DRUM *and* CROAKER

A Highly Irregular Journal for the Public Aquarist

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http://commons.wikimedia.org/wiki/File:Artemia_salina_4.jpg#mediaviewer/File:Artemia_salina_1.jpg

DRUM AND CROAKER 50 YEARS AGO

Excerpts from 1965(2), Edited by John H. Prescott, Curator of Fishes, Marineland of the Pacific)

Richard M. Segedi

NEW ENGLAND AQUARIUM -THE PROPOSED SEA WATER SYSTEM
Dave Miller

The proposed systems supporting the marine exhibits at New England Aquarium can be divided into three major and essentially independent sections. The first segment is concerned with the intake and processing of raw harbor water, and backwash supply. Dual intake lines running some 300 feet out into the harbor are proposed for alternate use to avoid fouling problems inherent in the area. The intake pumps (maximum capacity of 2,000 G.P.M.) will supply raw water for backwashing the sand filters connected with the other two systems. During processing, water will be pumped into two 18,000 gallon tanks, aerated and filtered through two vacuum D.E. units until acceptable water clarity is obtained. Water will then be transferred to a 55,000 gallon storage tank which supplies the make-up for the ocean tank, and exhibit tank systems. Run at maximum capacity the treatment system can supply the two exhibit systems with 100,000 gallons of sea water per day. Normal make-up is estimated at 25,000 gallons per day. The ocean tank system, straightforward in concept, is a sand-filtered, semi-closed unit (200,000 gallons) with maximum make-up proposed at 10% of the entire volume per day. Water is pumped from the basement to a fourth level gravity tank which feeds into the centrally located 170,000 gallon display tank. This tank drains to rapid sand filters on the basement level. The main recirculating pumps return the filtered water to the gravity tank. Filter area is designed so that filtration rates of one gallon per square foot per minute can be achieved at maximum pumping capacities. The exhibit tank loop is again a semi-closed system and distinct from the ocean tank.

AQUARIUM OF NIAGARA FALLS
William E. Kelley

Aquarium of Niagara Falls, a new, privately-owned, $1-1/2 million "inland oceanarium" in Niagara Falls, N. Y. is described. A three-level circular building totaling 33,000 square feet under roof houses some three dozen exhibits of 200 to 100,000 gallon capacity. Dolphin and electric eel demonstrations emphasize the performance of these animals in nature.

The use of air-lift pumps to move up to 5,000 gallons per hour of water is explained. All systems achieve turnover rates through filtration better than once per hour. Fiberglass is used in the construction of coral and rock formations for dioramic backgrounds.

Tank arrangements inhibiting "queuing up" of people in display areas have been successful. Photographic facilities for each tank are built-in key-operated. Emphasis is on marine creatures and they are presented in artificial sea water.

A PLANNED ONE-LEVEL AQUATIC ANIMAL SYSTEM FOR THE CLEVELAND AQUARIUM
D. H. Moreno

Money has recently been made available for the construction of an addition to the Cleveland Aquarium. Although the plans are still in the early preliminary stages of development, the architect and the aquarium staff feel confident that certain features will be adopted in the final plans. One of the important features will be the arrangement of all tanks and equipment on one floor. The building will be octagonal in shape, with six large hexagonal tanks nested together and facing outward, toward six of the inside corners of the building. This radial arrangement will spread the visitors viewing each tank into a roughly fan-shaped area, which will not overlap that for the adjacent tanks. Individual, pie-shaped reserve tanks with air-lift operated biological filters will be located directly behind the exhibit tanks they serve, with provisions for running them independently or in tandem with the exhibit tank. Service for these tanks and filters will be from catwalks running just above the water surface.

IN-HOUSE LIVE *Artemia* CULTURE: A SIMPLE, LOW-COST, LOW-MAINTENANCE DESIGN

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Summary

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 purchasing live adult *Artemia* for feeding newly acquired and/or wild-caught specimens, or for supporting high-maintenance species like syngnathids.

Since 2006, the North Carolina Aquarium at Fort Fisher (NCAFF) has successfully sustained live brine shrimp production using a low-maintenance design that incorporates a simple system of hatch-out buckets, grow-out tubes, and harvest barrels. This setup currently provides an average of more than 1 lb (0.5 kg) of enriched adult *Artemia* per week, yet only requires about 3-4 hours/week for maintenance. The total yearly cost of in-house production is estimated to be about 35% that of importing the same amount of adult live brine shrimp from standard suppliers. The level of production can be easily adapted to individual aquarium live food demands, and the design is especially advantageous for facilities dealing with floor space limitations.

This paper provides detailed instructions on how to set up and maintain a highly productive live brine shrimp culture system, and assumes that the user already has a basic understanding of *Artemia* life cycles and general culture techniques.

Materials and Methods

200g of dry *Artemia* cysts are first decapsulated using standard protocols and then stored in 50cc (13 dram) plastic prescription vials in the refrigerator for up to 2 weeks. For the production of a single batch of *Artemia*, two vials (~80g wet-weight) are added to a 5-gallon bucket filled with ~4.5 gallons of artificial seawater (Crystal Sea Marine Mix, ~30ppt). Two open airlines (weighted with stainless steel bolts attached by small cable ties) provide strong aeration to oxygenate the water and keep the eggs in suspension. A 100W submersible heater set at ~83°F (28°C) is added to speed up hatch time and improve total hatch success.

After 18-24 hours, most of the eggs have hatched into nauplii and are ready for transfer into a grow-out tube (Fig. 1). The airlines and heater are removed to allow the bright orange nauplii to collect at the surface, and any dark brown unhatched eggs to settle to the bottom. Nauplii are strained through a 100μm nylon mesh net, while remaining unhatched cysts are retained in the bucket with clean saltwater to allow an additional 12-24 hours of hatch time (heater is removed). Late-hatched nauplii can be added to the tube, or can be used for other purposes. Ongoing high percentages of unhatched eggs may indicate a bad batch of cysts or a failed decapsulation.

Figure 1: Part of the live brine shrimp culture setup at the North Carolina Aquarium at Fort Fisher. One of the white hatch-out buckets for nauplii is shown in the middle, with one of the harvest barrels (gray trash can) at the far left. The different colors of the growout tubes indicate different stages of *Artemia* – the whitish tube second from left contains 48-hr nauplii fed with enriched unbleached flour only; darker tubes indicate later stages fed with flour and *Spirulina*. The darkest tube (orange) is about 12 days old and ready for harvest at the small adult size.

The basic unit of the culture system is the grow-out tube (Fig. 1). These standard culture tubes are made of transparent fiberglass (18"d x 5'h) that is very durable, lightweight, and easy to clean (http://www.solar-components.com/). A ½-inch drain valve is plumbed at 90° to the bottom of each tube with a nipple on the end for a hose to be attached for harvesting the adult *Artemia* (Fig. 2). The tubes are supported and elevated by a 12-inch stand made of treated wood and FRP grating that prevents damage of and allows easy access to the drain valves. Brine shrimp cultures have no special light requirements to grow so ambient lighting is the only source.

For a single batch, a clean grow-out tube is filled with about 55 gallons of artificial seawater (at ~30ppt), and an airline is added. Strong aeration is very important, and will not harm the brine shrimp. (Regardless of the size or shape of the tube you choose, do not fill it completely so that there is room for food addition over a 14-day period. We fill to within about 10-12" from the top.) Next, a 5L plastic measuring container is filled with standard iodized table salt that is added to the tube and allowed to dissolve completely. This is the most important step in the process, because it **raises the salinity to over 60ppt**, in which *Artemia* can exist without

difficulty, but predators and pathogens cannot. (We have found that salinities below 60ppt still allow some bacterial mats to form that will eventually cause a crash of the entire tube.) Once the salt has dissolved, the harvested nauplii are added to the grow-out tube.

Figure 2: A close-up of the 90° drain valve plumbed into each grow-out tube.

Tubes and harvest barrels are fed 2x/day, in the early morning (~8am) and late afternoon (~4pm). Splitting the feeds has been found to optimize ingestion rates while reducing the amount of waste. Tubes less than 4 days old are fed unbleached enriched all-purpose flour, while tubes older than 4 days and harvest barrels are fed a mix of both flour and *Spirulina* algae powder. Each tube receives an average of 1 – 1 ½ tsp (3-5g) of flour and/or ½ tsp (1-3g) of *Spirulina*. However, as more tubes near harvestable size, more *Spirulina* is added until a 1:1 ratio is reached. (It should be noted that these numbers are estimates, as exact amounts are not measured for each tube due to time constraints.)

To feed the entire system at once, the same 5L plastic measuring container is filled with artificial seawater that is used to dissolve the food prior to feeding. A heaping tablespoon of flour is dissolved into ½-liter of the seawater using a commercial blender (~15 seconds on high), and the mixture is added back into the container total and stirred. Approximate 500mL portions of the flour-only mixture are removed from the container and added to flour-only tubes (~500mL/tube). A heaping teaspoon of *Spirulina* powder is then blended with a small amount of seawater and mixed into the water remaining in the 5L container. That mixture is divided between the rest of the tubes and harvest barrels; more or less may be added to each tube depending on the density of the culture, the stage of development of the nauplii, and the desired rate of growth (what constitutes an appropriate amount is learned mostly through experience or

trial-and-error by the aquarist over time). If there are no flour-only tubes, the flour and *Spirulina* may be blended at the same time.

Observing grow-out tube water clarity over time can help in determining whether tubes are being overfed or underfed. If the visibility through a tube is less than six inches across, change 30-50% of the water and reduce the daily amount being fed. (The appropriate amount of salt must also be added after the water change to maintain 60+ ppt salinity.) If a culture appears to be growing more slowly than usual, the food amount can be increased (this should be done incrementally to avoid crashing the culture). For reference, healthy and productive *Artemia* cultures should reach small adult size within 10-14 days and full (reproductive) adult size within 21 days. Submersible heaters set at 80-82°F can be added to tubes to increase growth rate, but this may lead to increased food demand and risks potential overfeeding and culture crash, so proceed with caution. At this time, our setup does not utilize heaters - except during the hatch-out process - and cultures are maintained at ambient temperatures.

To harvest a grow-out tube, one end of a ¾-inch clear vinyl hose is attached to the drain valve nipple and the other end positioned over a standard brine shrimp net (or fine to semi-fine mesh if harvesting sub-adult sizes) secured at the top of a large bin that overflows to the drain (Fig. 3). (When initially opening the drain valve, there will be a large plug of sludge that comes out – this should be put directly into the drain before moving the hose over to the net.) Harvested brine shrimp are transferred into a 55-gallon harvest barrel (Fig. 1) filled with 40 gallons of clean artificial seawater and ~4L (dry-weight) of iodized salt (or enough to raise salinity to 60+ ppt).

On average, the brine shrimp are transferred from grow-out tubes to harvest barrels at around 12 days for small adult size, but 14 days is the maximum, unless large water changes are performed. (Letting cultures go longer than 14 days in the tubes greatly increases the risk of waste build-up and subsequent culture crash.) The small adults are then made available for enrichment and harvest over a week's time, or are grown out in the harvest barrel for an additional week to reach full (reproductive) adult size. For propagation needs, the cultures can be transferred to a harvest barrel at any desired size for enrichment and continued grow-out.

We currently maintain 8 grow-out tubes and 3 harvest barrels that provide a large amount of live brine with a variety of sizes available for propagation, but the system design is easily changed to fit individual facility requirements. A single tube can also be used to grow a double batch for the first 4-5 days and is then split into a second clean tube using a hose attached to both drain valves. Clean water and additional salt is added to both. "Head-starting" batches this way improves the production efficiency by significantly reducing wait time for access to full adult sizes. This method is especially helpful when the demand for live food increases from larval/juvenile fish propagation and/or the acquisition of new/wild-caught fish, especially during the summer collection season.

Figure 3: Harvesting a grow-out tube: the hose transfers the harvested brine shrimp into a net while a collection bin keeps them suspended. A second net with smaller mesh is used prior to draining to catch any smaller sized brine shrimp that may have passed through.

Acknowledgements

The authors would like to thank Ricardo Aguilar-Duran, Technical Manager of the Veracruz Aquarium (Acuario de Veracruz), for significant contributions to this protocol and especially for the insight of using extreme hypersalinity to maintain monocultures.

AQUATIC-FOCUSED EXPERIENTIAL LEARNING

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The Toledo Zoo has embarked on an expansion of its "experiential learning" opportunities; where the public and staff alike "learn by doing". It has long been understood that these methods of active learning are far better for most people than is learning by rote or typical "instruction by demonstration". The Zoo has long had internship programs which are perhaps the classic example of experiential learning. However, the number of students able to participate in this sort of activity is resource-limited. Additionally, internships are often not very structured, and some students do not excel without some structure. A new model arose; structured practicums of a finite time frame where the students underwrite the costs. A pilot program was designed and implemented that demonstrates the viability of this idea.

It has been known for many years that larval tropical fish are transported northward along the Atlantic coast of North America by the Gulf Stream current. Towards the end of the summer, some of these larvae have settled out in the shallows around Long Island. These juvenile fish are destined to perish when the water temperature begins to drop in late October. Aquarists, knowing this have harvested these fish for aquarium exhibits, as a more sustainable means of acquiring specimens than taking animals directly from reefs. As the Toledo Zoo was in the midst of stocking its new aquarium, undertaking such a collecting trip would help that process. A proposal was submitted to the Toledo Zoo's program committee and once approved, a budget was developed and then a prospectus was sent out to the Bowling Green State University marine lab. Of the twenty students who applied, six were selected to participate in the collecting trip. Most of these students opted to also receive credit from BGSU which considered the trip to be a one credit field course.

The student program began with lectures about collection planning and animal ethics, as well as tours of the Toledo Zoo's aquarium holding facility and the new aquarium that was under construction at the time. The subsequent six day collecting trip was led by the aquarium curator, an aquarist and a Toledo Zoo biologist. The participants drove a van to Long Island, and along the way stopped to tour the Pittsburgh Zoo, a tropical fish wholesale firm and then the Long Island Aquarium. Once on site, we met up with Todd Gardner who held a collecting permit, and knew the best sites for collecting the tropical waifs. We spent the next four days collecting, visiting the marine labs of two local colleges, as well as a nature center.

The most productive collecting was done by seine, in shallow sea grass flats. There was also some limited diving and snorkeling, as well as night-lighting while wading. The students learned how to properly handle the fish, and to identify common species. The curator selected specimens that fit the institutional collection plan, and those were retained while the other animals were safely released.

We ended up bringing back 53 fish and 20 invertebrates comprising 20 different species. Only one small goby was lost during transport and acclimation back to the Toledo Zoo.

Most of the students elected to enroll in a one credit hour independent study course afforded them by Bowling Green State University (who partnered with The Toledo Zoo on this project). Additionally, the student participants each paid a fee to the Zoo which covered all costs of the trip with the exception of a portion of the staff's time. That cost was offset by the concurrent staff development, as well as the exhibit value of the animals that were brought back to the collection. For future trips, it will be a simple matter to make these wholly self-supporting by:

- Utilizing Zoo vehicles as opposed to a rental van
- Reducing Zoo staffing by one person
- Increasing number of participants from six to eight students
- Increasing the cost to students by 10% (we had 14 students on a waiting list, so the cost threshold had obviously not been reached).

After the trip, we solicited feedback from the trip participants in order to make adjustments for future trips:

Positive comments:
"It was a great experience that I will never forget about.'
"This trip was more than what I expected"
"The wholesale place was surprisingly interesting. They had a lot going on there and it was fun to poke around."
"As you might have guessed, I loved the trip!"
"The trip was an amazing experience and so much fun. I made some great friends and it really strengthened my belief that marine biology is what I want to do for the rest of my life…"

Suggestions made by students:
"Make designated roommates instead of letting people choose"
"Maybe pick out restaurants ahead of time"
"Maybe you could shorten the pit stops and lunch breaks."
"You could allow more time to relax in the hotel because I felt like we barely had down time."

From this feedback it appears that we offered the students a valuable learning experience, but that we underestimated the role that interpersonal relationships play in the success of a trip such as this. Social media interactions will probably need to be restricted on future trips; students were frequently accessing their smart phones during scheduled activities such as tours, and this likely detracted from their overall learning experience.

All in all, this was a very successful trip for the Zoo and students alike. We intend to expand our offerings for experiential learning to include adult education trips, as well as collecting trips to other locales. An adult experiential learning trip to Trinidad is scheduled for the spring of 2015. Other ideas include local collecting trips, as well as major collecting trips to tropical areas utilizing boat-based venues.

Students and Toledo Zoo staff retrieving a seine in the sea grass flats of Shinnecock Bay, Long Island.

WHEN DO GIANT FLASHLIGHT FISH (*Anomalops katoptron)* LIGHT-UP THE MOST AND WHAT MAY CAUSE IT?

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Abstract:

Anomalops katoptron or giant flashlight fish are found in lower lit areas of the ocean in the Indo-Pacific region. Little is known about these flashlight fish since they live 200-400m (650-1400ft) deep, typically in caves. They can be noticeable in dark waters due to a bioluminescence patch called a photophore under their eye. There are theories that the fish use their photophore to search or hunt for food and possibly for communication or mating. This study was conducted to observe *Anomalops katoptron* behaviors and quantify their light-ups or "blinks". The results reveal that flashlight fish blink their photophores and light up the most during feeding times.

Introduction:

Flashlight fish can grow up to 35cm (14in) and have a black body with a blue hue to its dorsal and caudal fins. They are closely related to the lantern fish belonging to the family Anomlopidae, order Beryciformes. They have a split dorsal fin and a light-emitting organ (photophore) under each eye. The bean-shaped photophore glows due to the bioluminescent bacteria that inhabit the light organs.

The light organ in the flashlight fish's eye is always on, but the fish can choose to hide the light organ by tucking it down and away in a pocket to appear "off". When the light is showing, it is considered a "light-up" in this study.

Bioluminescence is a product of a chemical reaction in an organism. The oxidation of luciferin, catalyzed by the enzyme luciferase, results in oxyluciferin and light. Adenisine triphosphate (ATP) provides the energy to produce the luciferin.

$$\text{Luciferin} + \text{ATP} \rightarrow \text{luciferyl adenylate} + \text{PPi}$$

$$\text{luciferyl adenylate} + \text{O2} \rightarrow \text{oxyluciferin} + \text{AMP} + \text{Light}$$

Methods:

In an exhibit at the Aquarium of the Pacific in Long Beach, CA, there are 19 flashlight fish in an 80 gallon tank. The dimensions of the tank are 213.4 cm (7 ft) long X 61cm (2 ft) wide X 91.4cm (3 ft) deep. The fish were acquired by an overseas collector so age and sex of fish are unknown. The décor of the tank includes 6 tall thin rocks of varying heights and black sand. The tank turns over at a rate of 55 gallons per minute and water is supplied through the bottom right side of the tank through a spray bar. Their diet consists of frozen mysis shrimp, small krill, herbivore gel, and live mysis shrimp. The fish are fed 2-3 times per day. The light source of the tank comes from 2 LED red lights which automatically come on from 7:35 AM – 6:00 PM.

Observations of the flashlight fish were recorded on video for 5 minute intervals throughout the day. Observations were recorded during feeds, rests (non-feeding and non-cleaning periods), baseline rest (1st rest of the day), cleanings, and tank distractions (external noises or light disturbances). Behaviors were defined as follows:

Rest behavior: All the fish were schooling in an approximately 35.6 cm (14 in) diameter on the right side of the tank close to the bottom where the tank's water supply jet is located.

Feeding behavior: Fish were fed 2 times per day and were scattered throughout the exhibit.

Cleaning behavior: The fish were dispersed vertically and moving away from the tools being used to clean the tank.

Distractions: When a camera or phone flashed, the fish would disperse every direction for a few seconds, and then come back to their resting pattern after 20-30 seconds. When there was a sound distraction behind the exhibit, some fish would travel to the top of the tank (possibly looking for food) and then return to a schooling pattern.

The observations were narrated on video and notes were recorded in a journal. Each video clip was reviewed in order to count the number of light-ups per frame. For every 5 minute video, the number of light-ups was counted every 5 seconds. The number of light-ups was recorded in a spreadsheet to calculate the average for each 5 minute interval and averages were calculated for each activity (all feedings, all rests, and all cleanings). *(Editor's Note: units throughout are therefore equivalent to "average events per 5 minute interval.")* Light-up averages were compared among the various activities in order to determine when the flashlight fish light-up the most. This method of observation and recording was repeated in 94 videos over a 4 month period.

Results:

Rest behavior: The rest counts ranged from 0.3 to 2.3 throughout the day and night. The average of all the rest counts was 1.05. The baseline rest average (first rest period of the day) was 1.61.

Feeding behavior: During a feeding, the fish show their lights quite frequently and only hide their photophore for a split second. The flashlight fish light-up the most during feedings with a light-up average of 3.79. There is a measurable count that drops off after the feeding is over (to an average of 1.68) and it may continue to drop over time (Table 1).

Cleaning behavior: During a daily tank cleaning, the number of light-ups averaged 2.85. This is higher than a baseline rest by 43.4%. However the cleaning count was lower than a feed count by 33%.

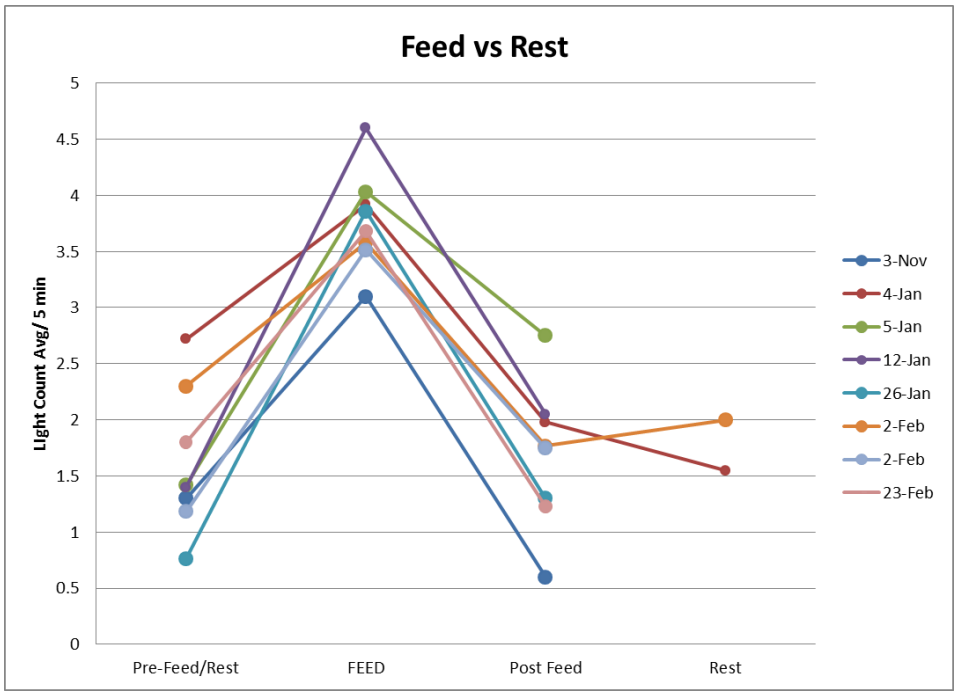

Table 1: Feed vs. rest light-up averages.

Rest over a 24 hour period: When examining the number of light-ups during the rest periods throughout different times of day, the counts varied widely (0.3 to 2.3). When reviewing the data on a scatter plot, there was no obvious correlation to one particular time during the day. However, the higher light-up rest counts were at the typical aquarium feeding times of 8:30 AM and 3:30 PM (Table 2). This result may suggest the fish were expecting to be fed as if learning the scheduled feeding times. Also, the fish appeared to be more active in the morning. This may be due to the 12 hour interval of fasting during the evening and the fish were more excited to eat during the morning. Their light-ups are noticeably less frequent after the aquarium closed (lowest count of .30).

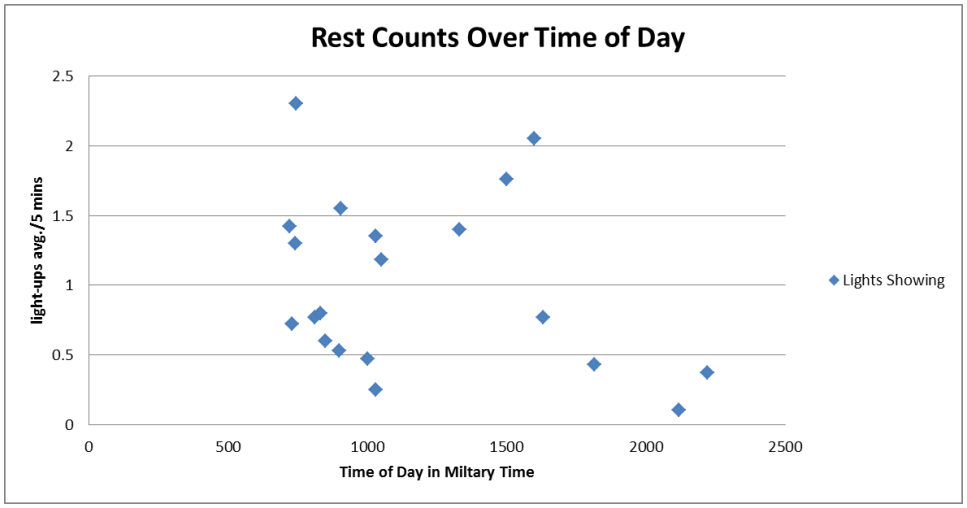

Table 2: Rests over a 24 hour period, 5 minute average intervals.

Schooling Behavior: Although the light-up counts varied, the behavior of the fish does seem different at different times throughout the day. During early morning, the fish are schooling but spread out in a wider school about 61cm (24in) across, and seem to be quite active. During the midday and evening period (when the gallery is open to guests), the fish swim in a tighter school and mainly hover in the lower right corner which is the darkest area of the exhibit. When people came up close to the tank, the fish would light more frequently and tighten up their formation. At night, the fish were in a much tighter school (20.3cm or less).

Diet: Live mysis had the lowest light-up counts at 2.08. The fish seemed to turn their light off more frequently during live mysid shrimp feedings than when eating frozen foods. The highest light-up count occurred during the frozen mysis feedings at 4.03, then small krill at 3.92, followed by herbivore gel at 3.58. The light-up count of frozen mysis was almost double that of the live mysis light-up count. The fish seem to be more active and excited the longer the time is between feeds.

During any activity in the tank such as cleaning or a feeding, the fish would move faster than a rest period and light-up more frequently. After any activity within the tank, the fish would eventually group back together into their "normal" resting school.

Conclusions:

The hypothesis was that the flashlight fish would light-up the most during a feeding time as flashlight fish supposedly blink/ light-up to attract their prey or find their food. The hypothesis was supported because the data showed the highest number of light counts on average during feeding at 3.79 (Table 3). The feeds had the highest light-up counts out of all the different activities or scenarios. The feeding light-up counts were noticeably higher than the baseline rest counts on the same day (135% on average). When graphed, the data showed a "peak" from baseline rest up to feed count and back down to post feed (Table 1). The average decrease is -55.68% post feed.

Activity	Avg/5 min intervals
All feeds	3.79
All cleanings	2.85
All baseline rests	1.61
All rests	1.05
All night rests	0.3

Table 3: Average light-up count per 5 min intervals.

The cleaning count versus the baseline rest count was 43.4% higher on average. The fish light-up more during a cleaning than a rest, but not as much as a feeding. The fish may light up more due to lack of space with tools in the exhibit. On a plot chart, the light-up counts for all rests varied throughout the day. However, some of the higher points were around usual feeding times, so the fish may have been more active and expected to be fed. The flashlight fish may burn lots of energy lighting up and have a high metabolism in which they need to eat often.

At night after the gallery closes, the fish light-up count did drop to the lowest light-up average of 0.30. The observations showed the lowest light-ups occurred when all the fish did not light-up over a period of time. There appears to be a "sleep mode" when the fish are not fed or distracted late at night. Typically, 4 fish would point their heads into the center, while the rest of the school would be huddled tightly around them in a sphere-like shape.

A possible hierarchy was observed in these fish. There were fish acting as scouters that would check on surrounding noises or distractions, parting from the rest of the school, and would swim around other parts of the tank. During video recordings, there were also less dominant fish that swam above the school and didn't light-up as much. Possible "leaders" would normally group at the front of the school of fish and would light-up more frequently.

The fish would typically school in the darkest part of the tank. During the day, the darkest part of the tank was the lower right corner. After 9:00pm, when the gallery lighting was dimmed, the darkest part of the tank was the left lip of the tank and the fish would move there. A dark tarp was moved in front of the tank to test the darkest spot theory and to create darkness in certain spots of the tank. The fish would always go to the darkest part of the tank.

Acknowledgements:
The author would like to thank Dee Ann Auten, Aquarist II for her mentoring and patience with the many videos; and the curator of fishes and invertebrates, Dr. Sandy E. Trautwein for supporting this project.

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General Information for

RAW 2016

New Orleans, LA, USA

(note that Registration for RAW 2015 at the Monterey Bay Aquarium has CLOSED)

Host institution: Audubon Aquarium of the Americas – New Orleans, Louisiana

When: March 21-25, 2016 (TAG meetings Monday March 21st)

Conference Hotel: Astor Crowne Plaza New Orleans
739 Canal St. New Orleans, Louisiana (504) 962-0500

Rates: *rooms must be booked by February 19, 2016 to get conference rate.*

Single	**$149.00/night**
Double	$149.00/night
Triple	$169.00/night
Quad	$189.00/night

Tentative Schedule for the week:

TAG Meetings	March 21
General Sessions	March 22-25
Ice Breaker	March 22
Aquarium Evening Event	March 23
TBD night events	March 21 & 24

Abstract Deadline: February 10, 2016

For more information, see www.rawconference.org after RAW 2015 (Monterey, CA) has concluded in March

Registration Fee: *Before March 1, 2016*

$75 – Public Aquarium or Non-profit affiliate

$125 – Commercial affiliate

Late Registration and Commitment Form:

The form for 2016 will be located on RAW website (after RAW 2015 concludes in March). 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 fee to:

$100 – Public Aquarium or Non-profit affiliate
$150 – Commercial affiliate

Airport & Other transportation options:

The conference hotel is located 15 miles from Louis Armstrong New Orleans International Airport (MSY). This airport is a hub for Southwest Airlines and flights are pretty reasonable. Baton Rouge Metropolitan Airport (88 miles) and Gulfport-Biloxi International Airport (77 miles) are also options but can be slightly pricier.

Sample Southwest Airlines Air Fare Estimates to New Orleans for Budgeting (as of 2014):

NY LaGuardia	$190
Newark	$219
Chicago	$260
Atlanta	$192
Ft. Lauderdale	$170
LAX	$208
Dallas	$91
Charlotte	$154

Drive time Estimates to New Orleans:

Atlanta	6 hrs 37 mins
Houston	5 hours
Jacksonville	7 hrs 40 mins
Charlotte	10 hrs 15 mins
Chattanooga	7 hours

Transportation Estimates between Louis Armstrong New Orleans International Airport and the Conference Hotel (as of 2014):

Taxi	$33 ($14 for each additional passenger)
Airport shuttle	$20 ($38 round-trip)

For questions regarding RAW 2016
Please email Dee Murphy Dmurphy@audubooninstitute.org

RAW 2014 ABSTRACTS

Regional Aquatics Workshop, April 22 - 25
North Carolina Aquarium, Wilmington, NC, USA

Tuesday, April 22
Propagation/Conservation/Collecting

Animal Professionals

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

Shark Ray Breeding - Instructions Not Included

Mark Dvornak, Jolene Hanna, Scott Brehob and Jen Hazeres
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On 24 January 2014, Newport Aquarium's adult female shark ray "Sweet Pea" gave birth to a total of seven pups. Having confirmed the pregnancy just 16 days before, the birth that quickly followed was a surprise to the husbandry biologists. Raising the pups has been an extremely challenging experience, especially in a land-locked aquarium. This presentation will outline not only the birth itself, but the many challenges and frustrations that followed. What aquarium biologists have learned - and what is still unknown - also will be discussed.

Makin' Babies - Captive Rearing of Echinoderms

Jordan Salyers
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Public aquariums often seek cost-effective and sustainable methods of obtaining and maintaining invertebrate populations to support the industry's mission of conservation. In addition, recent limitations of available healthy wild stocks make acquiring and maintaining echinoderm collections challenging. For over 30 years, many species of echinoderms have been successfully and efficiently reared in research labs using culture methods that can easily be adapted by public aquariums to create a sustainable and cost-effective in-house stock. The methods to produce and rear echinoderms from gamete to adult will be reviewed and applications for public aquariums will be described.

From Egg to Bay: Adventure Aquarium's Horseshoe Crab Head-Start Program

Matthew Ferroni
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The American horseshoe crab (*Limulus polyphemus*) is of great ecological importance to the Delaware Bay, as its eggs provide a vital source of food to migratory shorebirds. Hemocyanin extracted from horseshoe crabs is used by the biomedical industry to manufacture LAL, making the crabs incredibly valuable to humans as well. The Adventure Aquarium started its Horseshoe Crab Head-Start Program in June 2011, with the intent to collect naturally fertilized eggs, rear the offspring under captive conditions, and release them back into the bay once they had reached a suitable size. Each year, naturally fertilized eggs are collected from multiple nest sites on Kimbles Beach in Cape May Court House and transported back to Adventure Aquarium for hatching. They are kept in a closed system containing synthetic seawater, with temperature and salinity maintained at 79°F and 30ppt, respectively. After hatching, the crabs are divided into groups and placed in floating containers with 1000µ nylon mesh bottoms. They are fed twice daily with enriched *Artemia* nauplii, Cyclop-eeze, and New Era Marine Pellets, based on instar stage. Water changes are performed weekly and growth rates and survivorship are recorded. These culture techniques developed over the last several years have resulted in accelerated growth rates and increased survivorship. The American horseshoe crab is known to molt five to seven times in its first year of life under natural conditions. Under captive conditions, however, Adventure Aquarium has reared individuals reaching an equivalent size in only five months, and growing as large as their eleventh instar in only one year. In the summer of 2014, Adventure Aquarium plans to release the 2011 and 2012 groups of crabs back into the Delaware Bay. Pending the results of a tag retention study, set to commence in early 2014, these animals maybe tagged using coded wire tags.

Sponsor Presentation – Aqua Logic

Sponsor Presentation – Dynasty Marine

Catching Lightning in a Jar: Preliminary Success of Breeding *Pomacanthus sp.* at The Columbus Zoo and Aquarium

Aaron Jeskie and Ramon Villaverde
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The Discovery Reef habitat at the Columbus Zoo and Aquarium currently houses four different species of *Pomacanthus*, each with at least one breeding pair. During the summer of 2013, 12 juvenile *Pomacanthus* sp. were raised from eggs collected from Discovery Reef using devices designed by Rising Tide. Eggs were rinsed with clean sea water and separated using various sizes of micron mesh. Initial attempts to raise several different batches of eggs all resulted in total mortality after several days. After hatching, several unidentified species of fry

also were seen in addition to *Pomacanthus*, but none survived past the first few days. In further attempts to raise fry from collected eggs, multiple variables were adjusted, including egg density, food density, salinity, airflow and container size. Ultimately, using a plastic pretzel jar with a healthy population of copepods and green water yielded the 12 larvae that would survive. During the first critical days after hatching, the fry progressively fed on copepod nauplii, adult copepods, and then *Artemia* nauplii. Despite this success and our continued efforts, we have been unable to duplicate our results. Nonetheless, this singular success demonstrates that any facility with a breeding population of fish has the potential to set up hatching and rearing vessels at little to no cost.

Conditions for Captive Spawning of French Grunt, *Haemulon flavolineatum*

Matthew Dawson, Larry Boles, Stacy Knight, Matthew Wittenrich and M. Andrew Stamper
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In order to reduce the environmental impact and increase the sustainability of the aquarium trade, more ornamental species need to be captive-bred. One such species that is found in many large aquariums around the world is the French grunt, *Haemulon flavolineatum*. Little information is known about their natural reproductive behavior, making captive breeding for French grunts difficult. While culture of larvae in other *Haemulon* sp. has been documented, volitional spawning of *H. flavolineatum* has never been documented in a captive setting. At The Seas with Nemo and Friends® at Walt Disney World Resort®, 48 adult French grunts were stocked evenly into six 650-L recirculating seawater systems, each supporting two 250-L tanks. Within two months of stocking, egg production began. Environmental conditions such as temperature, salinity, dissolved oxygen, and pH were recorded daily. Photoperiod was maintained on a 12L:12D light cycle. Eggs (mean diameter = 0.95 mm) from each system were collected and quantified daily for 216 days. Total system fecundity ranged from 769,000-2,510,000 eggs over the entire spawning period and was poorly correlated with temperature ($R^2 = 0.345$), although a slight positive trend was noticeable. A sex ratio for each tank was also determined by sampling gametes using a catheter insertion method. The results of this study demonstrated that captive breeding of French grunts is possible under ideal conditions. However, more research is needed to determine the optimal environmental conditions that will maximize fecundity and egg viability for sustainable, cost-effective production of French grunt larvae for the aquarium industry.

Asexual Propagation of *Acropora cervicornis*

John Than
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What do you do when you only have one genotype to release and there are no other genotypes available to cross with it? Well, if you are Ken Nedimyer and are part of The Coral Restoration Foundation (CRF), you have options. During the coral spawn of 2012, The Florida Aquarium and its partners encountered this issue when they had only one genet fire off and none

to cross. With a special activity permit in hand, and the nature of how the CRF is licensed with its corals, the ability to physically remove "bundles" from another coral of a different genet became an option. Included in this presentation is a review of the events and the technique used to retrieve the bundles to achieve fertilization, as well as a summary of the successes, failures and plans to use this technique in future coral spawning events.

Professional Development Session – Separate registration during lunch

Sponsor Presentation – Cairns Marine

São Tomé and Príncipe – The West-African Opportunity

João Correia, Nuno Rodrigues and Telmo Morato
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São Tomé and Príncipe is a Portuguese-speaking archipelago in the Gulf of Guinea, off the western equatorial coast of Africa. Its geographical characteristics result in a peculiar marine fauna and some unique endemic species. Flying Sharks' privileged relations with African Portuguese-speaking countries resulted in a three-week expedition to São Tomé, with the main goal of collecting as much information as possible to evaluate its potential as a new operations base. While in São Tomé, the team documented local marine life by underwater photography, visited local fish markets and talked to members of the fishing community. These explorations revealed that local artisanal fisheries are an important resource for the islands' population, providing 60-70% of animal protein for human consumption. Unfortunately, this trip also revealed a borderline catastrophic state of fish stocks, with local artisanal fishermen catching almost exclusively juvenile specimens, and law enforcement being virtually inexistent.

Collection and Acclimation of Deep-Water Teleosts Without Ever Getting Wet...Almost

Frank Young
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This presentation will review the process of collecting and acclimating deep-water benthic teleosts from depths of 250-700 FSW using recreational fishing gear. It will discuss the selection of fishing locations and the proper gear to use, with additional techniques for actually hooking and landing the fish described in further detail.

Once the fish are brought to about 100 FSW, a diver enters the water to de-hook and vent the fish. It is then slowly brought to 30 FSW where they are vented again and hung in a bag for decompression. After the decompression is complete they are brought to the surface and placed in cool water for transfer to the holding facility. The specialized holding facility for these deep-water fishes also will be highlighted.

Determining the Life History and Spawning Cycle of the Grooved Brain Coral *Diploria labyrinthiformis*

Bob Snowden
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Little is known about the spawning cycle or life history of the grooved brain coral, *Diploria labyrinthiformis*. The Pittsburgh Zoo & PPG Aquarium has been working cooperatively with Caribbean Marine Biological Institute (CARMABI) in Curacao, Netherlands Antilles and the SECORE Foundation to determine the spawning cycle of a *D. labyrinthiformis* population (n=40) located at Holiday Beach in Curacao. Repeat observations of this population were made throughout last year, and gametes were collected, fertilized, reared through embryogenesis into larvae and eventually settled onto ceramic tiles. This year we will be putting 2-year-old *D. labyrinthiformis* colonies back out onto the reef for the first time. Detailed analysis of the embryogenic cycle was documented as well as the formation of asexual larvae from the breaking apart of some embryos at the prawn chip stage of development. This species – like others in the same genus – is a broadcast spawner, but differs by spawning while the sun is still up. It was thought that this species was spawning like this throughout its entire range, but was not proven until also observed in Puerto Morelos, Mexico in August 2013. This species has now been observed spawning in May, June, July, August and September. This breakthrough in understanding the life history of *D. labryinthiformis* is an important step towards the conservation of the species and has not been described in detail previously.

Sponsor Presentation – CaribSea

Do Public Aquarium Live Coral Exhibits Grow Under Cannon Type LED Lights?

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In order to address heating and energy conservation concerns, LED performance was evaluated on a 2,000 gallon coral reef exhibit in the Marine Cove at the Sertoma Butterfly House. The goal of the study was to determine if coral health could not only be sustained, but thrive and flourish under an all-LED environment. The aquarium system used in the study is 120 inches long, 72 inches wide and 36 inches deep. The exhibit is comprised of many small polyp corals at varying depths as well as large polyp corals on the sand bed. Four hundred-watt metal halide fixtures were exchanged for various cannon-form LED lights. The LED cannons consisted of four major manufacturers of the same wattage and composition style. All four were compared by measuring the PAR levels, spectrum, and spread of the LED cannons. Other aspects considered included heating and cooling benefits, the effects of focused light into the exhibits, and increased budget.

Displaying the Sexy Beast: Collection and Transport of Large *Carcharhinus albimarginatus*

Lyle Squire
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Silvertip sharks (*Carcharhinus albimarginatus*) are an enigmatic, heavy-set species of requiem shark that attain a length of 10 feet. They are unmistakable with the iconic white blazes on the tips of every fin that appear to glow as they cruise the steep, deep, outer ledges of coral reefs and atolls. In the tropical Indo-Pacific, silvertip sharks have long been revered by divers as one of the most impressive apex predators to be encountered. Despite these striking attributes, silvertip sharks have remained unrepresented in public aquarium collections until recent times, with Cairns Marine completing the world's first air transport of silvertip sharks from the Great Barrier Reef (GBR) in 2010. Four years later, and with nearly a decade of fieldwork and research, a suitable location and window of time have been identified for the collection of silvertip sharks for display. This presentation - with a substantial video component - charts the journey from collection on the remote Ribbon Reefs of the GBR (located 250 nautical miles north of Cairns) to the eventual transportation of animals on standard commercial passenger aircraft. The husbandry and holding procedures onboard Cairns Marine's flagship *Sharpshooter* are discussed, as well as the care at the land-based facility where preparation for shipping takes place. Recent groundbreaking shipments of these animals have now helped to answer the questions of how large a silvertip shark can be and still be safely transported, and what duration of time can be achieved without compromising animal health.

How Enriched *Artemia* Nauplii Affect the Development of White-spotted Jellyfish (*Phyllorhiza punctata*)

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The white-spotted jellyfish *Phyllorhiza punctata* is exhibited in aquariums all over the world. Its growth and overall development has been shown to be extensively dependent on its feeding regime. In order to study the effects of different types of enrichment diets on the growth rate of *Phyllorhiza punctata* ephyrae, an experiment using bioencapsulation of *Artemia* nauplii was conducted. In the comparison of different enrichments, results showed that the highest growth rate was promoted by the SELCO enrichment. Observations also indicated that adult *Phyllorhiza punctata* increased in size and displayed improved overall condition when this type of enrichment was used.

Boring but Important: Supply Chains Defined

Laura Simmons
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Every wild-collected aquarium animal originates in a fishery, travels within a supply chain and is provided by a supplier, but fisheries, chain of custody and suppliers vary greatly in quality, ethics, size and type. The first concept in understanding source and supply is that all wild-caught aquarium animals come from fisheries. Fisheries are becoming a primary concern for aquarium collections, as they are the first step in every supply chain. Connecting the fishery to the aquarium requires a chain of custody that can be very simple or quite long and complex. Single or short, traceable supply chains originating in established, well-managed fisheries are the most sustainable, but also the least common. The majority of aquarium animals distributed globally come from regions of least management and the supply chains are long, involving collectors, middle men, exporters and importers. The supplier is the primary point of contact for all aquaria when acquiring animals. It is a mistake to assume that knowing the supplier means that an aquarium can trace the origins of its animals. In fact, in most instances, the supplier is simply a wholesaler or trans-shipper at the end of a very long line of actors in the supply of the animals. This presentation will define the fisheries, supply lines, and suppliers from which aquarium animals come, and attempt to give a clearer understanding of the misunderstood reality of animal sourcing.

Wednesday, April 23

Veterinary Session

Sponsor Presentation – Red Sea

Discospondylitis in a Green Moray Eel (*Gymnothorax funebris*)

Dr. Shane Boylan, Jennifer Oliverio and Jason Cassell
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A green moray eel of 12+ years presented with a rapid onset of positive buoyancy and inappetence. The animal was floating horizontally with a 90° bend at the swim bladder with the tail pointing ventrally. Blood-work, GI endoscopy and ultrasound revealed no abnormalities while radiography, confirmed by CT, detected spinal compression cranial to a hyper-inflated swim bladder. Conservative therapies of time, vitamins, glycoaminoglycans and steroids did not improve the condition after several months. Alternative therapies were then chosen, and twice weekly near-infrared/cold laser therapy was conducted for two months. Habituation to handling allowed for laser therapy to occur without sedatives. Laser therapy continued once weekly for nine months with improvement in sinusoidal body movement, but buoyancy remained positive with intermittent control of orientation. Experiments with swim bladder deflation followed by anticholinergics +/- oxytetracycline failed to effectively prevent swim bladder inflation. However, four months of normal buoyancy on display were achieved with aspiration of gas and

an intraluminal injection of hetastarch and 100mg/ml enrofloxacin. After positive buoyancy returned, we attempted a surgical approach to inject cyanoacrylate into the swim bladder to permanently prevent gas release, with gas volume controlled by aspiration/injection. For an additional month, buoyancy was controlled, but orientation remained inconsistent. After two years of therapy, the eel was humanely euthanized under MS-222 anesthesia to evaluate the swim bladder and spine. In conclusion, improvements in movement and appetite suggest some benefit to the therapies. In addition, the location of the swim bladder rete was unknown until necropsy, and current anatomical knowledge will greatly help future cases.

Fish Anesthesia: Alternatives to MS-222

Dr. Kate Bailey
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The necessity for fish anesthesia on a daily basis is a reality for many of those working in the aquarium setting. This necessity should produce a driving force for the exploration of more predictable ways to produce anesthesia, both for animal welfare, and the peace of mind of people providing care. There is a paucity of literature investigating reliable and predictable anesthetic agents in fish. Currently, the only FDA-approved fish anesthetic agent is MS-222 (tricaine methanesulfonate), a sodium channel blocker in peripheral neural tissue, but has no known mechanism of action for production of general anesthesia. Due to our lack of knowledge surrounding MS-222, it is logical to investigate alternative anesthetic agents with known mechanisms of action. Potential alternative anesthetics include alfaxalone and propofol, both of which are GABA agonists that are used routinely as injectables for general anesthesia in companion animals. Fish have GABA receptors in their brain, and the findings from initial investigations using koi carp are promising. Alfaxalone has been investigated for both immersion anesthesia and intramuscular injectable anesthesia. While immersion provided rapid and reliable anesthesia, intramuscular injection produced variable results, and resulted in an unacceptable incidence of morbidity and mortality. Propofol was investigated as an immersion agent, and also provided rapid and reliable anesthesia. While this is useful information for small and easily confined fish species, it is much less useful for large and hard to confine species. Other drugs such as ketamine and dexmedetomine are used for injectable anesthesia with varying success. Further studies are needed to investigate alternative options for injectable anesthesia, especially in larger fish, as the options currently available are frequently unreliable and variable in efficacy and utility.

Public Display Aquatic Invertebrates: Medical Advances

Dr. Gregory A. Lewbart
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Invertebrate animals comprise over 95% of animal species, yet non-parasitic invertebrates are vastly underrepresented in the typical veterinary school curriculum. This presentation will provide a brief introduction to the more prominent aquatic invertebrate groups

(coelenterates, mollusks, crustaceans, echinoderms, horseshoe crabs) and introduce some recent medical advances that apply to maintaining these species in captivity. Specific topics for review include: white patch disease of sponges, eversion syndrome of jellyfishes, aspergillosis of soft corals, white and brown jelly syndrome of hard corals, growth anomalies of *Acropora* spp. and *Montipora* spp., acroporid serratiosis, green algal disease and pharmacokinetics in horseshoe crabs, diet-induced shell disease of crustaceans, and pain recognition in shore crabs. The emerging and important topic of animal welfare for aquatic invertebrates also will be reviewed.

Voriconazole Toxicity in African Penguins (*Spheniscus demersus*)

Dr. Craig Harms and Dr. Rebecca Wells
NC State University College of Veterinary Medicine, Center for Marine Sciences & Technology (Harms) - Raleigh, NC
Bayside Hospital for Animals (Wells) - Fort Walton Beach, FL
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Penguins are popular exhibit animals, and traveling exhibits allow institutions without penguin facilities to capitalize on their conservation messaging and revenue potential. Prophylactic antifungal treatments are commonly administered during shipping to prevent aspergillosis, a primary cause of mortality in captive penguins. From April – September 2012, four African penguins were housed in a traveling exhibit at the NC Aquarium at Pine Knoll Shores. The penguins arrived already on a treatment of voriconazole at 20 mg (= 6.0 – 6.9 mg/kg for 2.9 – 3.3 kg birds) once daily x five days, and transitioned well into the exhibit. All four birds had prior histories of suspected aspergillosis, so in case of clinical signs developing while veterinarians were away, voriconazole was prescribed to have on hand for administration following veterinary consultation by phone. Extrapolating from the prescription label recommended dosage of one 50 mg tablet (15 mg/kg) once daily for chickens, it was decided to cut the dosage in half for prophylactic treatment during shipment at the end of their stay. Penguins received 25 mg once daily for three days, 50 mg for one day, were anorectic for three days, and then received one more treatment at 25 mg. After that, the medication was discontinued and supportive care instituted. One penguin died five days later, while the other three survived. Liver enzymes were markedly elevated, and blood voriconazole concentrations five days after the last treatment exceeded 10 µg/ml, which is considered toxic. The main histopathology finding was severe hepatic lipidosis. Even the lower extrapolated dose turned out to be too high for penguins, and miscommunication led to an additional overdose.

Sponsor Presentation – Quality Marine

Sponsor Presentation –Ecoxotic

A Customized 3D-Printed Splint for Stabilization of an Open Front Flipper Fracture in a Green Sea Turtle (*Chelonia mydas*)

Dr. Emily F. Christiansen
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Sea turtles frequently present to veterinarians and rehabilitation centers with traumatic injuries caused by boat strikes or similar impacts. These injuries commonly include fractures of the extremities, with or without open wounds. While sea turtles have an impressive capacity to heal without significant intervention, the preservation of maximal function in these flippers is a priority for successful return to the wild. Various forms of surgical and external coaptation have been attempted in sea turtles, with limited success. Their natural saltwater environment is not conducive to typical bandaging and splinting materials, and the forces applied against a flipper in the aquatic medium are substantial enough to cause most types of surgical fixators to fail, even in smaller individuals.

In July 2013, a juvenile green sea turtle was found floating at the surface in near-shore waters of central North Carolina. The turtle had sustained an open fracture of the right radius and ulna with ventral exposure of bone fragments, along with other injuries. The flipper exhibited severe dorsoflexion at the fracture site, but the tissue distal to the injury retained adequate circulation and nervous function. A 3-D printer was used to create a customized form-fitting plastic polymer splint designed using modeling software around CT-derived tridimensional renderings of the fractured and intact flippers. The splint was generated in a two-piece clamshell design with multiple perforations, with a hinge along the cranial edge, and included a large window over the open wound to allow for cleaning and monitoring. The splint was initially applied under sedation, and secured onto the flipper with synthetic suture material. The turtle tolerated the splint very well, and showed increased swimming confidence within minutes of being placed in the water. The splint remained in place for 40 days, providing considerable external stabilization with no obvious side effects.

Whole Blood Transfusion in a Cownose Ray (*Rhinoptera bonasus*)

Dr. Alexa McDermott, Dr. Cara Field, Dr. Tonya Clauss, Lynda Leppert, Nicole Hatcher, Jeffery Ingle and Mayela Alsina
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A female cownose ray (*Rhinoptera bonasus*) presented for extreme lethargy and pale coloration. Examination revealed a heavy *Branchellion torpedinis* leech load in the oral cavity, and the leeches were manually removed. A blood sample taken from a ventral wing vein showed hematocrit (HCT) levels at 1% (normal individual at 20%). Due to severe anemia and weakness the prognosis was poor, so a whole blood transfusion was carried out in an attempt to save the ray. A 20 kg cownose ray with a HCT of 35% served as the blood donor. Sixty ml of whole blood was collected from the donor into heparinized syringes at a ratio of 1:10 heparin to blood. The solution was then delivered directly to the recipient via a ventral wing vein, administered over a 25-minute time frame. The recipient received a prednisolone injection to minimize

possible transfusion reaction, as well as antibiotics and supportive iron dextran and Vitamin B complex injections. Post-transfusion, a major cross match was performed with no signs of agglutination. One day following the transfusion, the recipient's HCT had increased to 4.5%. Four days post- transfusion, the ray's coloration had drastically improved, and the HCT had increased to 9.5%. Over subsequent days the ray began swimming with more vigor, and the HCT continued to increase. At 55 days post transfusion, the ray had a HCT of 22% and was behaving normally.

Common Causes for Mortality Events in Captive Sand Tiger Sharks (*Carcharias taurus*)

Dr. Robert George
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Sand tiger sharks, *Carcharias taurus*, are the most common large sharks displayed in public aquariums. In the wild, female sand tigers mature at 9-10 years of age, while males reach maturity in only 6-7 years. Exhibited sand tigers often live in captivity for 10-15 years, and in some cases survival times extend well over 20 years after capture have been reported. With exhibit sand tigers routinely reaching geriatric ages, it would be expected for such animals to eventually show systemic problems associated with age-related organ failure. In addition to organ failure as a cause of death, a variety of physical issues such as prolapse of the spiral valve, gastric prolapse, gastro-intestinal foreign bodies, and reproductive disorders can occur. This presentation will review some of the most common causes of mortality for these sharks.

Successful Treatment of *Neobenedenia* Monogeneans in Quarantined Teleosts

Dr. Catherine Hadfield, Dr. Leigh Clayton and Ashleigh Clews
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Neobenedenia are capsalid monogeneans that infect the skin of marine teleosts and are a common cause of recurrent morbidity and mortality in aquarium systems. The life cycle is direct and eggs can survive in the environment for extended periods. Twenty-two shipments of tropical marine teleosts from Queensland, Australia were received by the National Aquarium between March 2012 and May 2013; *Neobenedenia* was identified on fish from most of the shipments. Infection was often subclinical, but disease was observed in two outbreaks, with surgeonfish and tangs particularly susceptible. Early signs consisted of subtle changes in behavior, reduced appetite, and brown coalescing spots on the dorsum and pectoral fins. Later signs consisted of dark coloration, intermittent flashing, clamped fins, ulcerative keratitis, lethargy, and mortalities. Praziquantel immersion was the most effective treatment, at a dosage of 4 mg/L every four days for at least 15 doses. Outcomes were monitored using visual exams, skin scrapes, opportunistic necropsies, and fine-mesh egg traps. All systems remained on treatment until the fish were moved to exhibit (a minimum of 60 days after parasites were last seen), and all fish received freshwater dips prior to transfer. Ongoing monitoring shows no recurrence of *Neobenedenia*. Although the quarantine cost was high, the potential cost of managing *Neobenedenia* on exhibit would have been higher. Quarantine protocols for incoming Indo-Pacific fish now include

praziquantel immersion on arrival (5 mg/L for 2 hr after acclimation), copper sulfate (0.18-0.20 mg/L for 21 d), with praziquantel (4 mg/L q 4 d for 8 tx) starting during copper therapy, and freshwater dips prior to transfer.

Metabolomics in Fish Health Management

Dr. Michael Stoskopf
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Advances in the management of fish health over the past two decades have enhanced longevity for many species and provided the basis for earlier recognition of several frequently occurring syndromes. Improved knowledge of fish physiology and anatomy combined with better approaches to husbandry, routine monitoring, and more reliable delivery of therapy have been key to these advances. However, the development of diagnostic techniques for earlier and more accurate diagnoses of disease conditions is needed. Metabolomics offers the opportunity to obtain quantitative information about a wide array of biochemical pathways important in the homeostasis of teleosts utilizing relatively noninvasive samples and techniques. Currently in the development phase, metabolomic diagnostics is already providing important insights into the etiology of complex conditions, and holds significant promise to revolutionize fish health management. In this talk, sampling considerations and techniques are reviewed, and potential future applications for this emerging technology are discussed.

Thursday, April 24
Animal Training

Sponsor Presentation – Living Color Enterprises Inc.

Capturing Elasmobranchs Using Behavioral Husbandry Training Techniques to Reduce Stress in Transports and Medical Procedures

Nicole Leier
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This presentation will break down the processes by which we were able to condition these animals to being captured. It will also compare and contrast pre-training blood values against the blood values received after implementing the training techniques. As a result of these training methods, we are able to reduce stress and more easily perform animal moves and preventative health checks on our elasmobranchs.

The Successful Training, Capture and Transport of a Goliath Grouper (*Epinephelus itajara*)

Amy Reierson
The Seas with Nemo and Friends, Walt Disney World Resort - Orlando, FL
amy.l.reierson@disney.com

The Seas with Nemo and Friends® at Walt Disney World Resort® is home to a 5.7 million-gallon, multiple-species exhibit. For many species, behavioral husbandry is a key tool to ensure good overall health and safe capture techniques. For a resident goliath grouper (*Epinephelus itajara*), primary and secondary reinforcements were used to desensitize the animal to enter a series of enclosures, including crates, bags and pipes. The animal's voluntary participation in this training program enabled aquarists to successfully capture and transport him to a new environment. This presentation will review the key factors that helped make this training a success as well as the challenges that were overcome along the way.

Can You Teach an Old Nautilus New Tricks?

Gregory Jeff Barord
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Living nautiluses are members of an ancient lineage that can be traced back nearly 500 million years, long before mammals, dinosaurs, insects or sharks were even around. However, even after nearly 500 million years of environmental change and evolutionary adaptation, living nautiluses still closely resemble their fossilized ancestors and are thus called "living fossils." The closest living relatives to nautiluses are octopuses, squid and cuttlefish but there are significant differences between them. Aside from the presence of a shell, the nautilus nervous system is less developed than that of other cephalopods and is a primary reason why nautiluses have been labeled as unintelligent or just plain "dumb." However, recent evidence suggests that nautiluses possess similar learning and memory capabilities as other cephalopods. In this presentation, we show that nautiluses use visual cues within their environment to navigate an experimental maze and change their behavior depending on the location of a "homing beacon." The nautiluses were conditioned to locate the beacon during five training trials. Next, a sixth "probe" (or test) trial was conducted by either removing the beacon or shifting the beacon 45° or 90° from the original location. During each probe trial, the behavior of the nautiluses changed as a result of the change in beacon location. This suggests that nautiluses are capable of different types of learning strategies and have the behavioral flexibility to alter their actions based on their environment. These results shed light on the evolution of the nervous system in cephalopods, but also may provide relevant information related to their captive care.

Training of Marine Eels: How to Make Feeding Easier

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A multi-species eel display is a popular exhibit at The Seas with Nemo and Friends® pavilion at the Walt Disney World® Resort. The 260-gallon cylindrical exhibit was home to six snowflake moray eels (*Echidna nebulosa*), two zebra moray eels (*Gymnomuraena zebra*) and four spotted snake eels (*Myricthys maculosus*) when it opened in 2006. This diverse collection, however, proved to have challenges when feeding due to the eels' natural foraging behaviors, resulting in unpredictable swimming patterns and in-tank aggression. In order to create a more controlled and efficient feeding environment, a training and enrichment plan was created to improve and enhance the welfare of these animals by reducing aggression and controlling diets. It was also thought that this plan could provide opportunities to easily capture the animals for medical procedures or get daily visual assessments for body condition. We also wanted to demonstrate to guests the ability of fish to respond to behavioral management. The snowflake moray eels were trained to swim into clear 2-inch PVC pipes where they were offered food from the top. The spotted snake eels were trained to come up onto specific artificial plate corals at the top of the reef work of the exhibit where they were individually fed using either short feeding sticks or by hand. The zebra moray eels were baited out of the holes in the reef work of the exhibit using a long feeding stick. To date, the greatest challenge has been dealing with aggression among conspecifics and our greatest success has been working through the challenges to achieve full participation from all of our eels in our various training techniques. The progression of this project has taken several years to arrive at our current training strategy; its continued success is due to regular readjustments based on the results of the implementation as well as the changing population dynamics.

Thursday, April 24

Exhibitry/Animal Health/Quarantine

Sponsor Presentation – Piscine Energetics

Attempted Treatments for *Eimeria southwelli* in Cownose Rays (*Rhinoptera bonasus*)

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Cownose rays (*Rhinoptera bonasus*) are one of the most commonly exhibited elasmobranch species in public aquaria. This species is commonly afflicted with the coccidian parasite *Eimeria southwelli* that is associated with (though not definitively the cause of) chronic fatal disease in an aquarium setting. The three North Carolina Aquariums at Fort Fisher, Pine Knoll Shores and Roanoke Island maintain cownose rays in their aquarium collections; veterinary care is provided by the North Carolina State University, College of Veterinary Medicine, Center for Marine Sciences and Technology. From 2008 to 2012, several medical

treatments were attempted to reduce or eliminate the parasitic loads in cownose rays that were confirmed positive with moderate to heavy infestations of *Eimeria southwelli*. Four separate trials were performed, including ponazuril paste (administered orally), and clindamycin, sulfamethoxine and enrofloxacin - all injected intracoelomically. None of these attempts was successful in reducing or eliminating the coccidian, and the sulfamethoxine treatment regimen appeared to contribute to the fatality of a few animals. Throughout all of the treatments, optimal routine husbandry, especially nutritional support, was paramount in delaying the onset of clinical symptoms and maintaining clinically healthy rays.

Degradation of Formalin – Evidence for Changes to Long-Term Formalin Treatment Protocols

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Pharmaceuticals are commonly used for the treatment of disease in the aquarium and aquaculture industries. Formalin has been used as a standard treatment for ectoparasites and fungi for nearly 80 years, but little is known about the effects of formalin on saltwater recirculating systems or the rate of formalin degradation in saltwater. This study documents the effects of a five-day long-term formalin treatment (25ppm) on saltwater aquaria. The response of the biological filter, formalin degradation rates, and the effects of fish biomass on formalin degradation were examined. Throughout the experiment, the biological filtration function remained unaffected. Formalin concentrations regularly decreased from 25 ppm to 0 ppm in less than 6 hours, and fish density had no effect on the degradation rates. The dynamic degradation of formalin suggests the need for frequent testing and follow-up doses during treatment cycles to ensure the maintenance of therapeutic concentrations and to prevent recurrence of pathogens.

Aulani Marine Teleost Quarantine - Part Deux

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At RAW 2012, Eric Curtis reviewed pre-opening quarantine procedures for fish in the 167,000-gallon snorkel-lagoon at Aulani, a Disney Resort and Spa, in Hawaii. This paper is a follow-up to his presentation with a discussion of our updated quarantine procedures. Quarantine protocols had to be modified when we discovered that fish were not completely free of parasites after treatment. By increasing chloroquine dosage and decreasing salinity, we were able to successfully bring our fish through quarantine. Keeping fish free from subsequent infections is also a major concern, since "biosecurity" is an issue when hotel guests snorkel in the ocean-side lagoon and then snorkel in Rainbow Reef. However, no significant disease outbreaks have occurred at this time. We will present several hypotheses to try to understand this situation.

Sponsor Presentation – Reed Mariculture

Professional Development Session – separate registration over lunch

RAW Business Meeting – Bid for 2016 Conference Host

Sponsor Presentation –Brightwell Aquatics

How Water Quality Influences the Captive Husbandry of Hellbenders, *Cryptobranchus alleganiensis bishop*

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The St. Louis Zoo Wildcare Institute's Ron Goellner Center for Hellbender Conservation is home to the largest collection of Ozark Hellbenders (*Cryptobranchus alleganiensis bishop*) in the world - approximately 4,200 specimens. The Center's focus is to breed and head-start these unique, fully aquatic salamanders for eventual release in order to augment remaining populations. Water quality has played an important role in our accomplishments to date, influencing decisions pertaining to enclosure design and maintenance, breeding, larval rearing, feeding strategies, etc. We are constantly monitoring and adjusting water quality parameters to ensure our continued success with this species.

A Survey of Voltage in Aquaria Housing Elasmobranchs

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This is an update of a talk originally presented at the International Elasmobranch Husbandry Symposium in November 2013. It is well known that elasmobranchs interpret faint electric fields, which aids in navigation and prey location. Components within aquaria usually operate with some measure of errant voltage, but how this may affect captive elasmobranchs has not been thoroughly investigated. This project examined whether stray voltage may warrant being a regular husbandry consideration along with tank size, substrate and life support. Institutions participating in this survey were given instructions describing how to test voltage with a voltmeter and then asked to complete a questionnaire regarding the system(s) tested and the species they housed. The data to be presented provides a baseline of typical voltage variances within aquaria and presents possible avenues of further investigation into the effects of stray currents on captive elasmobranchs.

Sponsor Presentation –Hayward Flow Control

Sponsor Presentation – YSI/Xylem

When a Dock is More Than Just a Place to Tether Boats: Incorporating Learning Laboratories on a Floating Surface

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Bringing students and the community closer to the ocean was the driving force behind a comprehensive plan to replace the Ocean Institute's 1970s-era dock. By 2000, the entryway to our floating, mobile classrooms was in dire need of repair. Decades of wear in Dana Point Harbor and the tugging of large vessels had taken its toll; the dock facility was beyond the point of salvage. When plans to build the landside educational facilities were developed, a new dock was to be included, but budgetary constraints delayed its construction. By 2011, the plans had blossomed into a trove of interactive educational stations, including a Biological Diversity Laboratory. Utilizing floating platform technology, a sturdy surface incorporates plumbing and electrical chases for the life-support system as well as hidden features for educational discovery. Not to mention the Ocean Science Landing is engineered to keep all the aquaria level with changing tides and surges. Built right on the water in mid-2013, the Maddie James Seaside Learning Center now introduces thousands of K-12 students and public visitors to current and emerging issues in oceanography, environmental science and maritime history.

1985 Called - They Want Their *Artemia* Methods Back

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Artemia production is one of the most important aspects of a successful hatchery, playing a crucial role in larval rearing protocols. For public aquariums, they are the go-to feed for jellyfish. Yet when was the last time your institution updated its protocols? Do you still use oil-based enrichments? In the Roger Williams University/New England Aquarium larval rearing workshops, one of the most popular topics among participants has been the best method of *Artemia* production. These workshops demonstrated that most participants wereunaware of key factors, including: 1) the damage they were doing to *Artemia* during harvesting; 2) how to separate the hatching envelope from nauplii; 3) how to properly enrich *Artemia*, and; 4) how to cold-store *Artemia* to reduce costs, space and labor. This talk will review the latest and most up-to-date *Artemia* protocols, and will challenge institutions to question their current methods.

Pest Management in a Semi-Open Freshwater Multi-Taxa Exhibit

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Pest control in an aquarium or zoo setting is never an easy task. Many of the conventional baits available are also harmful to fish, amphibians and reptiles, making it difficult to find effective bait that is also safe for aquatic environments. One of the "Maryland Mountains to the Sea" gallery exhibits at the National Aquarium had an infestation of cockroaches that was overtaking the exhibit. Traditional pest eradication techniques such as many pesticides and traps could not be used, as they posed a threat to the animal collection and were visible to the public. So creative and new approaches to managing this pest issue were used to control and mitigate the issue. These methods included researching and choosing bait that did not pose a threat to the animals, training the turtles to eat from a targeted area and not scavenge food off the bottom, and finally, an extensive clean-up of the exhibit. Biological Programs staff and Exhibits & Design worked together to repair and patch holes, seal cracks and lay out bait with the active ingredient Dinotefuran. After only a few weeks of intensive measures, the infestation was not only controlled, but was completely eradicated while the exhibit remained open to the public with all the animals on display. Ongoing measures include targeting animals and laying out bait on a regular basis to keep the cockroach infestation under control.

Friday, April 25

Acquisitions and Collections Management

Sponsor Presentation – New Era

The Collection and Husbandry of
Mahi Mahi (*Coryphaena hippurus*) at Adventure Aquarium

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Coryphaena hippurus - better known as the common dolphinfish or mahi mahi - are well known for their striking coloration and fast growth rate. Mahi mahi are found worldwide in subtropic, tropic and offshore temperate waters. They are popular in both the food industry and game fishing. September 2013 was the start of an exciting couple of months at Adventure Aquarium. The beginning of September kicked off the first of two successful collecting trips for mahi mahi off the coast of New Jersey. In total, Adventure Aquarium successfully collected and transported 18 mahi mahi back to our 44,000-gallon quarantine system. After two months in quarantine, they were introduced into our 760,000-gallon Ocean Realm exhibit over the course of two weeks. While transferring this species onto exhibit presented a few obstacles, we continuously modified the plan for introduction. Even though our introduction of the mahi mahi to the exhibit was successful, we continue to address challenges of feeding, predation, dive operations, parasites and wound treatment, which will be presented here.

Rockfish Eye Surgery and New Techniques in Prosthetics

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Fish in captive settings are prone to eye disease, and rockfish in particular are commonly afflicted with a disease known as exopthalmia. Eye disease in fish is known to cause decreased feeding activity, poor growth, development of secondary illness and increased mortality (Nadelstein and Lewbart, 1997; Hargis 1991). In some cases, removal of the diseased eye in combination with medical treatment may resolve the issue and allow the fish to return to normal health. Over the past decade, Seattle Aquarium veterinarians and aquarists have performed multiple surgeries to remove eyes from rockfish, using various techniques. Additionally, value has been shown in researching techniques to install artificial eyes in these fish. The placement of a prosthetic eye in a one-eyed rockfish allows aquariums to accomplish three goals: 1) continue to display a fish that may otherwise be euthanized or taken off display due to aesthetic concerns; 2) provide the fish with a convincing replacement to potentially reduce fish aggression, and; 3) refine a technique that has yet to be implemented as commonly in fish as it has in mammals and birds. In addition, removal of the eyes allows vets to establish diagnostic tools to assess the cause of the eye issues. This presentation summarizes the work performed at the Seattle Aquarium with respect to rockfish eye removal and prosthetic installation.

Managing Mycobacteria in Syngnathid Systems

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The fact that non-tuberculosis mycobacteria (NTM) is a group of one of the most troublesome pathogens in the aquatic industry is well known. Those of us with syngnathids in our collections also know that, for some reason, this family of fish is particularly susceptible to these infections. This presentation will briefly revisit what we know, or think we know, about *Mycobacterium* spp.; as well as review some of the changes in basic husbandry that the Seattle Aquarium has used to significantly reduce the number of NTM breakouts and mortalities in our syngnathid populations.

Sponsor Presentation – Reef Brite

Sponsor Presentation – Living Color AMS

Rehabilitation of a Sea Turtle with a Serious Skull Fracture

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The sea turtle rehabilitation facility located at the North Carolina Aquarium on Roanoke Island is a small center managed in cooperation with a local non-profit organization, the Network for Endangered Sea Turtles. For many years, the facility primarily has been a receiving location for cold-stun events occurring along the northeastern North Carolina coast. In 2013, the center accepted a boat-strike turtle with deep wounds to the carapace, as well as a head wound that exposed the brain case. In conjunction with the NC State University College of Veterinary Medicine, it was decided that this turtle was a viable candidate for rehabilitation. Although there is little literature available concerning the survivability of these types of cases, anecdotal evidence from past cases demonstrated a proclivity to euthanize such patients. Working with the knowledge that few such injured turtles have survived non-surgical rehabilitations, we chose to proceed. Nearly one year after admission, results have been excellent, adding to the list of successfully treated sea turtles with skull fractures penetrating the brain case.

Majano Anemone Infestation and Control at the National Aquarium

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The National Aquarium recently experienced a large majano anemone (*Anemonia majano*) infestation in its 6,400-gallon (24,227-liter) Pacific Coral Reef Exhibit. These small anemones covered large expanses of live rock and corals, resulting in the partial or complete mortality of several coral colonies. Although much information is available about effective techniques for controlling *Aiptasia* anemone infestations, there is less information in the hobbyist literature about majanos, especially for the treatment of an infestation of this magnitude. Many techniques for removal were tried, including mechanical, chemical and biological treatments. The best solution was found to be manual removal where possible, followed by treatments with calcium hydroxide. At this time, the infestation is under control, with majano populations at manageable levels and the appropriate majano predators added to the system and included on our stocking lists for the future. The National Aquarium now has a plan of action for future majano infestations.

The Jack Track: A Fish Transportation System

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The Birch Aquarium at Scripps needed a way to move a couple dozen jack mackerel, *Trachurus symmetricus*, down from our two-story kelp tank to a ground-level outside holding area, so we came up with the idea of a fish "slide." We adapted the idea from fish returns that

had been used years before at Hubbs-Sea World Research Institute to return 3”-6” white seabass to raceways after tagging. Although many other fish species were transferred in buckets, the fast moving jack mackerel never would have made the two-flight trip in a bucket, and the narrow stairwell made stretchers a poor alternative. Come see this fast-paced session to see how we moved these fish and many others quickly and easily.

Interview with a Vampire Squid: Advances in the Collection and Display of Deep-Sea Cephalopods

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In preparation for a show featuring the diversity of cephalopods, MBA’s husbandry team has worked its way down an ambitious list of target species. The challenges associated with exhibiting cephalopods are well known in the aquarium world, but the most daunting and unorthodox of our goals was to display deep-sea cephalopods. To develop a husbandry protocol for a type of animal few people have ever seen, let alone tried to keep alive in captivity, we’ve had to get really creative. Over the last 18 months we’ve supplemented our own knowledge with lots of reading, a fair bit of trial and error, and a growing partnership with our sister institution, the Monterey Bay Aquarium Research Institute (MBARI).

Our Aquarium’s founder David Packard had a particular fascination with the deep sea, and so he supported the formation of MBARI in 1987. One of the founding members of the institution, deep-sea pioneer Bruce Robison, remains the head of the Midwater Laboratory at MBARI. Using remotely operated vehicles (ROV), his team observes and collects inhabitants of the Monterey Submarine Canyon from as far down as 3,000 meters. When we approached them with the proposition of collaboration, we learned that Robison’s current post doctorate fellow happened to be an expert in deep-sea cephalopods, a perfect match for our collecting goals.

Since 2012, MBA’s husbandry team has assisted with and eventually begun leading ROV expeditions to collect deep-sea squids and octopuses. Our current record for longevity of an animal is 28 days, with a specimen of *Vampyroteuthis infernalis* that fed in captivity on multiple occasions in mid-2013. We have experimented with many different collection, transport and husbandry techniques, and to date have kept over 30 individual animals from 10 distinct species. This past January, one of our *Opisthoteuthis* or “Dumbo” octopuses laid the first documented clutch of cirrate octopus eggs in captivity. This demonstrates how our progress benefits not only the aquarium community, but the research community as well.

This talk will outline the inception, progression and current status of our deep-sea cephalopod program, including a discussion of life support, display techniques, species descriptions, and of course, lots of sweet footage of deep-sea animals.

Changing Feeding Strategies in a Large Multi-Species Exhibit

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The National Aquarium's 335,000-gallon Atlantic Coral Reef Exhibit houses 67 species with a total of 620 specimens. In 2013, feeding strategies for teleosts in the exhibit were revised in an effort to improve both nutrition and sustainability. Moving from a primarily thawed, frozen-food diet to a primarily pellet-based diet resulted in reduced costs, reduced food preparation times and reduced actual feeding times. In addition, the pellet-based diet appeared to improve overall animal health, as indicated by body condition and coloration. Improvements in water clarity and water quality were also observed, especially in reduced nitrate levels.