. Concurrently, behavioral management of other exhibit species were also considered in order to prevent interference during training sessions with the groupers. Grouper training was built upon stationing that was previously established with a visual queue at the water's surface. The location of the target was relocated to a feeding platform that provided easier access to the water. Tactile training was introduced and approximated longer holds at the water's surface. Stretcher and net desensitization sessions were conducted submerged underwater at varying depths, with the ultimate objective to have the grouper station at the surface of the water, over a submerged open net and stretcher. Will discuss successes, roadblocks and logistical considerations.

Growing Corals on Trees: A New Technique to Better Utilize All Three Dimensions of a Tall Coral Propagation System

Steve Hartter
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Georgia Aquarium Inc

Typical coral propagation systems incorporate a high surface area to water volume ratio to maximize bio density and light penetration. Various coral grow out trays have been utilized at the Georgia Aquarium in our 1055 gallon (188” x 36” x 36”) propagation vessel, but none allowed for a more efficient use of all three dimensions. In

December of 2011, after viewing a technique developed by the Coral Restoration Foundation, we implemented the use of coral trees into our propagation system. The trees allowed corals to be stacked on top of each other, while still affording adequate light and water motion even to the bottommost corals. The corals fragments grown on the trees developed rapidly into unique 360 degree colonies. I will outline the construction, maintenance, and results of our propagation efforts utilizing coral trees. With square footage at a premium in the Public Aquarium realm, the utilization of coral trees in a tall propagation system provides another means to achieve efficient coral propagation and support of our reef aquarium displays.

Drum and Croaker Editor's Note

The content of original manuscripts submitted to *Drum and Croaker* is not generally edited, although I will fix obvious problems if I find them. Articles not sent in the proper format (described in the "Hey Authors" section on the web site) are subject to more tinkering to "fix" them. Figures, tables and photos may be compressed, cropped or otherwise modified to save physical or file space, and photos, tables, and figures not referred to in the text may be omitted for the same reason.

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I assume that all submissions have been authorized by all original authors or co-authors, and have successfully completed any internal review process required by your institution.

ABSTRACTS OF THE FIRST GIANT PACIFIC OCTOPUS (*Enteroctopus dofleini*) WORKSHOP

The Seattle Aquarium, Seattle, WA
17 March 2012

Organized, Convened, and Edited by Roland C. Anderson and Shawn E. Larson
The Seattle Aquarium

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Introduction to the First Giant Pacific Octopus Workshop

Roland C. Anderson and Shawn E. Larson
The Seattle Aquarium

Welcome to the first giant Pacific octopus workshop! Thank you to both the audience and the presenters for coming here today, and to the Seattle Aquarium for hosting this event. Thanks to Director Bob Davidson for his gracious introduction!

One of our colleagues, Dr. Bill Summers of Western Washington University proposed 25 years ago that we should organize a Pacific Northwest cephalopod meeting, we meaning either him or me. We are both retired now and, unfortunately, he is unable to attend this event. I'm sorry we couldn't arrange such a meeting back then but here we are now. This meeting is more focused than the cephalopod meeting we envisioned as Bill and I were interested in the many species of squid, particularly stubby squid as well as octopuses. By the way, we use the term octopuses for the plural, since octopus is based on a Greek rather than a Latin root.

We are here at the Seattle Aquarium for several reasons. One, the body of water the aquarium sits on is home to the largest species of octopus, and two, because the aquarium has a long history of exhibiting octopuses. At least one of the attendees is taking advantage of that fact to film some footage of GPO sucker movements as part of his research.

Henry Lee said back in 1875 that an aquarium without octopuses was like a plum pudding without plums. We concur. After Henry Lee began exhibiting octopuses at his aquariumin Brighton UK in 1867, octopuses became more popular after he published his book. He had a good publicist that reported to the local newspaper and there were frequent articles about what the octopus did, like escaping to a next-door tank to eat the fishes kept there, and then returning to its home tank. Yes, there is a basis for that rumor that now seems to be attributed to every large public aquarium keeping GPOs. Henry Lee's time, Victor Hugo published his lurid novel "Toilers of the Sea," wherein he describes a fictional attack by an octopus on a sailor. Such accounts were tremendously popular at the time and they contributed to the popularity of octopuses today.

We are here today to hear about current research on GPOs, *Enteroctopus dofleini*. This is the world's largest species of octopus. Although there have been contenders for the title, no other octopus species has been found to be bigger. One was accurately weighed at 71 kilograms or 156 pounds. There have been even larger GPOs reported but there is a question about the accuracy of the weighing. One was taken from Puget Sound and reported to be 210 pounds. One from California was reported to be 400 pounds and several were reported to be between 400-500 pounds in Murray Newman's book "My Life in a Fishbowl." Newman was the first director of the Vancouver Aquarium. These very large GPOs are reportedin Jim Cosgrove's book on GPO's titled "Super Suckers.

Largely because GPOs are the largest octopus species, they are the most common octopus exhibited in public aquariums so they are a good species to observe behaviors in aquariums. Lately we that octopuses are the smartest of the invertebrates. They can learn to open child-proof pill bottle to get at food inside and learn simple mazes. They even recognize their human keepers, and perhaps most astounding, they exhibit simple play behavior.

Today, we're going to hear some interesting papers describing cutting-edge research on GPOs. Some of this research is so new that the presenters were just finishing up their analyses this week and some of them are still working on their abstracts! Today we're going to hear about how temperature affects GPOs, whether GPOs contribute to the general ecology of their cohorts in the Salish Sea, physiological studies such as genetics and steroid hormones, how they use their suckers, and how and where they live around the north Pacific. This afternoon we'll lead a round-table discussion on where the knowledge gaps are in our understanding of these fascinating invertebrates and what further research needs. Thank you again for attending the first giant Pacific octopus workshop hosted by the Seattle Aquarium.

The Legacy of Olive

Guy Becken
Diver and Seattle Aquarium Volunteer
Des Moines, Washington

Olive was a giant Pacific octopus who for several months in 2001 lived the end of her life cycle including laying her eggs at a popular dive site in Seattle's harbor. She was visited regularly by local divers, especially by a group of Seattle Aquarium divers every Tuesday night. In the end she was successful by guarding and tending to her eggs to hatching, despite many adverse visitations by naïve divers.

Olive was a pretty much non-descript female GPO that arrived at the popular dive site in West Seattle, part of Seattle's harbor sometime in late 2001. Local divers reported her taking up residence in one of two sets of pilings lashed together and resting on the bottom in about 90 feet of water – straight out from the fishing pier at Sea Crest's Cove 2.

At first she was shy and reclusive. As time passed, she came to recognize and accept handouts from many divers that visited her den. Her life, mating, and guarding of eggs thus completing her life cycle was no different than what has been played out by other octopuses in the region for generations. However, this time, this one octopus's life was watched and talked about by hundreds of humans. What started out as some friends diving together on a Tuesday night grew into a popular story creating a legacy highlighting the life and death of Puget Sound giant Pacific octopuses for local divers.

The approach we used to create this legacy was simple. We met to dive every Tuesday, we dove, we took pictures, and we met afterwards to talk about and revisit our experiences. The people who joined and experienced Olive and her legacy would end up being forever touched.

What we didn't know at the time was that our circle of friends would expand as the number of people who were interested in Olive and her legacy grew. What was a relatively unheralded event shared by a few divers in 2001 became a story that would be etched in our minds, told again and again. Today with each new group of divers and with each new den found and watched, we still remember Olive and can't help but wonder if it is one of Olive's babies.

Giant Pacific Octopuses and the Seattle Aquarium

Kathryn Kegel, Biologist, Seattle Aquarium, k.kegel@seattleaquarium.org

The Giant Pacific Octopus (*GPO*) has always been one of the most popular animals at the Seattle Aquarium. The 3,000 gallon exhibit designed to hold two GPO's at once, separated by a removable barrier. It is an open flow through system, with water coming from the Puget Sound, being passed through a sand filter then pumped into the exhibit and then back out to PugetSound. The water temperature and salinity vary with what is happening in the Sound. Two dens were built into the rockwork of the exhibit to give the animals a place to den up if they choose. To help show how amazing these animals are and to educate the public, two daily talks and attempted feedings are done daily. As part of our collections plan, we are permitted to take up to 6 GPO's in a 12 month period. These animals are collected locally and typically spend anywhere from 6 months to 1 year on site. We release our GPOs at the end of their life cycle, when they start showing signs of being ready to mate.

Enrichment is an important part of our husbandry care of GPO's. We offer of variety of enrichment items from different foods, food puzzles, floating objects, to diver interactions. We continue to try and come up with new ways to enrich our GPO's with several of our interns coming up with new ideas, such as dripping water on the surface and bucket training to help reduce the stress on the animal during weightings'.

One of the biggest events put on by the Seattle Aquarium is dedicated to Giant Pacific octopuses. Octopus Week happens every February and is kicked off by our Octopus Blind Date, where we remove the barrier between a male and female GPO in hopes that they will mate. The week continues with live octopus releases, divers swimming with octopuses and educational activities centered around octopuses.

One of our more frequently asked questions about GPO's is, how many Giant Pacific Octopuses are there in the Puget Sound, and how is the population doing? To try and begin to answer that questions the Seattle Aquarium started its annual Giant Pacific Octopus census in February 2000. The census goal was to help determine if the population of Giant Pacific Octopus (GPO) was healthy and if there are fluctuations in the population from year to year. Establishing a baseline of how many GPO's there were in the Puget Sound was also a goal of the census. Beyond gathering information on our local octopus population, the census also hoped to become an effective outreach to our local dive community.

Close Relatives of the Giant Pacific Octopus

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The giant Pacific octopus (GPO) belongs to a group of octopuses that are most comfortable living in cold seawater. For the American GPO, the maximum temperature is around 17°C (63°F). This group seems to have originated in the northern Pacific area and has gradually spread world wide. The first clue to this origin is in the similarity of the internal organs of male octopuses of the genus *Enteroctopus* and closely related octopuses found around the northern half of Japan (Gleadall, 2004). It is also informative to consider a group of octopuses without an ink sac, apparently lost because these octopuses live at depths where little light penetrates, so releasing ink serves no purpose for them.

The inkless octopuses, most of which are mesobenthic and live in water at around 4°C (40°F), used to be described as species in the genus *Benthoctopus* but this name was misused because it belongs to a small, more distantly related group of bathybenthic octopuses in the genus *Bathypolypus*. Most former "*Benthoctopus*" species are now identified as members of the *Muusoctopus* / *Vulcanoctopus* species complex.

Inkless octopuses have long been considered difficult to identify but careful morphological study can distinguish them using characters such as the number of suckers on the hectocotylus of males; relative proportions of the arms; the size and appearance of the funnel organ (the latter being a 'W' or 'V V' shaped gland on the inside of the funnel in incirrate octopuses); skin colour and patterns; and presence of counter-pigmentation (dark ventral surfaces and pale dorsal surfaces, which is opposite to the typical pigmentation distribution). The inkless octopuses are of special interest because they all have large eggs, typically around 25 mm long. The young hatch directly into benthic-dwelling octopuses without a planktonic paralarval stage (which the GPO has), and this means that for inkless octopuses there is only one main method of dispersal: walking (since most benthic octopuses seem to spend little time swimming unless distancing themselves from a predator).

Studies using molecular data have shown recently that some Japanese species (with a functional ink sac) diverged early from the *Enteroctopus* / *Muusoctopus* group, and that *Enteroctopus* then split away from the *Muusoctopus* / *Vulcanoctopus* group, most of which subsequently became inkless. Since this separation, *Muusoctopus* species appear to have spread south along the western side of the Americas and are now dispersed widely, including around Antarctica and also in the other oceans. The pattern of distribution seen from the molecular data shows some apparently strange relationships. Two species found in the Northeast Atlantic, *M. normani* and *M. johnsonianus*, appear to be only distant relatives. So, for example, *M. normani* is more closely related to *M. yaquinae* from the Pacific US than to *M. johnsonianus*, which itself is closer to the Antarctic species.

The relations of *M. normani* to other *Muusoctopus* species can be understood more clearly, however, in terms of (i) its status as a junior synonym of *M. januarii* (the type species of the genus *Muusoctopus*) and (ii) biogeography. *Muusoctopus januarii* appears to be very widely distributed throughout the northern and central Atlantic and its ancestor was probably was the first *Muusoctopus* species to colonize the Atlantic. It seems very likely that it entered the Atlantic through the Atrato Seaway, which used to be a deep sea passage between North and South America. This passage closed completely about 3 million years ago (Mya), but began closing due to tectonic uplift from about 15 Mya. Using the timing of this event, along with the timing of the splitting of two recently identified subspecies from South American waters (*M. longibrachus longibrachus* and *M. longibrachus akambei*), it is possible to estimate when *Enteroctopus* separated from the inkless octopuses (Gleadall, in revision). This in turn should soon provide a better understanding of the timing of the separation of the different species of *Enteroctopus*.

In Japan, research on *Enteroctopus dofleini* has been progressing slowly over the years. Basic information has been obtained about the timing of maturity and mating; and the movements of octopuses in the vicinity of northern Honshu and the island of Hokkaido have been investigated by Kyosei Noro in Aomori, in co-operation with Professor Yasunori Sakurai of Hokkaido University. Some of the results from data published in Japanese by these researchers will be summarized here in English to provide a basis for some comparisons with the American GPO.

Finally, a little information is provided about a current boom in interest in octopuses in Japan. This involves the use of octopuses as a source of food, particularly 'takoyaki,' which is currently very popular with people of all ages. The Japanese GPO is not involved in this (although the Japanese fishery for *E. dofleini* is worth more than a hundred million dollars a year). Takoyaki is based almost entirely on *Octopus vulgaris*, which is prized around the world for its good taste. The Japanese and American GPO's and their inkless cousins are relatively safe for the moment as they are not considered tasty enough to be used to make the incredibly popular takoyaki.

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Evidence for Inter-Sucker Coordination during Different Arm Movements in the Giant Pacific Octopus

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Octopodes are able to use their appendages to manipulate objects with a virtually infinite number of degrees of freedom. The mechanisms by which they achieve fine and forceful control of grasping are realized through the coordinated action of the suckers and the arms, a complex distributed sensory-motor processing system that has received little attention. Coleoid cephalopods (octopodes, squid and cuttlefish) have suckers on their arms and tentacles. Squid and cuttlefish use their suckers primarily for adhesion by suction. Octopus suckers are morphologically different from those of squid and cuttlefish due to the presence of extrinsic muscles, which allow for more elaborate functionality (Kier, 1982; Kier & Smith, 1990). The flexibility and behaviors mediated by the octopus arms are well known (Packard, 1988), but the mechanisms underlying sucker control are just beginning to be understood. Recent evidence (Grasso, 2008) supports the hypothesis that the suckers of the octopus arm, acting as part of this hyper-redundant system, are not passive agents, but actively contribute to grasping and manipulation. In this study, we hypothesized that motions of neighboring suckers would be more coordinated during goal-directed arm movements compared with passive movements. To examine this hypothesis in closer detail, we tracked the movements of suckers from digitized video footage of single arms of *Enteroctopus dofleini*, while they employed those arms to achieve different goals. In quantitative analysis, we calculated movements of sections of the arms carrying the suckers. We subsequently deducted those arm movements from sucker movements to estimate the individual sucker motions independent of the arm. We computed the pair-wise cross-correlation between the movements of 15-36 suckers along the arm. Significant correlations and anti-correlations ($p<0.01$) were found that demonstrated neighborhoods of coordinated movement both locally and at distant intervals along the arm. The delays between these correlations were less than 10 milliseconds (<10.0 ms) and therefore unlikely to have resulted closed-loop communication between the sucker control circuitry and the brain. It appears most likely that they are generated by local circuitry. These patterns of arm-independent sucker activity varied with the task to which the octopus applied the arm. These results provide functional evidence for the neuroanatomically demonstrated connectivity within the arm and define an explicit expected role of the information passing between suckers that connectivity formerly implied. Further investigations of the mechanisms underlying sucker functionality will help to better understand octopus behavior as well as the phylogenetic differences among octopodes, squid and cuttlefish.

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Seattle Aquarium Research of Captive Giant Pacific Octopuses *Enteroctopus dofleini*

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The captive husbandry of giant Pacific octopuses *Enteroctopus dofleini* is well understood, but their endocrine signatures are not well documented. The major vertebrate reproductive hormones—estrogen, progesterone, and testosterone—and the stress-related hormone, corticosterone, are relatively well known for many vertebrate species. However, few studies on these hormones within invertebrates have been conducted. Our hypothesis was that endocrine signatures within octopuses are similar to those found within vertebrates in response to reproductive activity and stress. Using standard immunoassay techniques, we measured fecal steroids within fecal samples collected from five female and three male giant Pacific octopuses housed at the Seattle Aquarium. The mean estrogen level ranged from 3.67 to 99.39 ng/g of feces, progesterone ranged from 44.35 to 231.71 ng/g feces, testosterone ranged from 9.30 to 18.18 ng/g feces, and corticosterone ranged from 10.91 to 22.14 ng/g feces.

Average progesterone and estrogen levels within all female octopuses were similar with progesterone levels higher than estrogen levels except for within one female, FO5. Two female octopuses were observed mating with males during the study (FO1 and FO5). Estrogen levels in vertebrates tend to surge during reproductive events such as estrous or mating but they generally last only as long as the active receptive mating period (or estrus) and the peaks of estrogen can be easily missed if a sample is not collected during the time of estrus which is the reason why were only able to document the increased estrogen associated with breeding in FO5. (Norris 1996; Larson, Casson and Wasser 2003). Progesterone levels tend to be more sustained as they maintain egg development and pregnancy and may be easier to document during random fecal sampling (Norris 1996). Octopuses are egg layers and progesterone is thought to be linked to the preovulatory phase of egg development in the ovaries which may occur when the octopuses are smaller, younger and before the animal's senesce, mate and die (Norris 1996; Anderson 1997). This pattern of sustained elevated progesterone in the animal's ovarian development and rise of estrogen only during mating behavior within the female octopuses is similar to that found within egg laying vertebrates (Norris 1996). .

Changes in corticosterone levels associated with stressful events were clearly documented. All three stressors that FO3 was exposed to resulted in an increase in measurable corticosterone, including exposure to the adrenal gland stimulator, ACTH. It is unknown at this time what tissue or organ in the octopus is similar to the pituitary or the adrenal gland in vertebrates that would secrete ACTH or the glucocorticoids in response to a stressful event. However the data suggests that octopuses may have a stress response similar to vertebrates. Future work may document changes in fecal stress levels leading up to the onset of octopus senescence. Perhaps a significant change in fecal corticosterone levels at this critical time may allow biologists the opportunity to plan for the octopuses last phases of the life cycle such as mating, senescence and death.

Conservation Genetics:

Several *E. dofleini* samples have been collected from Oregon Coast (Seaside), Neah Bay (Washington Coast) and Puget Sound for population parameter estimates. In addition we have samples from a female and several hundred eggs from Vancouver Island, BC for paternity estimates. We are currently testing 6 markers developed in *Octopus vulgaris* (Greatorex et al. 2000), and 20 markers developed here from *E. dofleini* for population parameters of Puget Sound, outer coast of Washington, and Oregon coast octopuses. We are still in the optimizing primer phase so to date we have no reliable genetics data for statistical analysis.

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The Phylogenetic Placement of *Enteroctopus dofleini* within Cephalopoda (Mollusca)

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The cephalopod family Octopodidae (Mollusca: Cephalopoda) contains some of the most well-known representatives of intertidal and deep-sea octopods including the common shallow water species (*Octopus bimaculoides* and *O. vulgaris*), blue-ringed octopus (*Hapalochlaena*), and the Giant Pacific octopus (*Enteroctopus dofleini*). The Octopodidae is also a very large and morphogically diverse family containing approximately 90% of all incirrate octopus species, whose members range in size from the pygmy squid "*Octopus*" *wolfi*, which weighs under one gram), to *E. dofleini*, which can reach weights in excess of 150 kg. The high degree of morphological variability seen in the Octopodidae has led to a substantial amount of taxonomic confusion and disagreement, both at higher and lower levels. Earlier works (e.g. Robson, 1929; Voss, 1988) have divided the family into as many as four subfamilies based on the number of sucker rows on the arms, plus the presence/absence of an ink sac and crop diverticulum (Table 1).

Subfamily	Genera*	# sucker rows	Ink sack	Crop diverticulum
Octopodinae	*Amphioctopus, Benthoctopus, Callistoctopus, Cistopus,* ***Enteroctopus****, Hapalochlaena, Octopus,*	2	●	●
Bathypolypodinae	*Bathypolypus*	2	○	reduced/○
Eledonin subfamilies (1-3)	*Eledone, Graneledone, Megaeledone, Pareledone Thaumeledone, Velodona*	1	●/○	●/○

Table 1. Primary subfamilies, genera and morphological character states for the family Octopodidae. ●=present; ○=absent. *Only genera included in this study are listed.

To better understand the evolutionary history of *Enteroctopus dofleini*, a robust phylogeny is needed. Although some morphological studies have suggested the Octopodidae to be monophyletic (e.g. Voight, 1988), current molecular and combined analyses have demonstrated that neither the family Octopodidae, nor the genus *Octopus*, are monophyletic (e.g. Carlini et al., 2001; Strugnell et al., 2011), suggesting a very dynamic evolutionary history. The present study aims to provide new hypotheses regarding both higher-level relationships among incirrate octopods, as well as within the family Octopodidae, with special focus on the phylogenetic position of *Enteroctopus dofleini*. Here we assembled a data set that includes genetic data from ten molecular loci for a broad sampling of octopods representing five of the seven incirrate octopod families. We then conducted a number of phylogenetic analyses to generate a novel phylogeny for the octopods.

Our findings yielded several interesting results (Fig. 1). From a morphological standpoint, all octopods with suckers in a single row form a monophyletic group, albeit with somewhat low support (57%), indicating a single transition from the ancestral two-row state. The other two characters generally used to divide subfamilies of Octopodidae, ink sac and crop diverticulum, have been lost independently indicating that they may not be appropriate to use to separate higher taxonomic levels.

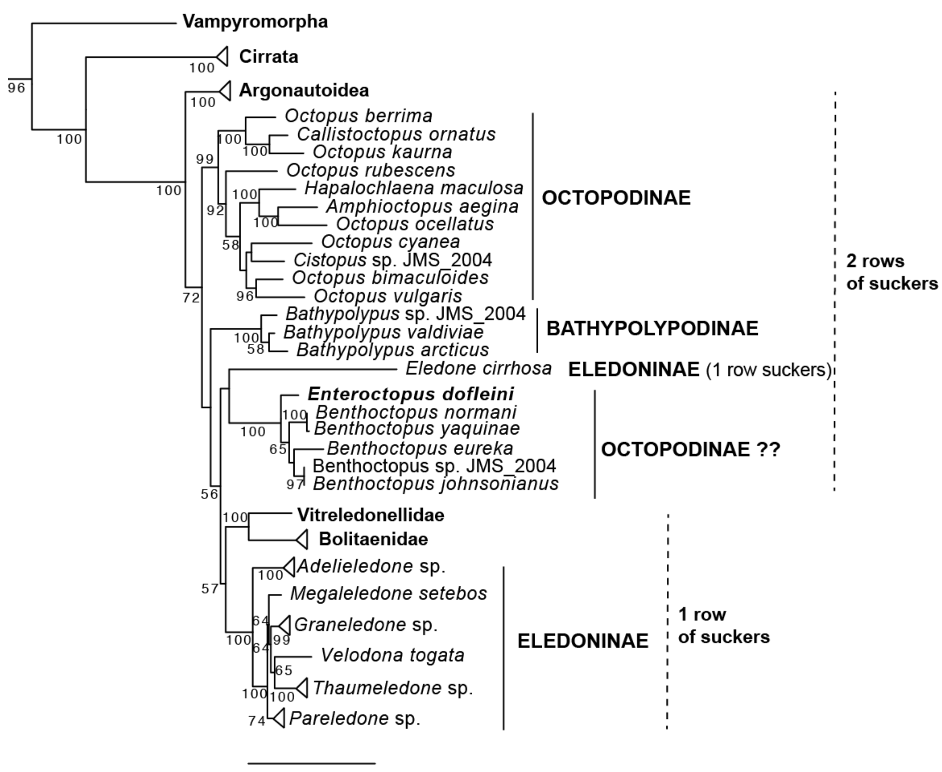

Figure 1. A molecular phylogeny of Octopodiformes. Higher-level groups are **bold**. Sub-families of Octopodidae are listed in capitalized **BOLD**. Triangles at tips represent multiple species. This topology was generated in the maximum-likelihood program RAxML from a larger data set of 178 cephalopods represented by 4-10 molecular loci.

All families aside from Octopodidae are recovered as monophyletic, as was the superfamily Argonautoidea (with 100% of bootstrap replicates supporting this clade), and a clade containing Vitrelledonellidae and Bolitaenidae (100%). The Octopodidae is divided into four major groups: the first containing several genera of Octopodinae (including *Amphioctopus, Cistopus, Callistoctopus, Hapalochlaena,* and *Octopus,* 99%), the second representing Bathypolypodinae (100%), the third forming a clade that contains *Eledone cirrhosa, Benthoctopus* and *Enteroctopus* (100%), and the fourth containing the subfamilies Eledoninae and Graneledoninae (100%). The placement of the bolitaenid clade between the octopidin and eledonin groups renders Octopodidae non-monophyletic.

Our phylogeny recovers a high degree of support for the clade containing *Enteroctopus dofleini,* which is sister to the genus *Benthoctopus* (100%). It is worth noting that only a single species of *Enteroctopus* was included in this study. Hudelot (2000) found substantial support for the validity of *Enteroctopus*, but several species have yet to be fully examined. Also, no morphological characters are presently known to unite *Benthoctopus* and *Enteroctopus*. Further morphological examination of all genera and species is warranted to identify potential diagnostic features before taxonomic changes can be undertaken.

Several important groups of octopods are non-monophyletic, suggesting that the taxonomy of all families within Incirrata, particularly Octopodidae and the subfamily Octopodinae, need to be further examined. New specimens must be collected and vouchered so that they can be studied to help resolve taxonomic issues using comprehensive molecular and morphological study. With a more stable octopod taxonomy, issues of species complexes, population-level variation, and broad scale evolution can be better addressed.

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Is the Giant Pacific Octopus (GPO) the Octopus Archetype?

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Because of its size, lifespan and visibility, *Enteroctopus dofleini* (or the GPO) is 'the' octopus in many aquarium displays and the minds of the general public. But does it deserve this reputation—how representative is the species? For one thing, most of the members of the family are in the genus *Octopus*, and *dofleini* was removed from that genus (see the presentation by Lindgren et al). But apart from that, how does its natural history reflect that commonly found in the group?

The GPO has a fairly wide range, around the North Pacific, from Hokkaido to southern California, typical of species with planktonic paralarvae, not as wide as *O. vulgaris* but it is probably a species complex across different areas. Like other shallow-water octopuses such as *joubini* and *briareus*, it is probably limited in its distribution by the availability of shelter (Hartwick, Ambrose & Robinson, 1984), It is found mainly in rocky habitats, though opportunistically might utilize large rocks or human structures such as concrete mooring or piers to extend its range into other habitat types. Like other octopuses, it consumes much of its prey in the home, leaving a midden of shell remnants outside.

The GPO is like most octopus species in being nocturnally active, though with some small activity in the daytime (Mather, Resler & Cosgrove (1985), also see the presentation by Scheel). We do not know whether this activity pattern is flexible as in *vulgaris* or quite fixed like *macropus* and *joubini*, and while the zeitgeber or cue for activity is probably light, this has not been experimentally determined. Within this activity pattern, there are clear periods of sleep. When this was suggested first over 20 years ago for *vulgaris*, reviewers were unwilling to allow the term as octopuses did not have a mammalian cortex. However, recent studies of behavioral sleep in animals of several phyla have persuaded the skeptics.

Like most octopods, the GPO has a semelparous life cycle. The sexes are permanently separate and the lifestyle solitary, with sexual maturity postponed until the end of the lifespan. Males probably seek out females (the cues are only speculated on) and pass spermatophores (Mann, Martin & Thiersch, 1970) along the groove in the third right arm (each one is about a meter long and mating takes four hours) where they burst and sperm are stored in the female's spermatophoric sac. Females lay tens of thousands of eggs the size of rice grains in strings attached to some solid shelter, again typical of the octopods. With their long lifespan, animals may tend the eggs for almost six months and be visible to divers (see the presentation by Becken). Males go through a period of senescence at the end of their lifespan, probably exposing them to predators but making them a good display animal in the aquarium. The physiology of senescence has been minimally investigated, but there is likely a disruption of activity cycles and absence of pain responses.

Like most but not all species of octopus, the GPO is a generalist predator at the population level. They take a wide variety of crustacean and molluscan prey, differing by location, as is especially documented for *vulgaris*. But the selectivity of individuals is different from that of the population, some are specialists even on one prey species (Scheel & Anderson, in press), again like *vulgaris.* Prey animals are located by chemotactile exploration of likely habitats, again like most other octopod species, and digestion begins externally. GPOs and most octopus species have several techniques for prey penetration, including pulling shells of clams apart with the eight strong muscular arms, drilling with the radula and salivary papilla and chipping with the chitinous beak (Anderson & Mather, 2007).

Having no protective shell or exoskeleton, octopuses are vulnerable to many predators. Of the tens of thousands of eggs, only a few animals need to survive to maturity for the species to continue. They are taken by crustaceans and fish when small, and by fish and marine mammals when larger. GPOs have the usual octopod array of antipredator activities, ranging from hiding in the den to camouflage skin patterns and jet-propelled escape and ejection of ink when a predator approaches. Surprisingly, no systematic study of *dofleini* color patterns and changes has been done—they may be less variable than that of smaller species.

Finally, the GPO exhibits the common octopod centralized brain and intelligence. While no formal studies have been carried out, they have distinct personalities. They play, and solve ecological problems such as gaining access to clams, taking jar lids off to get access to prey inside and using water jets from the funnel as tools. These have also been seen in other octopods, though mainly tested in *vulgaris*. They move out to hunt and return to sheltering homes, showing spatial memory as does *vulgaris*, but because of their large size, GPOs have not been tested for this ability in captivity. Navigation may be a common problem for all mobile animals and that development of such ability may be a way to find cross-phylum comparisons of the evolution of intelligence.

In short, GPOs are typical octopods in many ways. Their most obvious difference from others is more, their long lifespan and large size. Perhaps, instead of calling the GPO an octopus archetype, we could define it as **ultraoctopus**.

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Escaping the Kraken's Grasp: A Sponge-Scallop Mutualism Possibly Maintained by Octopus Predation

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The association between some scallops and the sponges that overgrow their valves is known to occur in many locations across the globe and in represented in the northeastern Pacific by the scallops *Chlamys hastata* and *Chlamys rubida*, the valves of which will often be overgrown by the sponges *Myxilla incrustans* and *Mycale adherens* (Bloom, 1975). Sponges benefit from this association by the prevention of sediment accumulation when growing on live scallop valves, increasing the survival of the sponge (Burns and Bingham, 2002), and may additionally benefit from decreased nudibranch predation. The adapative value of this association to the scallops has generally been thought to be protection from sea star predation, but this hypothesis has not been well supported. Scallops have a highly effective swimming escape response and are rarely found to fall prey to sea stars in the field (Mauzey *et al.*, 1968). Additionally, the sea star *Pycnopodia helianthoides* appears to only be slightly less likely to consume sponge covered scallops than scallops with no epibionts in laboratory conditions (Farren and Donovan, 2007). Consequently, a clear benefit to the scallop to preserve the relationship is lacking. We propose that octopuses could provide the predation pressure to maintain this relationship. This hypothesis would predict that: (1) octopuses are an important cause of scallop mortality and (2) octopuses are less likely to consume scallops encrusted with sponges than unencrusted scallops. We found that *Chlamys hastata* may comprise as much as one-third of the diet of the giant Pacific octopus (*Enteroctopus dofleini*) and that *E. dofleini* is more than twice as likely to choose an unencrusted scallop than an encrusted one. While scallops are a smaller portion of the diet of the Pacific red octopus (*Octopus rubescens*), this species is five times as likely to consume scallops without sponge encrustment as those with it. These findings provide strong evidence that octopuses may provide an important adaptive pressure that maintains the scallop-sponge association.

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Anesthesia, Movements and Recruitment of
Giant Pacific Octopuses in Relation to Temperature

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The biology of giant Pacific octopuses (Enteroctopus dofleini) in relation to temperature is presumably well understood at the level of physiological tolerances and effects on growth (useful for husbandry, anesthesia and euthanasia) and behavior (driving biannual onshore-offshore migrations), but understanding is less satisfactory at the ecological levels (effects on population biology). Here I review our knowledge on the first two points and question its adequacy; and present new data on correlations of regional temperature changes and local population densities of giant Pacific octopuses.

Anesthesia

In the late 1990s, increased attention to cephalopod well-being and suffering led to the adaptation of cold-water anesthesia (4 to -1.9°C) as best practice for Enteroctopus dofleini. However, I found octopuses from Alaska waters remain responsive at 2°C to some stimuli, such as manipulation of the free edge of the mantle, and were unresponsive only when chilled to <1.5°C. At this low temperature, octopuses recovered from anesthesia slowly and some exhibited lower responsivity or respiration (also described for other anesthesia methods (Rigby, 2004); and other species (Agnisola, et al., 1996)), with abnormal posture and coloration following anesthesia. For Octopus vulgaris, minimizing handling time without anesthesia had fewer adverse effects than cold-water anesthesia, which led to reduced predatory response. My observations both during handling and on release indicated greater stress from chilling and prolonged handling than from minimum handling (no anesthesia with care taken to minimize all handling). In the absence of greater physiological knowledge about stress response in cephalopods, behavior remains a valuable indicator of welfare. Diver inspection of animals handled without anesthesia, as well as subsequent tracking data from attached sonic transmitters, indicated equivalent or lower incidence of abnormal respiration, coloration, posture, and fleeing behaviors. Animals without anesthesia adopted normal coloration and posture immediately post-tagging, respiration rates returned to pre-tagging rates within several minutes, and released animals fled with normal responsiveness. When handling is minimized and quick, it constitutes a temporary stress and discomfort for octopuses (for example, tag attachment is akin to piercing in humans), for which general anesthesia is inappropriate due to systemic adverse affects. Handling time increases animal stress; and anesthesia, while intended to lower stress, takes time. Elimination of anesthesia and minimizing handling time should be considered an alternative to anesthesia that both minimizes stress and avoids poor recovery from anesthesia.

Biannual inshore-offshore migrations

Substantial data are available to understanding movement ecology in the waters off Hokkaido, Japan (e.g. Kanamaru, Yamashita, 1969; Rigby, 2004), where a viable commercial fishery (and the possibility of passive tag-recapture studies) exists, although comparable data do not exist for northeast Pacific waters (Alaska, British Columbia, Washington, Oregon and California). Summaries of migratory patterns in the eastern Pacific are based on the movement data from Hokkaido Japan. In Hokkaido waters, Enteroctopus dofleini migrate inshore in early summer and in early winter and offshore to deeper water in later summer and again in late winter. These movements coincide with seasonal inshore temperature fluctuations from too cold (in late winter) to physiological optima for E. dofleini (early summer) to too hot (in late summer) and back again. However, den utilization and tracking studies (see Scheel, Bisson, 2012 for summary) in British Columbia and Alaska have not found patterns supporting a twice-yearly onshore-offshore migration as found in Hokkaido waters. Despite some northeastern Pacific studies with the potential to detect onshore-offshore movements, no published evidence exists that this migration pattern occurs in the northeastern Pacific. Movement ecology is central to defining populations, a concept key to fisheries policies. The assumption that giant Pacific octopuses in the eastern Pacific and waters of Alaska behave in the same ways as those in the waters of Hokkaido, Japan has not been supported by eastern Pacific data and is not warranted.

Correlation of regional temperature changes with local octopus density

I surveyed octopus densities in June at several intertidal sites in Prince William Sound, Alaska during most years 1995-2011. June temperature data were obtained from a west Orca bay oceanographic buoy (1995-2005) and from temperature loggers carried by scuba divers offshore of each survey site (2004-2011); both series were representative of regional temperature trends from multiple sources.

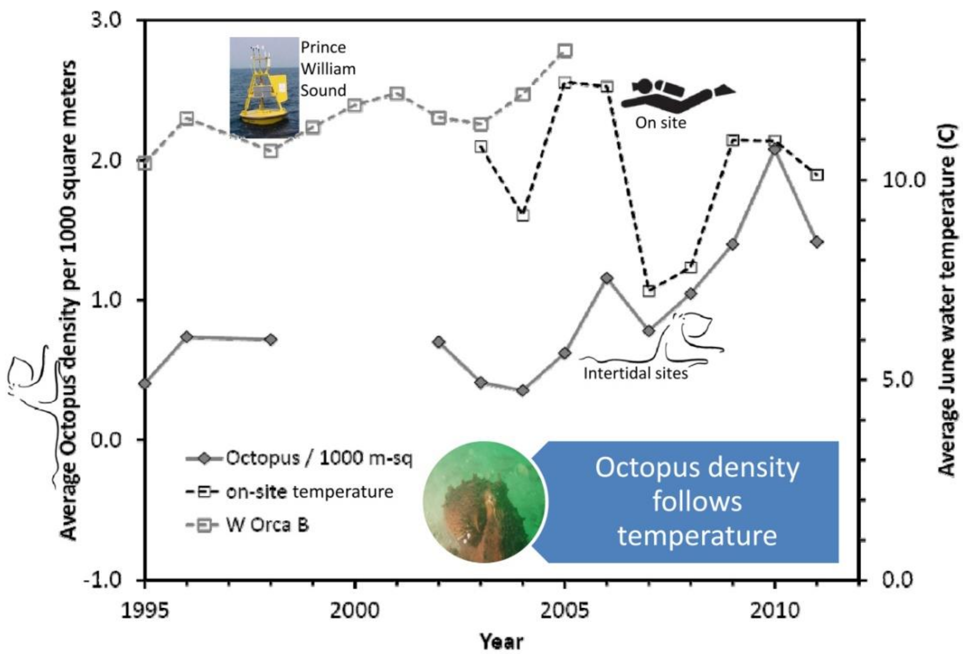

Figure 1: Correlation of octopus density with June average temperature measured by buoy or by divers.

Regional June average temperature trends and average local density of intertidal octopuses showed roughly similar trends, including stability during the period 1994 to 2004 (with some data gaps), termed the stable period; and an increasing trend 2005 to 2011 (variable period) with notable peaks of both warm years and higher octopus densities in 2005-2006 and 2010 (Figure 1). The inter-annual change in temperature was positively correlated with inter-annual change in octopus density (N=9, R^2 = 0.28, 1995-2011). During the stable period, with little trend in temperature or octopus density, octopus size distribution was bimodal, with peaks at <1 kg (recruitment) and at 3-5 kg (juveniles). In contrast during the variable period, with increasing trends in octopus density, size distribution was unimodal, with a larger peak at <2 kg (recruitment) and no frequency peak at 3-5 kg. Thus increasing local density was supported by increased recruitment in the smaller age classes.

Biology in relation to temperature

Uncertainty remains in our understanding of octopus biology in relation to temperature. While giant Pacific octopuses tolerate cold-water anesthesia well and the procedure causes no signs of distress, recovery is not universal, and particularly at the lower end of the temperature range long-term effects of cold-exposure are believed to occur. Minimizing handling overall may be preferred over anesthesia wherever possible.

Octopus behavioral response to temperature is variable and biologists should not assume octopuses from different thermal environments will respond the same to seasonal temperature changes. Bi-annual onshore-offshore migrations in the waters of Hokkaido Japan are correlated with temperature changes, and movements of octopuses are such that populations remain in physiologically optimal water temperatures. Evidence of similar behavior in eastern Pacific waters is weak or lacking, and such migrations cannot be assumed in the waters of Alaska.

Local octopus density and regional temperature patterns showed parallel trends: variable but warmer temperatures correlated with higher octopus density and with greater octopus recruitment. During the variable period, records also indicate higher incidence of Cancer productus in octopus middens from British Columbia to Alaska(Scheel, Anderson, In submission); and trends of octopus densities recorded in Alaska paralleled those in Puget Sound, WA (Seattle Aquarium annual octopus survey conducted each winter by scuba divers). This suggests an eastern north Pacific-wide signal, possibly affecting survival of mero-planktonic life stages of both crabs and octopuses, and leading to greater recruitment during years of average warming June temperatures. This change is reflected in greater octopus densities, and shifts in octopus diet toward larger prey species.

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Genetic Analysis of the Giant Pacific Octopus (*Enteroctopus dofleini*), Including the Development of Novel Microsatellite Markers, from South Central and Dutch Harbor, Alaska

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Multiple species of common, large octopus are known from north Pacific waters near Japan, but only one large species is known in the Gulf of Alaska and eastern Pacific, the giant Pacific octopus (*Enteroctopus dofleini*). The giant Pacific octopus ranges across the north Pacific (California north to Alaska and southwest to Japan), and is the most commonly encountered species in the eastern Pacific, found from the intertidal zone (Scheel 2002) to depths of 1000 m (Hochberg 1998). These octopuses make up a significant component of by-catch in Alaskan commercial pot fisheries (Reuter et al. 2010).

Although distinctive in the eastern Pacific, in the western Pacific, *E. dofleini* belongs to a larger group of common octopuses that may be morphologically difficult to distinguish. With the advent of molecular techniques, the taxonomy of north Pacific octopuses is currently being reexamined. In this study, we provide a genetically based description of *E. dofleini* from Prince William Sound to Dutch Harbor, Alaska (Figure 1).

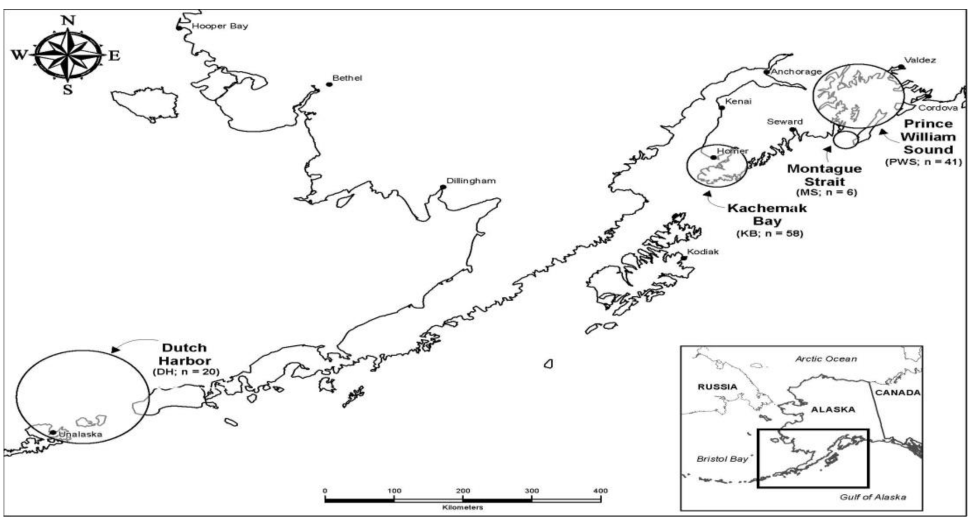

Figure 1. Study location areas (circles) indicating where tissue samples from the giant Pacific octopus (*Enteroctopus dofleini*) were collected, and number of octopuses sampled from intertidal (Prince William Sound, Kachemak Bay only) and subtidal waters (all sites).

We isolated and developed 18 novel microsatellite markers for *Enteroctopus dofleini* and examined them for 31 individuals from Prince William Sound (PWS), Alaska (Toussaint et al. 2011). These loci displayed moderate levels of allelic diversity (averaging 11 alleles per locus) and heterozygosity (averaging 65%). Seven loci deviated from Hardy Weinberg Equilibrium (HWE) due to heterozygote deficiency for the PWS population, although deviations were not observed for all these loci in other populations, suggesting the PWS population is not in mutation-drift equilibrium. These novel microsatellite loci yielded sufficient genetic diversity for potential use in population genetics, individual identification, and parentage studies.

In addition to the 18 microsatellites, we also used two mitochondrial genes (cytochrome b [CYTB] and cytochrome oxidase I [COI]) and three nuclear genes (rhodopsin [RHO], octopine dehydrogenase [OCDE], and paired-box 6) for phylogeographic and phylogenetic analyses. We found 8 of 42 octopuses sampled from Prince William Sound all shared (1) a CYTB and one of two COI haplotypes, and (2) a RHO and an OCDE allele, each of which occurred nowhere else among our 125 sampled octopuses from any location. Thus, there was concordance between the mitochondrial and nuclear signals that distinguished all sampled octopuses into one of two groups, (1) a rare type (n=8) found only in PWS or (2) common type (n>100) found in all sampled regions. These two types were distinguished from each other by a 4% sequence divergence in CYTB; however, within each type, haplotype divergence was <1%.

The common type COI haplotype was <1% divergent from published COI sequences of *Enteroctopus dofleini* from waters off British Columbia (Strugnell et al. 2009) and Japan (Kaneko et al. 2011), while the rare type COI haplotypes were 3 – 4% different from the same published sequences. Collectively, our results support the conclusion that octopuses with our widespread and common type are *E. dofleini* and that the rare PWS type may represent previously unknown cryptic diversity.

Excluding the octopus samples representing the rare type, no other significant, broad-scaled population structure was found from Prince William Sound to Dutch Harbor, Alaska (mtDNA: θ_{ST} = -0.009, P > 0.05 and F_{ST} = -0.009, P > 0.05; microsatellites: F_{ST} = 0.005, P > 0.05; R_{ST} = 0.003, P > 0.05; X^2 = 41.78, P>0.05; Bonferroni corrected for all comparisons); and thus, no support was found for population structure across intertidal, state (<3 miles from shore) or federal (>3 miles from shore) waters.

Preliminary genetic patterns found in this study indicate that *Enteroctopus dofleini* in southcentral Alaska are not sharply demarcated geographically across a 1300-km distance in the center of the species range. However, the unexpected discovery of cryptic diversity in PWS confirms a more complicated picture that remains to be explored, and emphasizes that our current understanding of the population biology of giant Pacific octopuses is based on limited data.

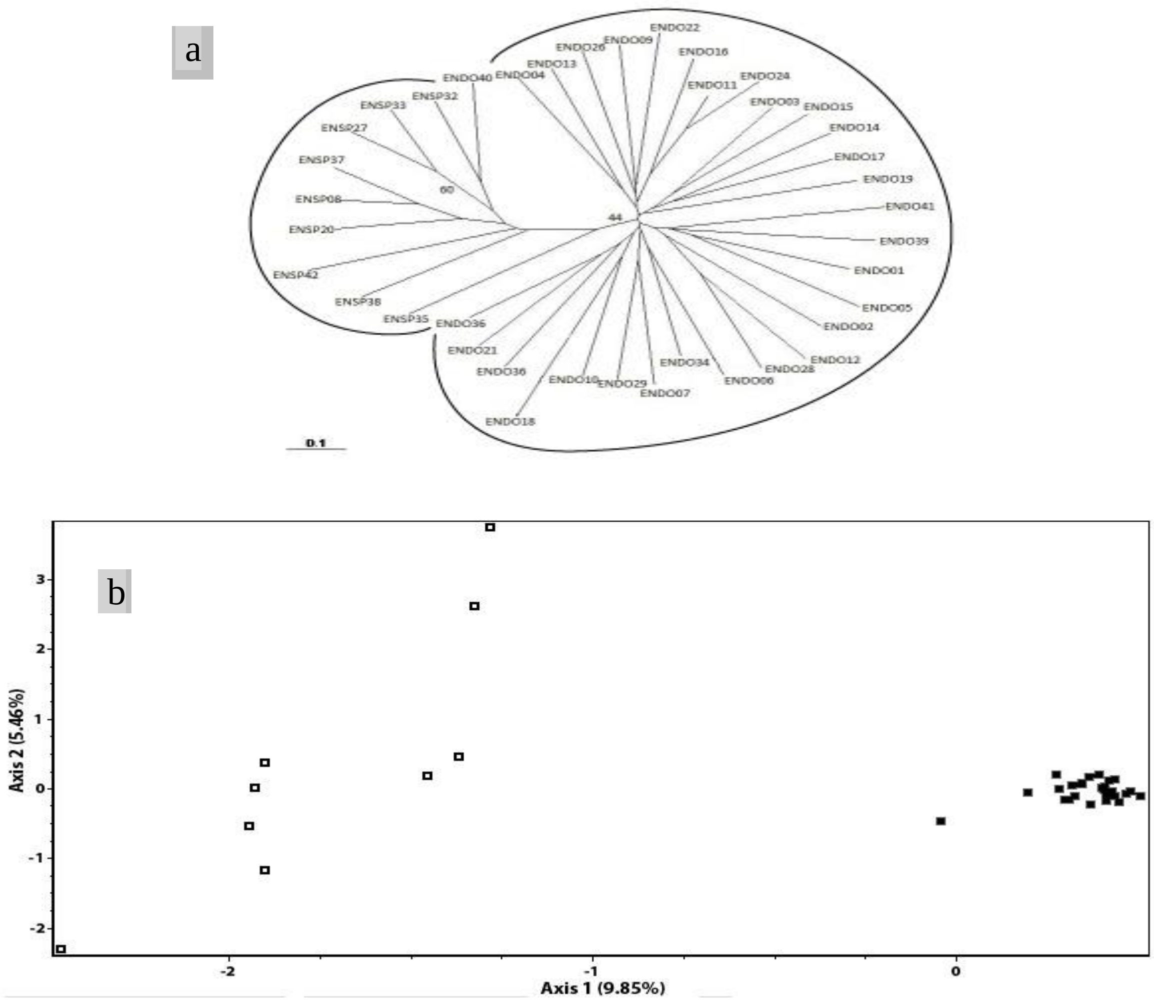

Figure 2 Shared alleles among giant Pacific octopuses from Prince William Sound, Alaska across 10 microsatellite loci. A neighbor-joining network (a) revealed two distinct groups (left and right sides emphasized by the external lines). The two groups consist of individual octopuses *Enteroctopus* (EN) possessing either the common haplotypes, *dofleini* (DO) or the rare haplotypes (SP) and a unique individual identifier. Bootstrap values of highly supported branches are marked at the supported nodes. A factorial correspondence analysis (b) separates the samples into the same two distinct groups. The solid black squares represent the common haplotype, *Enteroctopus dofleini* , and the open boxes represent the rare haplotype.

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University. Mention of trade names or commercial products does not constitute endorsement or recommendation for use.

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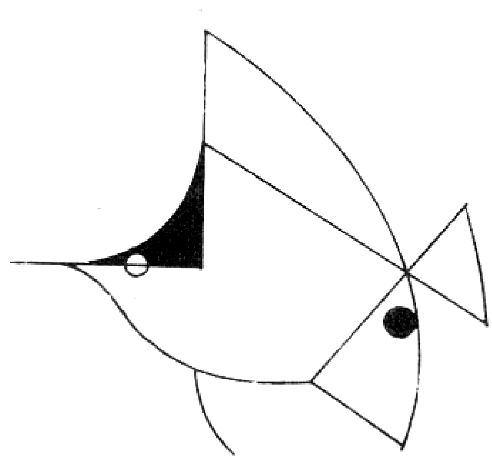

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