DRUM *and* CROAKER

A Highly Irregular Journal for the Public Aquarist

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Cover Photo: Bat Stars from B. Morrow's paper, p. 3
Interior Gyotaku: Bruce Koike
Interior Line Art: Craig Phillips, D&C Archives

DRUM AND CROAKER 50 YEARS AGO

(From the January 1971 issue)

Richard M. Segedi

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Aquarium Symposium - An End to the Confusion

Wm P. Braker, Director, John G. Shedd Aquarium

Two national meetings attracted a good representation of aquarists last year: the American Society of Ichthyologists and Herpetologists meeting in New Orleans and the American Association of Zoological Parks and Aquariums meetings in Buffalo. It was apparent, in both places, that there was much concern over the future meetings and permanent affiliations for our group.

After much correspondence and due deliberations, it is the consensus of this committee that we should all meet in Salt Lake City on September (?) 1971, with the AAZPA. *(Also see related topic on page 123 of this 2021 issue.)*

Phase-out of the National Fisheries Center and Aquarium

Bill Hagen, Assistant Director - NFC&A

The Fisheries Center, authorized by the Congress of 1964, and for which construction and operating appropriations have been provided, appears to be on the economy rocks.

The National Aquarium in the Department of Commerce Building will continue to function. Permanent personnel of the Fisheries Center presumably will be absorbed into the Bureau of Sport Fisheries and Wildlife.

Use of Laminated Plastic in Wood Aquariums

Herbert W. Reichelt, Millen National Fish Hatchery, Millen, GA

We have experienced some trouble in the aquarium at Bureau of Sport Fisheries and Wildlife Hatcheries with blistering and peeling paint in display tanks. These are of wood construction and painted with epoxy resin. To correct this, we have been gluing laminated plastic (Formica®) to the tank sides. Several companies make a smooth texture, solid color, varying in shades of green and blue, suitable for aquarium use. The glue is contact cement. A sufficient bond can be arrived at by sanding the rough areas and gluing directly to the old paint. Several tanks with the laminated plastic sides have been in use for over two years and no problems have been encountered.

Signs and Sense

George B. Rabb, Chicago Zoological Park.

The stereotypy prevailing in labeling exhibits in zoos and aquaria seems to stem from traditions of the natural history curiosity cabinet.

Thinking about the content of signs, rather than just consulting Axelrod and other bibles leads, to very fruitful thoughts about exhibits. The basic one is: why have a particular animal or exhibit at all? What biological, sociological or other point is being made? Are signs the way to communicate this point? Etc.

AQUACULTURE OF *Patiria miniata*

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Abstract

Bat stars, *Patiria miniata*, are a readily available species that are displayed in many public aquariums. While this animal has been captively raised before, it is not commonly done in the public aquarium setting. The aim of this paper is to condense the necessary information needed to raise these sea stars. This includes spawning methods, target sperm and algal concentrations, and an exploration into effective rearing vessels. *Patiria miniata* were cued to spawn and their larvae were raised in multiple styled bins with variable conditions to see what could be most effective. Small, static bins circulated by bursts of air have shown to be efficient vessels. Successful aquaculture and trade of these sea stars could reduce or eliminate the need of wild collection for this species, but the extended juvenile growth period does provide some obstacles that should be considered before you raise this species.

Introduction

The bat star, *Patiria miniata,* is a common temperate species exhibited by many public aquariums. They can be found in the Pacific Ocean ranging from Alaska to Baja California. The distribution of this sea star is likely related to substrate availability. They prefer areas with boulders (Schroeter et al., 1983). Stable substrates, like boulders, are also used by most adult kelp plants (Schroeter et al., 1983). Because of these preferences, Bat stars are often seen in kelp beds. These ecosystems are negatively affected by *Lytechinus* urchins. They limit kelp growth by grazing on the smaller life stages, reducing the overall recruitment rates of new algal species. Fortunately, *P. miniata* are omnivorous and predate upon these urchins so much so that adult *Lytechinus anamesus* actively avoid areas, or kelp beds, where these sea stars are present (Schroeter et al., 1983). While their ecological role might not always be positive, these sea stars do affect the health of the kelp forest environment.

A long-term warming trend from 1976 to 1998 occurred in southern California. Two years after this trend began, sea star wasting disease started to appear showing a relationship between the two (Eckert et al., 2000). This disease destroyed populations of several sea star species including *P. miniata*. With the scare of wasting disease in the past, the collection of sea stars, in general, has fluctuated. While *P. miniata* showcases lots of variety in color, it may not be an easy choice to collect from the wild forever as diseases and climate may change what we take for granted today (Eckert et al., 2000). The ability to captively reproduce these animals will help ensure their representation throughout aquariums without the need of wild collections. *P. miniata* can also be found with ripe gonads throughout the entire year unlike other stars (Strathmann, 2017). However, these animals take a long time to reach a displayable size. Therefore, attempts made should be effective enough to supply other aquariums that cannot dedicate this time or space. This paper will detail the successes and failures here in Omaha as well as a review of other used practices.

Spawn

When selecting stars to reproduce, animals with plump arms (Fig. 1) were chosen. This is a strong indicator that they are ripe with gametes. Once selected, stars were cleaned of debris, and all water used here after was filtered to 5µm to reduce culture contaminates. Spawning was cued through heat shock. Six stars were brought from their display temperature of 12.2°C and were placed into a container at 16.6°C. These stars were all in the same container because an individual spawning asteroid can stimulate others to spawn (Motti et al., 2018). However, a few minutes after a sea star began to spawn, they were moved to a new bin. One for females and one for males. This was done to provide greater control over sperm concentration for fertilization. You can distinguish female and male sea stars based on the size of their respective gametes. Eggs are much larger, heavier, and often yellow in color (Fig. 1a). Sperm is much smaller and white (Fig. 1b). In total, four females began to spawn after an hour, all within 15 minutes of each other. One male started spawning after 3 hours and one star never spawned.

Figure 1. a) Female *Patiria miniata* (Left) spawning; b) Male *Patiria miniata* (Right) spawning.

Additionally, stars can be induced to shed their gametes through the injection of methyladinine-1 (Wessel et al., 2010). To achieve this, inject a 25-ppm solution of Methyladinine-1 at 1 ml/50 grams wet weight into the celomic cavity of the star (Simon, 1974). Spawning should begin 30 minutes or more after the injection (Strathmann, 2017).

Fertilization

Once eggs were collected, they were fertilized by transferring 5 ml of diluted sperm from the male container (~10,000 ml) to the egg container and gently stirred with a pipette every 3-4

minutes for 20 minutes. Fertilization can be successful from 15°C up to room temperature (Wessel et al. 2010). The target sperm ratio is 1:10,000 (Ettensohn, 2004, Wessel et al., 2010). If you are privy to a microscope, optimal fertilization is achieved when the eggs are inseminated during metaphase 1. This is approximately 10-15 minutes after germinal vesicle break down (GVBD). However, eggs can be fertilized any time during meiosis 1, which lasts about 1 hour after GVBD. If eggs are in meiosis II, they are more susceptible to polyspermy (Wessel et al., 2010). Note that eggs here, were inseminated roughly 1.5-2.0 hours after they were shed showing quite a bit of flexibility in terms of fertilization timing. Once eggs were thought to be inseminated, it was time to move them. Stirring was stopped to allow eggs to settle to the bottom. They were then siphoned into a new container leaving most of the suspended sperm behind. Dead/decaying sperm can lead to bacterial blooms. From this container, eggs were divided up into their respective rearing vessels.

Containers

Containers were chosen of all shapes, sizes, and styles (Fig.2, Fig. 3). They were all placed in a wet table held at a constant 16.67°C. Rearing temperatures between 15°C and 20°C are optimal (Simon, 1974). The total system volume was 1,600 liters and was filtered to 5 µm. Several containers had 7.62 cm holes drilled through them. These holes were covered by 64 µm mesh and secured by Dowsil™ 795 silicon. These vessels were referred to as 'diffusion' bins because they passively exchanged with the surrounding bath water. Weight was added over the top of these vessels so that they did not shift around. The flow-through bins were the largest containers (Fig. 3). Water was directly pumped into these bins. Flow was constantly adjusted from 1-2 drops a second to 3-5 drops per second. It was increased and decreased based on water clarity. The static bin, unlike the rest, did not constantly exchange water. It received two 30-40% water changes daily through a sieve (Fig. 4). This helped protect the larvae during the water changes by dispersing the suction force. If the sieve were too small, larvae could be sucked into it and potentially damaged.

Figure 2. From left to right are the base dimensions of each container used. 18 qt container: 25.4 cm x 30.48 cm tall, White trashcan: 17.78 cm x 10.16 cm x 24.77 cm tall, Black trashcan: 15.24 cm x 10.16 cm x 24.13 cm tall, Static bin: 20.32 cm x 15.24 x 24.13 cm tall.

Figure 3. Two 58.42 cm x 43.18 cm tall flow-through bins, commonly referred to as BRT's. An external standpipe controls the water level. Water enters from above (not pictured) and must flow through a 64 µm screen before exiting.

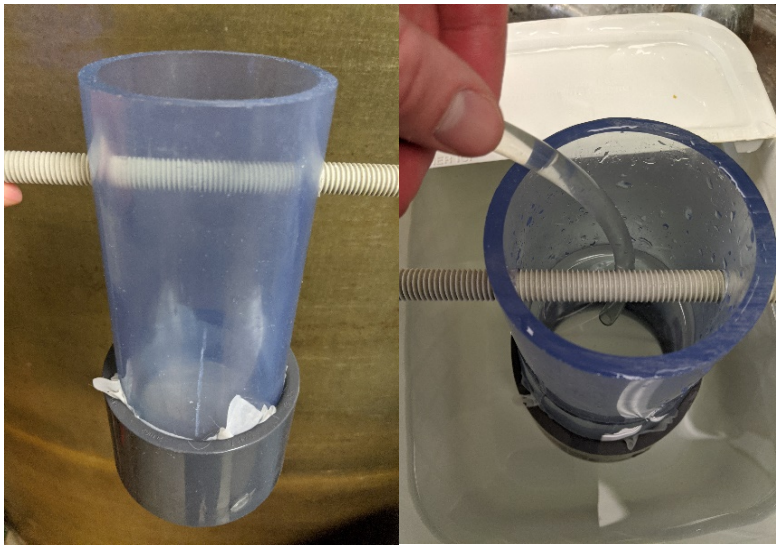


Figure 4. Standard sieve with 64 µm screen.

Egg Density

Fertilized eggs were separated into the different styled containers ranging from densities of 2-5 eggs/ml. However, a target density of 1 larva/ml is recommended (Ettensohn, 2004). The remaining zygotes, which made up the vast majority, were discarded. Eggs were then tumbled via constant air flow through 3.175 mm hard airlines with the goal of keeping them from settling to the bottom. The least amount of flow required to do this for each individual container was used. A Tetra® Whisper® 100 air pump was used to power the air manifold. After two days of tumbling, the eggs developed into bipinnaria (Fig. 5).

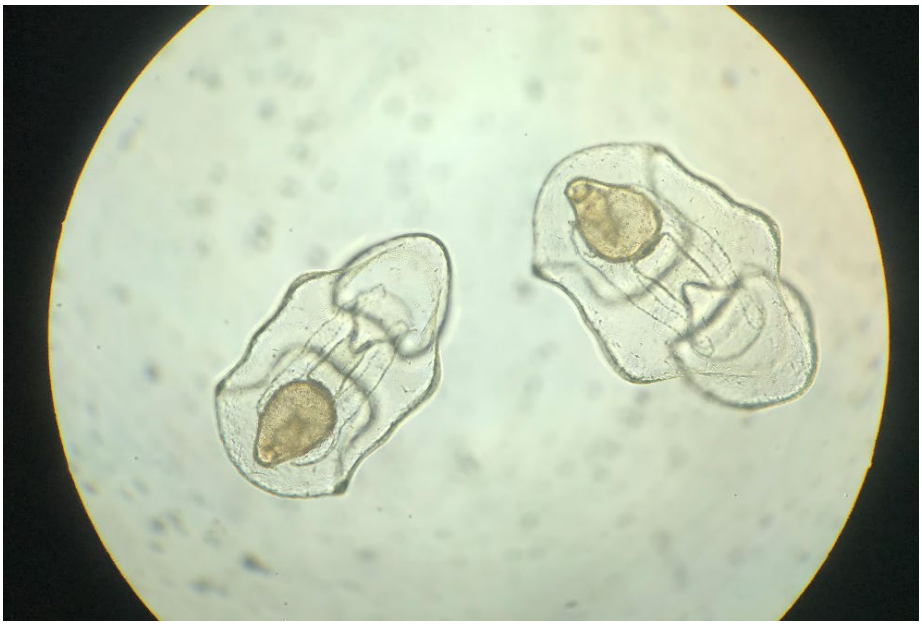
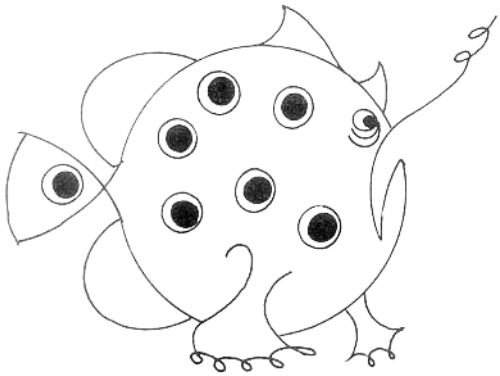


Figure 5. Two *Patiria miniata* larvae in the Bipinnaria stage 6 days post hatch. They are roughly 1 mm in length.

Growth and Care

Once the eggs developed, half of the containers were switched from constant flow to bursts of flow using Nearpow LLC timer switch outlets with the same goal of keeping larvae off the bottom. These outlets were set to 4 seconds on, 54 seconds off. Note that the air flow was higher for these when compared to containers with constant flow to counteract the time they were off.

Settled pockets of bad eggs and dead larvae were siphoned out 1dph (day post hatch). After the dead and decaying material was removed, small pockets of live larvae would form. Once or twice a day, the hard airline was moved to resuspend these pockets. Once the stars started reaching late brachiolaria stages (Fig. 6), as indicated by the presence of extended brachiolaria arms and visible adhesion disks (Strathman, 2017), this was avoided so that potential settlement would not be interrupted.



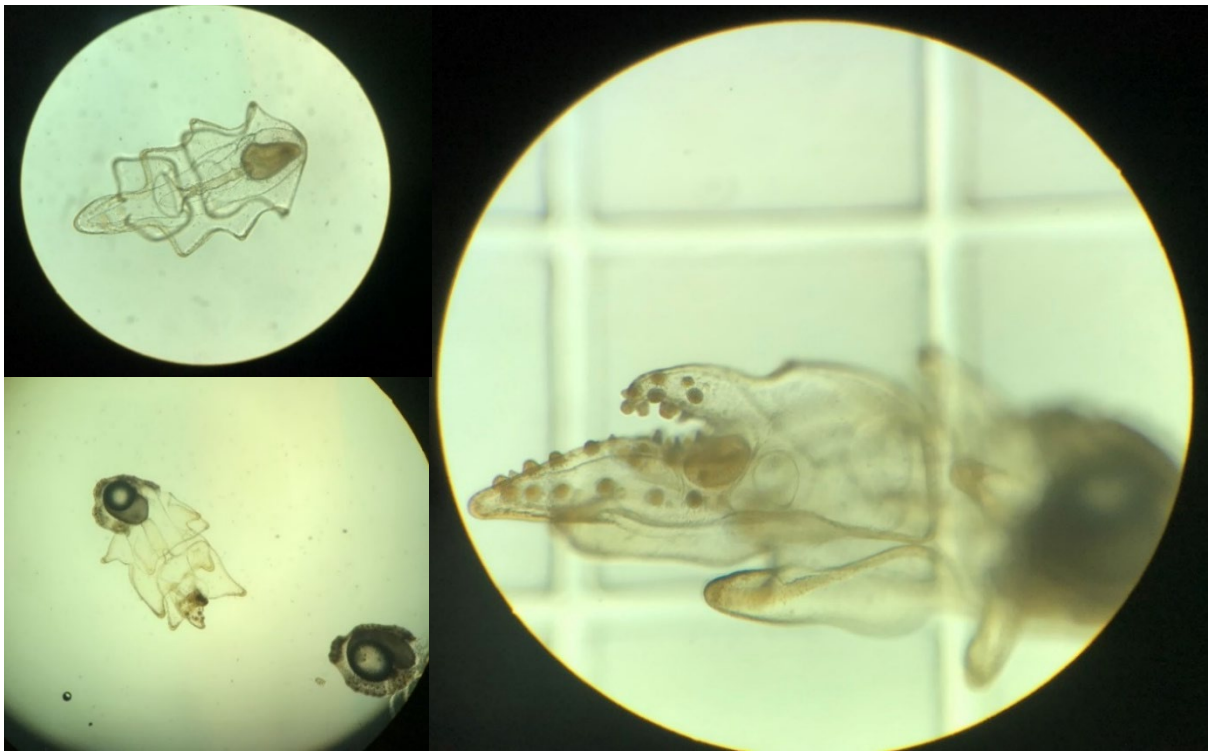


Figure 6. A 20-day old, immature *P. miniata* larvae in the brachiolaria stage (top left). A 33-day old, semi-mature, *P. miniata* larvae approaching the late brachiolaria stage by beginning to show visible adhesion disks on arms (bottom left). A 33-day old, mature *P. miniata* larvae (~2 mm in length) with fully extended brachiolaria arms with adhesion disks visible on the left side of the larvae (right).

Three algae species were used to feed the larvae approaching settlement. They were fed equal volumes of *Chaetoceros gracilis, Isochrysis tahiti,* and *Rhodomonas lens*. However, their algal densities were not equal (Table 1) therefore, the true ratio comparing cells/ml was 369:416:1, respectively. The achieved density in the bins (12,499 ± 1000 cells/ml) appeared as a slight tint from clear. This amounted to 20-160 ml per day depending on bin size. If bins were not completely clear the following day, no food was given, and flow was increased were applicable.

Offering more than one algae species is recommended (Ettensohn, 2004) but you can be successful feeding only *Rhodomonas lens* at 3,000 cells/ml (Cameron and Holland, 1983). If you do not have a cell counter, stomach contents can be easily checked via microscope. Several echinoderms can accelerate growth too fast and die at metamorphosis if they are fed too much (personal communication). This might not factor in for this species but be wary of overfeeding if larvae stomach contents are consistently full at any given time of day. Generally, larvae in the static bin had had 0-3 cells in their stomach before algae was added in the morning.

Table 1. The average harvest density of three phytoplankton species from their respective algae cultures.

Species:	*Chaetoceros gracilis*	*Isochrysis tahiti*	*Rhodomonas lens*
Density (cells/ml)	3.52×10^6	3.97×10^6	9.54×10^3

Once one star visibly settled, larvae in the static bin were placed into a larger BRT to increase available surface area. The diatom growth already present in all the bins can be a strong enough cue for settlement for certain species. Additional, common settlement cues were added the next day. This included live rock with several coralline algae species and 4ml of histamine (by Professional Formulas).

Settlement began at 33 days post hatch with most larvae settling at 55 dph. Note that some larvae were still in early developmental stages due to apical budding. Weeks after settlement, stars were relocated into the bath to increase the available surface area. This allowed more access to growing surface algae. Eventually, all juveniles were relocated, and the bins were removed. Juvenile bat stars were offered frozen *Calanus* sp. close to once a month but otherwise left alone with 24/7 lighting to grow algae. Once stars reached larger sizes (>7 mm); they were offered various mysid species multiple times a week.

Results

Roughly 2,000 sea stars were successfully settled. The static bin was the most successful bin for larval retention, experiencing minimal loss. Conversely, the larval density was halved in 30 days in the BRTs. All bins using constant airflow crashed two days post hatch. *P. miniata* juveniles showed recognizable pigment started at 104 days post settlement, and at 14 months these juveniles averaged 1 cm in width.

Discussion and Future Study

The burst timers were more successful across the board and could set a new standard in larval culture. Bin size and type are also important factors that could be fleshed out further. The small diffusion bins were comparable in size to the static bin, but they performed differently. One potential problem with the diffusion bins was the inability to hold algae cells long term. Likely, the food density dissipated too quickly starving the larvae in these bins early. However, when you compare only bin size, the smaller bins all performed better than the larger bins during the larval growth phases. This could be linked to the amount of airflow required to suspend the larvae. Bins with smaller base areas required less airflow to do this effectively. A brine cone could be an advantageous vessel considering these qualities.

Roughly 300-400 *P. miniata* juveniles were acclimated and moved into colder exhibits (8°C and 12°C) when they were 1 mm in length. These stars are no longer accounted for and presumed dead. Once juveniles reached 3-5 mm in length, they were acclimated and moved into the same exhibits mentioned before. These animals are currently alive 6 months later. Potentially, there is a critical size or growth benchmark in these juvenile stars the allows them to better tolerate changes in environmental conditions. Understanding these points could shed light on disposition timing and proper species management for the future.

Even though settlement numbers were high, finding a way to maximize post settlement growth would prove valuable for these animals. It took 14 months of dedicated space to have stars average 1 cm in width. If that could be improved, more institutions would likely be able to take on this project. With more efficient aquaculture and the amount of *P. miniata* in public aquariums, collection from the wild could be reduced to zero. Continual improvements should be made for the future of sea star aquaculture.

Acknowledgements

The author would like to thank Omaha's Henry Doorly Zoo and Aquarium for their continued support of aquaculture as well as Dr. Greg Barord and Andrew 'The Ghost' Hinrichs for their input and time reviewing this paper. Additionally, a special thanks to Kayla Ringuette for providing motivation and inspiration for the completion of this project.

Literature Cited

Cameron, R.A. & N.D Holland. 1983. Electron microscopy of extracellular materials during the development of a sea star, *Patiria miniata* (Echinodermata: Asteroidea). Cell Tiss. Res. 234: 193-200.

Eckert, Ginny L., J. M. Engle, and D. J. Kushner. 2000. Sea star disease and population declines at the Channel Islands. Proceedings of the Fifth California Islands Symposium. US Department of the Interior, Minerals Management Service, Pacific OCS Region 770 Paseo Camarillo Camarillo CA 93010-6064 USA, Feb 2000, p. 390-393.

Ettensohn, C. A. 2004. Methods in Cell Biology. Vol. 74, Elsevier Academic Press.

Motti, C. A., U. Bose, R. E. Roberts, C. McDougall, M. K. Smith, M. R. Hall, and S. F. Cummins. 2018. Chemical ecology of chemosensation in Asteroidea: insights towards management strategies of pest species. Journal of Chemical Ecology. 44(2): 147–177.

Strathmann, M. F. 2017. Phylum echinodermata, class asteroidea. *In:* Reproduction and Development of Marine Invertebrates of the Northern Pacific Coast Data and Methods for the Study of Eggs, Embryos, and Larvae. University of Washington Press. p. 535–555.

Schroeter, S. C., J. Dixon, and J. Kastendiek. 1983. Effects of the starfish *Patiria miniata* on the distribution of the sea urchin *Lytechinus anamesus* in a southern Californian kelp forest. Oecologia. 56(2-3): 141–147.

Simon, K. A. 1974. Effect of thermal stress on larvae of *Patiria miniata* (Brandt) including studies on induction of spawning, development and larval tagging. University of the Pacific, MS Thesis. 71 p.

Wessel, G. M., A. M. Reich and P. C. Klatsky. 2010. Use of sea stars to study basic reproductive processes. Systems Biology in Reproductive Medicine. 56(3): 236-245.

THE USE OF ANTIBIOTICS TO TREAT BROWN JELLY DISEASE WITHIN A CLOSED SYSTEM AQUARIUM

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Introduction

In the world of coral cultivation, it is a well-known fact that corals are very sensitive animals that can be easily stressed. Once stressed they are prone to diseases which could spell disaster for a collection. Brown Jelly Disease (BJD) is cited as being the second most common type of disease experienced by aquarists working with corals (Leewis & Janse, 2008). This infection can affect all species of coral, however is most commonly seen in species with large fleshy polyps, such as *Euphyllia* sp. and *Catalaphyllia* sp. Once a colony is infected, tissue necrosis occurs forming a brown jelly-like mass which gives the disease its name. This infection can spread rapidly along a colony as well as transferring easily onto close neighbors through the water column. Although the disease is well known, analysing the necrotic tissue shows that it is comprised of many species of bacteria as well as protozoans and viruses, several of which could be capable of being the primary cause of BJD. Initially it was thought that the disease was caused by protozoans, specifically the ciliate *Philaster digitformis* as the species was found on infected tissue samples but not on healthy corals (Sweet et al., 2013). Although the ciliates are found in high numbers within the necrotic tissue, there is no indication they are the primary cause of the disease. It is possible that they may be a secondary vector, facilitating the spread of the disease, or they are simply taking advantage of the disease, gathering en masse to feed on the dying tissue (Sweet et al., 2012).

Although many aquarists will have experienced BJD at some point or know of the problem, there appears no evidence that this occurs naturally in the wild. This could be down to natural flow taking the necrotic tissue away or the lack of the causing factor occurring in the natural habitats. There is evidence, however, that both wild and captive corals are affected by a similar disease known as White Band Disease (WBD) in wild colonies and White Syndrome in captive corals. Research into this disease has found the bacterial community found on infected colonies are composed of several species also found on swabs taken from corals affected by BJD (Sweet et al, 2013). Analysis of the infected areas in both diseases show the presence of ciliates as well as numerous species of bacteria that are the same. The concentration of these species was found in significantly higher volumes in the diseased sections of corals compared with control samples of healthy coral tissue (Sweet et al., 2013).

The coral system here at SEA LIFE London Aquarium consists of a 15 m long display tank, two 1,000 L cutting vats, sump and refugium, with a total volume of 25,000 L. The display houses over 40 species of both SPS and LPS corals as well as numerous other species of invertebrate and fish. One of the major theming features in the display is a rockwork arch, which is dedicated to species of *Euphyllia*. Over the first 18 months this area grew, in size and coverage, to such a point that additional theming was needed on the back wall of the section in order to house more colonies. (See figures 1 and 2.)

Figure 1. Arch of *Euphyllia* End of August 2020.

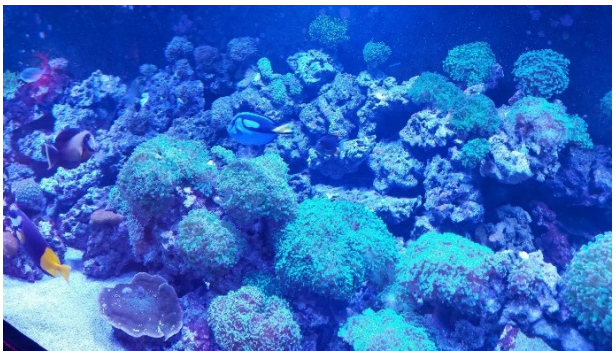

Figure 2. Arch of *Euphyllia* End of August 2020.

Back in September 2020, we started to notice a few heads of our *Euphyllia paraancora* section of the tank exhibiting signs of BJD, seen in Figure 3. We attempted to contain the outbreak quickly to limit the loss in livestock by removing the infected areas as and when we saw them. The removal was immediately followed by a dip in Lugol's iodine to remove any protozoans and clean the sections that had been cut. Unfortunately, this alone did not stop the spread of the disease and within a few weeks around 90% of the *Euphyllia* showed some sign of infection. The brown jelly also spread to three other coral species within the tank, some over 6 m away from the original infection site.

Figure 3. *Euphyllia paraanchora* with BJD before the treatments were carried out

Methods and Results

Removing Affected Corals from The Display Tank

The first step was to remove the infected corals from the display system in such a way that the disease didn't spread to nearby colonies. To achieve this, we first isolated the desired colonies from the rest of the tank by covering it with a fish bag. The bag was then closed around the base of the colony and removed, water and all. Once removed, any diseased tissue was fragged off using a bone cutting tool before transferring them into the treatment tanks.

For LPS species, such as *Euphyllia sp.,* this involved removing infected heads. In infected colonies of SPS, however, the colony was fragged a few cm past the spread of the disease, so that a small amount of healthy tissue was removed as well. This was done to reduce the continued spread of the infection. In areas where this technique could not be used (in areas of high coral coverage for example) the infected tissue was siphoned from the skeleton before removing the colony.

Phase One: Trialing Treatments

The first stage in treating the infection was to find a treatment that worked in slowing and stopping the spread of infection. This was easier said than done as the actual cause of the disease cannot be pinned down to a single species. Previous research into the treatment of acroporid species infected by white band disease revealed that the spread was halted using antibiotics (Sweet 2013). With white band disease and brown jelly disease being thought to have a similar cause (Sweet et al. 2013), the treatments used in treating WBD should, in theory, have a similar effect on BJD. To ensure that this was the case we ran three trials in a quarantine environment to determine the best course of action to take. Each trial consisted of frags of several species showing signs of infection including *Euphyllia paraancora, Seriatopora caliendrum, Leptastrea* sp. and *Acropora* sp.

The frags in the first quarantine tank were treated with Metronidazole, a prescribed antibiotic/antiprotozoal medication. The tank was dosed twice daily with 100 µg/l of Metronidazole and the tank was given a 50% water change each day, carried out over a period of 6 days. After the first round was complete, a second round of treatment was performed dosing the tank with 100 µg/l of Ampicillin, a broad-spectrum antibiotic. This was again dosed twice daily and a daily water change was performed. Each water change followed 5 hours after the first daily dose.

Corals involved in trial two were treated with both Metronidazole and Ampicillin at the same time. This reduced the treatment time down to six days in total as opposed to twelve and allowed us to see if there was any difference in treating together or separately. For example, to check whether dosing the two drugs together would have any negative impact on the corals or counteract each other. As with trial tank one, the dosages of both drugs were 100 µg/l and was dosed twice daily. A 50% water change was performed daily on the tank to ensure that any residual drugs in the tank were removed before redosing the system.

Finally, a third system was set up in which the frags were treated with MinnFinn™, an off-the-shelf medicated treatment used to treat a broad spectrum of diseases. This was done to see if BJD could be treated without the need for expensive, vet-prescribed drugs. The MinnFinn™ treatment consisted of two parts: part one administers the treatment and part two neutralizes it, removing the need to perform water changes. The treatment was performed as per the package instructions, with 4.5 ml of part 1 dosed per 38 L of seawater (10 US gallons). The treatment was left for 1 hour and then neutralized using 1.5 scoops of the neutralizing agent – part two. This was repeated every other day for three doses (6 days in total).

Water tests (Ammonia (NH^4), Nitrite (NO^2), Nitrate (NO^3), Salinity (PPT) and Temperature (°C)), were performed daily during all three trials to ensure that the water chemistry

did not deteriorate during or after the treatments and frags were checked multiple times a day to see if and how quickly the brown jelly was spreading throughout the treatment. Water quality checks were continued for a week after the treatments were finished to ensure there was no delayed impact after the treatment.

Observed Results Trial One

After the first doses of Metronidazole, we saw a dramatic difference in the state of the infected corals. Any brown jelly turned to white necrotic tissue and stopped degenerating overnight, shown in Figure 4. This showed that the ciliates were being removed from the system and were no longer eating through the infected tissue turning it to brown jelly. In the SPS, the increase in white banding slowed to minimal daily spread. Once the ampicillin was added, the spread of white banding and new LPS degradation halted after the first day. Any areas of degraded tissue were systematically removed to further limit the spread of infection.

Figure 4. *Euphyllia paraanchora* with BJD after being treated with Metronidazole

Observed Results Trial Two

Trial two showed the same results as the first trial. By the end of the trial there was no visible signs of brown jelly on any of the frags treated in the trial. A week after the end of the treatment however, we had a resurgence of the disease with a few heads starting to become infected. This was suspected to be due to latent bacteria that survived the initial treatment. To combat this, we removed the affected tissue and ran a second round of treatment and disinfected the trial tank to make sure we removed any bacteria found in the system. Since these steps were taken there has been no further observations of the disease.

Observed Results Trial Three

During the trial treatment of MinnFinn™, there was a delayed reaction to remove the problem. An improvement in the condition was observed after the second dose rather than the first seen in the other trials. After the recommended 3 doses the rate of infection slowed to a halt, however the infection reappeared around 6 days after the last dose.

Histological and Microbiological Analysis

Samples of infected *E. paraanchora* were sent for histological testing along with Microbiology samples before any treatments were carried out to ensure that we were treating the correct issue. The original histology found bacterial swarms and Scuticociliates across the infected tissue. The associate microbiology then followed this to show the bacteria *Comomonas testosteroni* along with a full sensitivity. After trial two, a microbiology sample taken across treated corals was sent for culture and returned as negative for bacterial presence, indicating the antibiotics had worked. Corals in Trial three which still showed some sign of infection were sent for a follow up histology and microbiology. These returned showing additional bacterial presence, fungal presence and ciliate presence. So, in this case, the MinnFinn™ had removed the originally identified bacteria but had not removed the ciliates or other bacteria which were then later found. From these results, alongside the observations made during the trials, we determined that the best treatment to proceed with would be from trial two, dosing Metronidazole and Ampicillin at the same time.

Phase Two: Testing the Treatment on a Mature Quarantine System

Before jumping straight into treating the main display we first tested the treatment on our coral quarantine system, a mature system of around 1,500 L. This was done to ensure that there would be no detrimental effects on any livestock beyond corals in the system, as well as what affect the treatments would have on the biological filtration. The system consists of a display tank containing around 20 species of both LPS, SPS and soft corals as well as other invertebrate species (shrimps, urchins, anemones) and several species of macroalgae. The system's LSS consists of two sumps, one with a deep sand bed filter and the other containing live rock, and a protein skimmer. The treatment was conducted as it was in phase one, with the Metronidazole and Ampicillin being dosed twice daily. However, seeing as the system was mature and had enough LSS, daily water changes were not performed. This also allowed us to mimic the process we would be carrying out on the main system. The same daily water quality tests were performed to see if the treatment caused any deterioration. In order to rule out any delayed drops in water quality, the tests were extended for a week past the end of the treatment.

One week after the trial had been completed there was no deterioration observed and no signs of stress in any of the systems livestock. These findings indicated that the treatment would not have any unwanted side effects to either the water chemistry of the display or the animals in it, so we decided to move ahead onto the next phase.

Phase Three: Treating the Main Display

In order to treat the main display, we had to modify the procedure considering the added LSS and the sheer volume of water being treated. Performing a daily 50% water change on a 12,000 L system would not only use up a massive amount of water, but would alter the water chemistry, especially as we run the Triton method on this tank, which advises minimal water changes. This would cause large amounts of unnecessary stress on the corals and other livestock. To minimize these risks, we isolated the main display tank for eight hours a day, during which the Metronidazole and Ampicillin were dosed as described in the previous phases. Once the eight hours were complete, we put the system back online overnight. This effectively removed the need to change water as the water in the tank was reconnected to the "untreated" water from the refugium and sump. Running the skimmers and UV systems overnight also ensured that any

residual antibiotics were removed. As with the previous phases, the water in the tank was tested daily to ensure that the water chemistry did not deteriorate and, as an added precaution, the temperature of the isolated tank was checked periodically to make sure it did not drop below safe limits.

From the beginning of the treatment the spread of tissue loss slowed and eventually ceased, as observed in the phase one trial. No further colonies were observed to be infected and there was no loss of tissue throughout the collection. In addition to the treatment, we performed a small heterotrophic feed at the end of each day. This was done to boost the energy available to corals to improve their immunoresponse and fight off any latent infections that may not have been impacted by the antibiotics. One-week after the treatment finished, there was no sign of reinfection in the system and the water quality held stable, indicating that the biological filtration was yet again unaffected.

Results Summary

As a very brief summary of the findings during our treatments, it does seem that we have been able to slow and stop the spread of the infection within our coral reef, without any detriment to the system. The main display has stopped showing signs of the infection and we have now started to repopulate the display slowly. Corals added to the display are showing no signs of stress and continue to be healthy and grow. Although we are still very early in the aftermath of our treatment, we are positive in our outlook and the outcome of the treatments. We will be using this experience to advance coral treatments within the industry, here in the UK, in the near future. We will also use our experiences here to create new protocols for arrivals from various sources to safeguard against this event in the future. Lastly, it has given us the insight to be more confident to trial other treatments in the future, if any bacteria which is resistant to Ampicillin is found.

Discussion

As previously mentioned, the antibiotics we used for this treatment had no detrimental effect on the biological filtration within any of the systems treated. This could be for a variety of reasons, but there are two main reasons that we considered before, during and after the treatments. Firstly, the denitrifying bacteria may be resistant to the antibiotics used, leaving them intact and still active throughout. Secondly the natural biological media used in the larger systems may have been too dense for the treatment to penetrate. This would also cause the bacteria to still be active throughout and not be impacted.

Also, during the final treatment trial of the main display, the main filtration system was not treated and was isolated during the day. As the antibiotics used have a particularly short life in water, the efficiency of the drugs may be dramatically reduced by the time the tanks were reconnected to the filtration system. Once the treatment was finished, protein skimmers, UV sterilisers and a high-quality activated carbon were all used to remove any residual drug.

Our experience using the MinnFinn™ has been incredibly limited on this occasion and we will be exploring it's use more in the near future. As it did have some positive impact on the issue at hand, the time sensitive issue we were facing in finding a solution for our main display, meant we were unable to explore the product in full. In no way do our findings account for the full

effectiveness of the product and we are aware of other aquariums that have used the product with success for other issues.

Our final note and bonus finding during this treatment has been the fast treatment of cyanobacteria. We had some issues with cyanobacteria within the main display system. Within two days of the treatment starting all cyanobacteria had been removed from the system with no additional effort needed. However, this did cause a huge leap in phosphate (PO^4) levels within the system which have been combatted with ROWA®phos.

References

Leewis, R.J. M. and Janse (Eds.). 2008. Advances in coral husbandry in public aquariums. *In:* Public Aquarium Husbandry Series, Volume 2. Burgers' Zoo, Arnham, The Netherlands. 460p.

Sweet M., R. Jones and, J. Bythell, 2012. Coral diseases in aquaria and in nature. Journal of the Marine Biological Association of the United Kingdom. 92(4): 791–801.

Sweet M., J. Craggs, J. Robson, and J. Bythell. 2013. Assessment of the microbial communities associated with white syndrome and brown jelly syndrome in aquaria corals. Journal of Zoo and Aquarium Research. 1(1): 20-27.

Atka Mackerel, Breeding Male. Bruce Koike.

COLLECTION, CULTURE, AND DISPLAY OF *Gonionemus vertens* AT THE MARITIME AQUARIUM AT NORWALK

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Introduction

Gonionemus vertens (the clinging jelly), is a species of invasive hydromedusa that is found widely around the globe. The original range of this jelly is unknown, although some hypothesize that it originated in the North Pacific around Japan (Tambs-Lyche, 1964). The cause of its spread also remains unknown, but most believe that the jelly was transported on the hulls of boats (Edwards, 1976). *G. vertens* is characterized by its ability to use its tentacles to cling to sea grasses and other surfaces in shallow coastal waters, instead of flowing freely with the ocean currents like most other jelly species. This sometimes poses a problem for beachgoers as their sting may be highly toxic to some (Pigulevsky and Michaleff, 1969). Clinging jellies are not a common display animal in aquariums. However, after finding several hydromedusae in our jelly cultures, we decided to try and grow this species for display in our summer "Living Lights" exhibit. Additionally, we were able to collect 62 wild specimens from the Connecticut coast with the help of local expert Dr. Paul Bologna from Montclair State University in Montclair, NJ. This review discusses our methods for collecting, culturing, and ultimately displaying *G. vertens* at the Maritime Aquarium over the course of several months.

Collection from the Wild

Wild medusae were collected from Mumford Cove in Groton, CT on July 14th, 2020. Samples were taken from eelgrass beds about 2 hours before low tide. Because of the potentially dangerous nature of the stings from *G. vertens*, full waders and gloves were worn. Samples were collected using large nets with a small mesh, about 61 cm in diameter, attached to 1.2 m long poles. Nets were scooped through the eelgrass beds, and the contents were brought back to the shore and dumped into a shallow bin filled with sea water. Samples were sorted either by (gloved) hand, or by using a disposable plastic 1 mL pipette to suction on to the bell and lift them out of the water. Once removed from the bin, the jellies were placed into glass jars with clean sea water. Jellies were identified by their subtle pulsing motions, and by the cross-shaped pattern of their gonads. Over the course of 3 hours, about 120 jellies were collected in total, and 62 were brought back to the Maritime Aquarium. The temperature and salinity at the collection site were 24.3ºC/31ppt respectively, and once at the aquarium, the jellies were acclimated to a large flowthrough tank held at 32 ppt salinity and 19ºC. The jellies were kept in this flowthrough at 19ºC for about a month before being acclimated down to 13.3ºC to meet the temperature of our cultured *G. vertens*.

Polyps/Collection of Hydromedusae from Polyp Bins

Our first sighting of a *G. vertens* this year was in one of our *Cyanea capillata* polyp bins during a weekly, routine scrubbing. These polyp bins were fed daily with 48-hour brine shrimp nauplii, water-changed twice a week, and plates scrubbed once a week to remove invasive hydroids and algae. We saw one tiny, clear hydromedusae clinging to the side of our acrylic polyp bin and identified it as a clinging jelly. In the past we had encountered some fully grown hydromedusae in our kreisel tanks, but we had never been able to determine where they came from and had never grown a small specimen to adulthood.

We still did not know what the polyps looked like, but at this point we at least knew that they had originated from our *C. capillata* polyp bin. We contacted local expert Dr. Paul Bologna, and were given a description and some images of what a *G. vertens* polyp should look like. We began searching through our petri dishes and on the sides/bottom of the acrylic *C. capillata* polyp bin for anything that fit the description. We were able to find some polyps that seemed to fit the bill and transferred them into a separate beaker. These specimens did not survive once placed in the beaker, and we were never able to confirm their identity.

For the next several months, we continued to find and remove *G. vertens* hydromedusae from our *C. capillata* polyp bin during routine maintenance. However, we were never able to truly confirm and isolate the *G. vertens* polyps in our bin. As a last resort, we tried using a blacklight in a dark room to examine the polyp bin for any polyps fluorescing green, as Dr. Bologna had told us that the polyps may biofluoresce. Unfortunately, this was an unsuccessful endeavor as it appears the polyp phase does not fluoresce as clearly as the hydromedusae phase does.

Growing Hydromedusae

We were not sure what culture method would work best for culturing the hydromedusae, so we decided to try the method that we use for the majority of our nettle ephyrae, and transferred the small hydromedusa into a 1000 ml beaker with a low bubbling air stick. Once the hydromedusae were transferred, we placed the whole beaker into our cold culture tray (56ºF/13.3ºC) (Figure 1). The beaker was outfitted with an air stick that bubbled at an extremely low rate, controlled by a gang valve. This allowed the *G. vertens* to cling to the side of the beaker without being blown around, but still produced enough circulation to keep the food suspended in the water column (Figure 2).

For feeding, we used the same schedule that we adhere to for our nettle ephyrae. The hydromedusae were fed with 48-hour live brine shrimp nauplii twice per day, once in the morning and once in the afternoon. Four days per week the beaker received a midday feed of moon juice (moon jellies squeezed through a fine mesh net). In order to keep the water quality in the beaker in good condition, each day the hydromedusae were transferred to a clean beaker of fresh seawater using a plastic, 2 ml pipette with the tip cut off. The clean beaker was prepared using filtered water from the same cold culture tray that the old beaker was sitting in. This ensured that the water in the new beaker had the same water parameters as the water in the old beaker (temperature, salinity, pH).

Once the jellies reached about 3-5 mm in diameter, they were transferred into an acrylic flow-through bin set up on our cold culture tray (Figures 3 and 4). The bin was outfitted with a curved, 200-micron mesh screen to prevent the jellies from flowing out into the tray. Additionally, the bin contained floating strands of fake plastic eel grass for the jellies to cling to. Flow entered the bin from a tube that was connected to a long manifold above the bin on our culture tray, and was kept extremely low with a valve, so as not to disturb jellies clinging to the wall or grass. The flowthrough was fed nauplii twice per day, and a midday feed of moon juice 4 days per week, with the addition of frozen, small mysis shrimp midday 3 times per week.

Figure 1. Cold Culture Table used for culturing *G. vertens*.

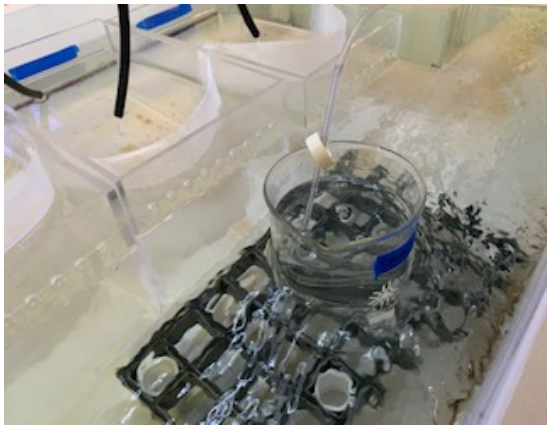


Figure 2. 1000 ml beaker with air stick located in our Cold Culture Tray used for the first stage of *G. vertens* growth.

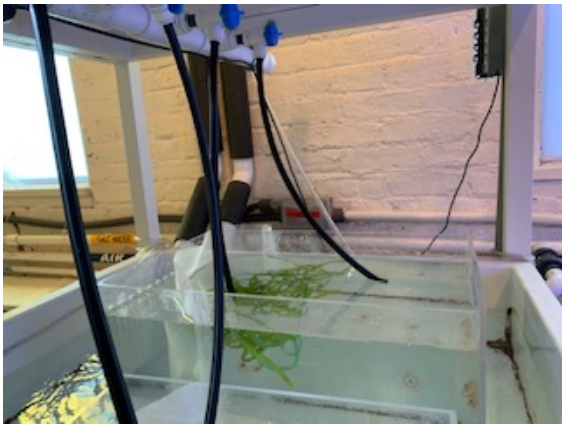


Figure 3. Side view of the flowthrough bin containing the *G. vertens* hydromedusae on the Cold Culture Table.

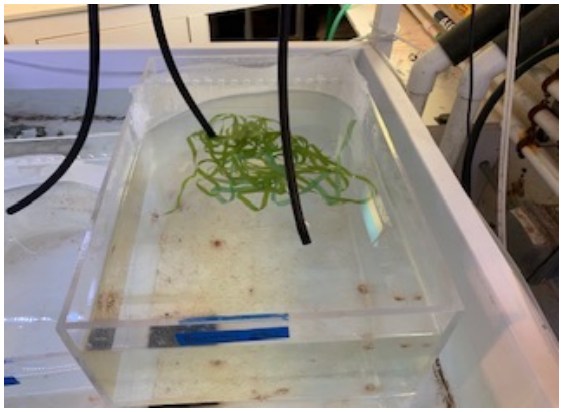


Figure 4. View of the flowthrough containing the *G. vertens* hydromedusae from above.

Husbandry of Fully Developed Hydromedusae

Once the jellies reached about 2 cm diameter, they were ready to be placed on display in our "Living Lights" exhibit. The tank was fed 48-hour, live brine shrimp nauplii twice per day. We initially removed the midday feeds that they were receiving in the culture tray to maintain water quality in this smaller system and decided to maintain them on this diet when we did not notice any negative impacts.

Method for Display

Fully developed hydromedusae were placed in an approximately 30-gallon shallow, square tank measuring 75 cm x 75 cm x 19 cm in size. This tank size and shape were selected because we needed the tank to fit in a specific location within our “Living Lights” exhibit. The tank had two returns that were located in the two front corners and two intakes that were located in the two back corners. Below the tank, each bulkhead was fitted with a valve. We placed a piece of curved, perforated PVC in front of the intakes and fitted it into the back two corners so that it stretched across the entire tank (Figure 5). This functioned as a screen and prevented the jellies from getting sucked into the filtration.

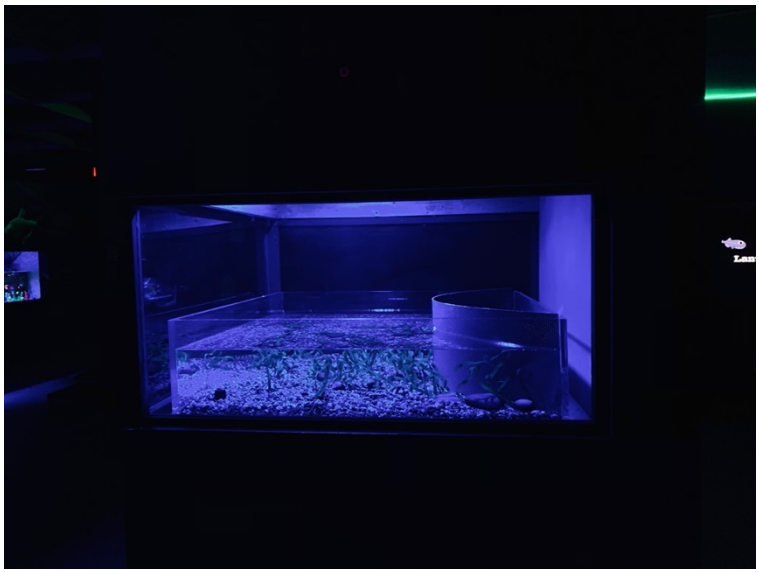


Figure 5. *G. vertens* "Living Lights" exhibit tank showing the perforated PVC screen on the right side.

Mechanical Filtration was located below the tank and included an Inland Seas cartridge filter with a 25-micron cartridge and a model MD-55RLT Iwaki Magnet pump, which was fitted with valves on either side. For biological filtration, plastic bioballs were placed on the intake side of the perforated PVC sheet. Additionally, the flow was greatly reduced using a ball valve placed directly after the pump. This low flow prevented the jellies from becoming stuck to the screen. We did not have flow meters to measure the flow rate, so we adjusted the valves on the pump and returns in order to achieve a flow that allowed the jellies to stay on the tank décor.

Tank décor included CaribSea Blue Ridge gravel substrate approximately 3 cm thick and the same plastic eelgrass strands we used in the culture lab. The strands of plastic eelgrass were glued onto medium sized rocks (2-3”) with DOW 795 silicone and then spaced throughout the entire tank. This provided ample surface area for the *G. vertens* to cling on to and provided a barrier to prevent jellies from becoming stuck to the PVC sheet.

The exhibit was designed to showcase the fluorescent green ring around the edge of the *G. vertens* bell caused by a Green Fluorescent Protein (GFP) specific to *G. vertens* named GvFP (*Gonionemus vertens* Fluorescent Protein) (Orologas, 2020). Initially we selected a blacklight as our exhibit lighting, as this is what we had on hand (approx. 365 nm). Though this spectrum resulted in fluorescence for some of our other display animals in the "Living Lights" exhibit (scorpion, tetras with GFP), it did not have the same effect on our *G. vertens*. We did some research and learned that 450-460 nm is a better wavelength for illuminating biofluorescent animals (aquaticinfo list serve, email from Pete Mohan), so we acquired a Wolezek 36W LED plant grow bulb and installed it in a standard fixture above the tank. This light not only resulted in a clear display of biofluorescence, but also did a better job of illuminating the entire exhibit.

Another aspect to note, this tank was near a large TV monitor that played a video on a loop. We noticed that the light from this screen made it difficult to see the jellies fluorescing; therefore, the side of the exhibit facing this screen was blacked out. The fluorescence on the jellies looked best when surrounded by subdued lighting (Figure 6) or no lighting at all and viewed through a yellow filter (Figure 7).

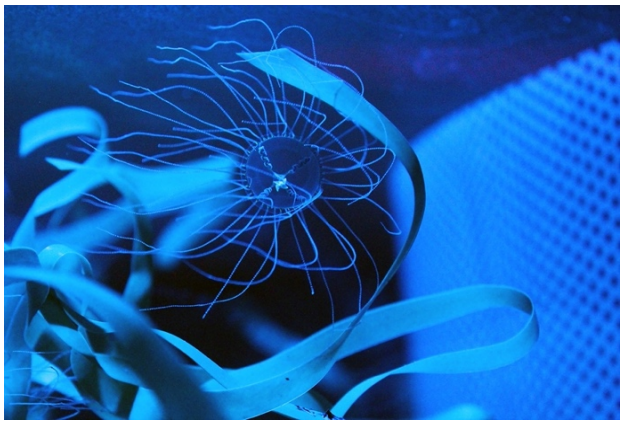


Figure 6. *G. vertens* as it appears on display in the "Living Lights" exhibit.

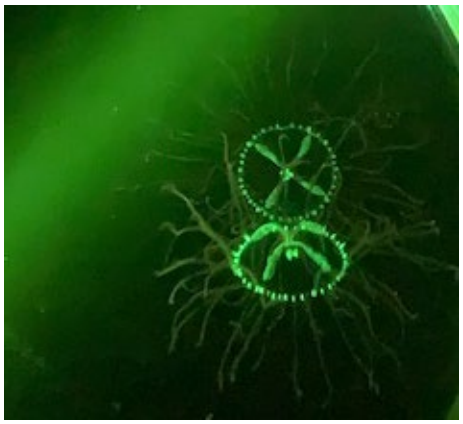


Figure 7. *G. vertens* as it appears under a black light, through a yellow filter, and in a completely dark room.

Concluding Remarks

Overall, we found *G. vertens* to be a very rewarding and low maintenance species to culture and display. From past experience and our communications from Dr. Bologna, we expected these jellies to be very short lived (<3 months), and did not expect the polyps to produce hydromedusae after the summer. Dr. Bologna also informed us that once summer temperatures get too warm the jellies disappear until the next year – this usually happens after June. However, at our temperatures we have been able to keep *G. vertens* alive for 9 months and were still pulling small jellies from our polyp bin as late in the year as October. Within the last couple months, we have collected fewer and fewer jellies, and do not expect to be able to keep this exhibit stocked until next summer.

Unfortunately, at this time, we cannot comment much on the care of the polyps due to the fact that we were never able to confirm their location within our bin. Hopefully, in the future we can locate the polyps and help to promote the presence of this species within other aquariums.

References

Edwards, C. 1976. A study in erratic distribution: The occurrence of the medusa *Gonionemus* in relation to the distribution of oysters. Adv. Mar. Biol. 14: 251-284.

Orologas, V. A. 2020. Cloning and expression of the green fluorescent protein (GVFP) from the clinging jellyfish *(Gonionemus vertens)* using RNA-Seq. Theses, Dissertations and Culminating Projects. 324. https://digitalcommons.montclair.edu/etd/324

Pigulevsky, S. V., and P. V. Michaleff. 1969. Poisoning by the medusa *Gonionemus vertens* in the Sea of Japan. Toxicon, 7(2): 145–149.

Tambs-lyche, H., 1964. *Gonionemus vertens* L. Agassiz (Limnomedusae)-a zoogeographical puzzle. Sarsia. 15: 1–8.

Festive Parrotfish. Bruce Koike.

Book Review

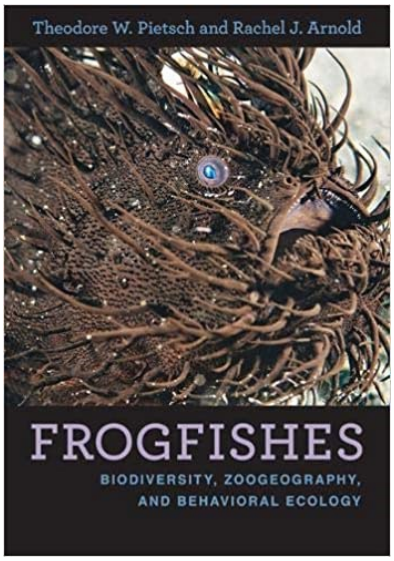
Frogfishes: Biodiversity, Zoogeography, and Behavioral Ecology
Theodore W. Pietsch and Rachel J. Arnold
Hardcover, 624 pages, 500+ Color Images. 2020. John Hopkins University Press.

Publisher's List Price $124.95 (USD), Amazon.com Price $113.12 (USD)

Review by: Barrett L. Christie, Maritime Aquarium at Norwalk

Despite the proliferation of high-quality online resources in recent decades, there is still a niche in science that can only be filled with a quality book. Far from being a vestigial appendage of days past, a high-powered scientific monograph or field guide can be one of the most powerful references used on a daily basis working with animals. Signal-to-noise ratio in the online world is extraordinarily high, and the value of a book in today's digital society is that it represents a curated collection of information assembled and vetted by an authority that the reader can rely upon for accuracy. The best examples are those remarkable works that serve as an extension and repository of the cumulative knowledge that has been painstakingly accrued by that expert over the course of their career.

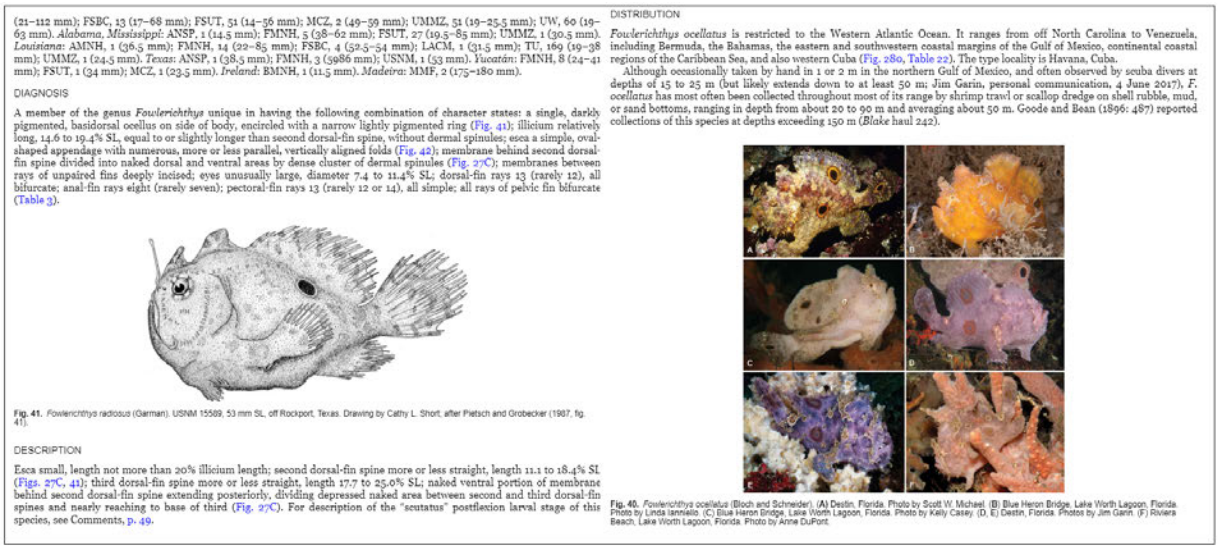
There are a handful of monolithic volumes that are conspicuous as the giants of their topic, many of which are familiar to the aquarium community. One thinks of John E. Randall's numerous tomes on the fishes of Hawai'i, Australia, and the Indo-Pacific, J.E.N. Veron's Corals of the Great Barrier Reef, Robert Rush Miller's Freshwater Fishes of México, Milton Love's Rockfishes of the Northeast Pacific, or Bigelow and Schroeder's Fishes of the Gulf of Maine, among others. These rare, remarkable books make one stop and marvel at the scope and depth of the information assembled. For the Antennariidae, the most comprehensive reference has been Pietsch and Grobecker's 1987 classic "Frogfishes of the World: Systematics, Zoogeography, and Behavioral Ecology", though this text has been out of print for decades, and has become increasingly rare and difficult to acquire.



In 2019, Dr. Theodore Pietsch (U. Washington) announced through a crowdfunding campaign that he and Dr. Rachel Arnold (Northwest Indian College) were compiling an updated volume, to encompass all of the new species described in the past 33 years with a phenomenal 500+ full color photographs. For anyone maintaining frogfishes, or possessing an interest in the taxon this volume will be an indispensable reference. These grotesque and wonderful fishes have been a staple in public aquarium collections for a long time owing to their capacity to inspire awe in the eye of the seasoned curator and the general public alike.

Having coveted the 1987 volume for many years, and never having been able to afford a copy of my own (as it routinely sells for $350-1,200 USD when available), I jumped at the chance

to pre-order a copy of the new version. My sole criticism of the volume is that the section on husbandry (written by Scott Michael) is a bit lacking in detail and rigor when compared to the standard set by the other chapters; it reads more as if written for aquarium hobbyists, though it still will likely be of some value to the professional aquarist. Otherwise, this volume is an essential compilation of the natural history and biology of these delightfully weird and amazing fishes.



(21–112 mm); FSBC, 13 (17–68 mm); FSUT, 51 (14–56 mm); MCZ, 2 (49–59 mm); UMMZ, 51 (19–25.5 mm); UW, 60 (19–63 mm). *Alabama*, *Mississippi*: ANSP, 1 (14.5 mm); FMNH, 5 (38–62 mm); FSUT, 27 (19.5–85 mm); UMMZ, 1 (30.5 mm). *Louisiana*: AMNH, 1 (36.5 mm); FMNH, 14 (22–85 mm); FSBC, 4 (52.5–54 mm); LACM, 1 (31.5 mm); TU, 169 (19–38 mm); UMMZ, 1 (24.5 mm). *Texas*: ANSP, 1 (38.5 mm); FMNH, 3 (59&6 mm); USNM, 1 (53 mm). *Yucatán*: FMNH, 8 (24–41 mm); FSUT, 1 (34 mm); MCZ, 1 (23.5 mm). *Ireland*: BMNH, 1 (11.5 mm). *Madeira*: MMF, 2 (175–180 mm).

DIAGNOSIS

A member of the genus *Fowlerichthys* unique in having the following combination of character states: a single, darkly pigmented, basidorsal ocellus on side of body, encircled with a narrow lightly pigmented ring (Fig. 41); illicium relatively long, 14.6 to 19.4% SL, equal to or slightly longer than second dorsal-fin spine, without dermal spinules; esca a simple, oval-shaped appendage with numerous, more or less parallel, vertically aligned folds (Fig. 42); membrane behind second dorsal-fin spine divided into naked dorsal and ventral areas by dense cluster of dermal spinules (Fig. 27C); membranes between rays of unpaired fins deeply incised; eyes unusually large, diameter 7.4 to 11.4% SL; dorsal-fin rays 13 (rarely 12), all bifurcate; anal-fin rays eight (rarely seven); pectoral-fin rays 13 (rarely 12 or 14), all simple; all rays of pelvic fin bifurcate (Table 3).

Fig. 41. *Fowlerichthys radiosus* (Garman). USNM 15589, 53 mm SL, off Rockport, Texas. Drawing by Cathy L. Short, after Pietsch and Grobecker (1987, fig. 41).

DESCRIPTION

Esca small, length not more than 20% illicium length; second dorsal-fin spine more or less straight, length 11.1 to 18.4% SL (Figs. 27C, 41); third dorsal-fin spine more or less straight, length 17.7 to 25.0% SL; naked ventral portion of membrane behind second dorsal-fin spine extending posteriorly, dividing depressed naked area between second and third dorsal-fin spines and nearly reaching to base of third (Fig. 27C). For description of the "scutatus" postflexion larval stage of this species, see Comments, p. 49.

DISTRIBUTION

Fowlerichthys ocellatus is restricted to the Western Atlantic Ocean. It ranges from off North Carolina to Venezuela, including Bermuda, the Bahamas, the eastern and southwestern coastal margins of the Gulf of Mexico, continental coastal regions of the Caribbean Sea, and also western Cuba (Fig. 28a, Table 22). The type locality is Havana, Cuba.

Although occasionally taken by hand in 1 or 2 m in the northern Gulf of Mexico, and often observed by scuba divers at depths of 15 to 25 m (but likely extends down to at least 50 m; Jim Garin, personal communication, 4 June 2017), *F. ocellatus* has most often been collected throughout most of its range by shrimp trawl or scallop dredge on shell rubble, mud, or sand bottoms, ranging in depth from about 20 to 90 m and averaging about 50 m. Goode and Bean (1896: 487) reported collections of this species at depths exceeding 150 m (*Blake* haul 242).

Fig. 40. *Fowlerichthys ocellatus* (Bloch and Schneider). (A) Destin, Florida. Photo by Scott W. Michael. (B) Blue Heron Bridge, Lake Worth Lagoon, Florida. Photo by Linda Iannello. (C) Blue Heron Bridge, Lake Worth Lagoon, Florida. Photo by Kelly Casey. (D, E) Destin, Florida. Photos by Jim Garin. (F) Riviera Beach, Lake Worth Lagoon, Florida. Photo by Anne DuPont.

Figure 1. An excerpt from the sample pages available on Amazon.com. Nearly every page bears at least one figure, and most species have full-color plates, often with multiple images to show wide variations in morphology.

A truly great book is one in which the author leaves the reader with a sense of not just the extent of our knowledge on a topic, but with a clear view of where the gaps in that knowledge lay. In doing so, we all may stand on the shoulders of these giants, in order to see a little further. This was one of the hallmarks of the 1987 original (see Smith-Vaniz, 1988), and the newest volume lives up to the eminence of its predecessor.

This work paints a clear picture of the roads not yet explored among the extensive summation of information, which is especially important, as many of these fishes are relatively easy to keep in captivity. We know so little about their reproduction, fecundity, growth rates, longevity, metabolism, *et cetera* that the aquarist should envision the possibilities for areas of research which may contribute to the biology of this taxon. As aquarists, we practice an applied science of husbandry based on a foundation of traditional biology, and we do not do nearly enough to advance the underlying science concerning the remarkable species in our charge.

If you have a penchant for the lophiiform fishes or related taxa, this book will be enlightening to your care of these species, and hopefully will inspire aquarists to answer some of the research questions remaining.

Literature Cited

Smith-Vaniz, W.F. 1988. Book Review: Frogfishes of the world. Systematics, zoogeography, and behavioral ecology. Science. 240(4,855): 1,075.

BEHAVIORAL RESPONSES OF BONNETHEAD SHARKS (*Sphyrna tiburo*) TO CHANGING EXHIBIT SALINITY

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Introduction

The New England Aquarium's Giant Ocean Tank (GOT) is a 200,000-gallon cylindrical exhibit, home to a diverse population of teleost, elasmobranch, and reptile species (Figure 1). Originally constructed in 1969, with renovations occurring in 1984 and 2013, the GOT is 40 ft wide and 23 ft deep with a large artificial reef structure in the center. This artificial reef provides vital habitat to a reef community of ~100 different species and a total population ranging from 800 to 1,000 individuals. The living collection has varied over the decades, but has primarily focused on tropical and sub-tropical Western-Atlantic species. Since its most recent renovation in 2013, the exhibit collection plan has been relatively stable and its current population consists of two species of sea turtle (*Chelonia mydas, Caretta caretta*), ~85 teleost species, two ray species (*Hypanus americanus, Rhinoptera bonasus*), and one species of shark (*Sphyrna tiburo*).

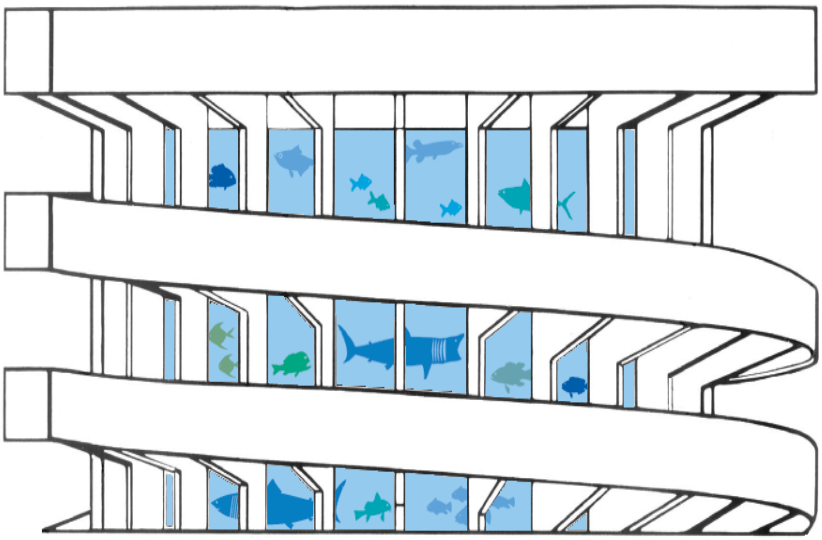

Figure 1. Diagram of Giant Ocean Tank exhibit structure.

One challenge often facing large aquarium exhibits is the management and treatment of parasite and disease outbreaks within the collection. The Giant Ocean Tank is no exception to this and has faced recurring outbreaks of *Cryptocaryon irritans* throughout the years. Treatment records dating back to 2006 show these outbreaks have been historically treated with copper sulfate immersion (therapeutic range: 175-200 ppb). During these treatments, standard protocol required all elasmobranchs to be removed from the exhibit and placed in holding tanks for concerns of potential copper toxicity (Grosell et al. 2003; Hadfield and Clayton 2011). Between 2006 and 2016, the GOT experienced 13 outbreaks of *C. irritans*, 11 of which were treated with copper sulfate. In 2017, hyposalinity was trialed as a treatment option and the exhibit salinity was reduced to 15 parts per thousand (ppt). After its success in managing the outbreak, hyposalinity became

the preferred option for treating the exhibit in subsequent outbreaks. The success of the hyposalinity treatment additionally started conversations about how reduced salinity could have the potential to prevent or subdue the frequency of outbreaks in the Giant Ocean Tank. Following these discussions, the exhibit transitioned to a brackish mix, with baseline exhibit salinity maintained at 22 ppt. The use of hyposalinity treatments in the GOT reduced the average length of *C. irritans* outbreaks, and allowed all ray species to remain on exhibit during treatment. During the 2017 and 2018 treatments, all bonnethead sharks continued to be moved off exhibit during times of hyposalinity. While maintenance of exhibit salinity at 22 ppt and a therapeutic salinity of 15ppt has failed to eradicate *C. irritans*, it has proved to be a viable parasite management strategy of the Giant Ocean Tank since 2017.

The Bonnethead shark (*S. tiburo*), is the smallest member of the hammerhead family (Sphyrnidae) and is often found in shallow, coastal areas ranging from the U.S. Atlantic coast and Gulf of Mexico to the Atlantic coast of Central and South America (Cortes and Parsons, 1996; Cortes et al., 1996). Their diet primarily consists of crustaceans, cephalopods and small fish, although recent research has also shown large amounts of seagrass ingested by bonnetheads through stomach content analysis (Leigh et al., 2018). While much of the feeding ecology, reproductive biology, and population demographics of this species have been well studied, a solid understanding of habitat use and residency has yet to be developed. As frequent visitors to estuaries and shallow coastal habitats, bonnethead sharks are exposed to large environmental fluctuations (i.e. temperature, salinity, and dissolved oxygen) and have been known to tolerate salinities ≤15ppt (Ubeda et al., 2009; Hyatt et al., 2018).

With this in mind, the New England Aquarium animal care team proposed keeping the three resident bonnethead sharks on exhibit during the 2019 hyposalinity treatment. Salinity was decreased to 15 ppt over the course of one week (1 ppt/day) from 22 ppt, with holding systems prepared to follow the salinity decrease with a 1–2 day lag behind the GOT salinity and ready to hold the sharks if needed as a contingency plan. Sharks were under careful observation during this time, with feeding behavior, body posture and swimming activity monitored closely for any signs of distress (i.e. pacing, mouth gaping, lethargy, and prolonged inappetence). Two of the sharks, Palmetto and Pigeon, fared well throughout the total 30-day duration of the treatment at 15ppt. Based on their overall welfare assessment and reaction to this treatment, the GOT team decided they would remain on exhibit when another *C. irritans* outbreak occurred in 2020.

It should be noted that the third shark, Largo, was removed 18 days into the 2019 treatment due to a sudden decline in appetite. This shark was transported out of the exhibit and held at 18 ppt until the treatment resolved and was then returned to the Giant Ocean Tank. As a precaution for the 2020 treatment, Largo was transported to a holding system prior to starting the exhibit salinity decrease. The holding system was slowly deceased to 18 ppt and held there to act as a contingency plan if the other sharks on exhibit needed to be transported. While in holding, it became apparent that Largo had several underlying medical conditions. She died two months later with abnormal medical findings becoming apparent during necropsy. The role that hyposalinity exposure played in the decline of this animal remains unclear.

In order to obtain more data regarding the behavioral response to treatment, the GOT team developed a method to assess swimming patterns and exhibit usage during the 2020 treatment. This study focused on Palmetto and Pigeon, both female bonnetheads estimated to be 8-16 years

old. Palmetto (120 cm TL, 8 kg) was originally acquired from the South Carolina Aquarium in 2014 and Pigeon (105.4 cm TL, 6.55 kg) was acquired from the Georgia Aquarium in 2012. Through this investigation, the team attempted to answer the following question: How does changing salinity alter swimming and feeding behavior in bonnethead sharks?

Materials & Methods

Salinity decreases for the 2020 hyposalinity treatment began on April 1st, with the target of 15 ppt reached on April 8th. GOT salinity was reduced by ~1 ppt/day by adjusting the salt and fresh water supply lines feeding the exhibit. The hyposalinity treatment was maintained for 52 days, with salinity beginning to transition back to 22 ppt starting on May 30th and reaching the target on June 12th (73 days beginning to end). The duration of treatment was contingent on visual observations of *C. irritans* infection on teleosts, with resolution of treatment occurring only when all signs of infection were resolved. Due to observed fluctuations in shark appetite during the salinity decrease, the increase was performed at a more gradual rate (0.5 ppt/day). Diet consumption was monitored for both sharks during the entirety of the treatment, while swimming activity records began once salinity was at 15 ppt and therefore only captured exhibit usage during the second half of the hyposalinity treatment and return to 22 ppt.

Swimming activity, used as a proxy for habitat use, was monitored by creating hypothetical "gates" for the sharks to swim through. Three gates were used to divide the perimeter of the tank, each with a corresponding activity zone. These gates were represented by visual markers at the surface of the exhibit to allow observers to tally when a shark was entering or leaving a zone (Figure 2). A fourth zone was created to encompass the shallow water above the artificial reef in the center of the exhibit. Observations were made three times each day (approximately 9:00, 12:00, and 16:00) with the observer tracking each shark's swimming activity for 10 minutes. Swimming activity records began on April 24th, 2020 (16 days after 15ppt had been achieved). To compare the statistical significance of shark swimming activity based on the observations in each zone, we performed a Mann-Whitney U Test (Wilcoxon Ranked Sum Test) using the software "R" (version 3.6.1, R Core Team, 2019).

Feeding response, monitored via daily food consumption and staff observation, was recorded throughout the hyposalinity treatment. Both bonnethead sharks are target trained and signaled to feed by the presence of an orange ball and in-water hammock (Figure 3). Each shark's daily diet is determined by body weight, Palmetto is offered 8 oz daily and Pigeon is offered 6 oz. While at 22 ppt the sharks are given a rotating menu of food items and vitamin supplementation to provide a balanced diet. Due to concerns that the hyposalinity treatment is a potential environmental stressor for the sharks, the menu was often altered to incorporate more "favorable" items based on each shark's established preferences to promote consumption, and salt tablets (500 mg) were supplemented as an alternative sodium source for osmoregulatory needs. Staff feeding efforts (meaning the amount of time spent feeding and/or number of feeding attempts) were often doubled or tripled as well.

Results

Swimming Activity

Cumulative swimming activity totals, regardless of salinity, showed that both sharks spent a majority of their time around the perimeter of the tank (Table 1). Summations of zone A, B, and C showed Palmetto spending 96% and Pigeon spending 94% of her swimming activity in these

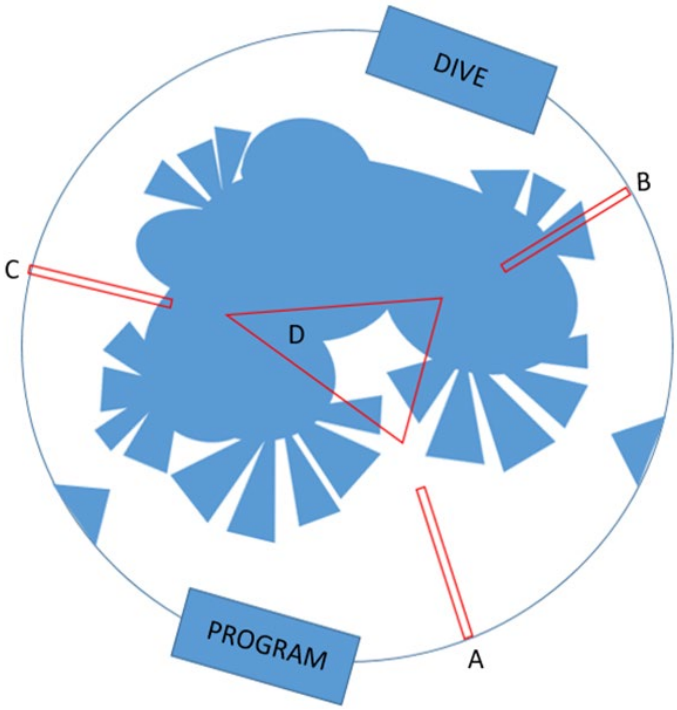

Figure 2. Aerial view of Giant Ocean Tank and distribution of activity zones A, B, C, and D ("gates" in **RED**). Solid **blue** shapes indicate surface platforms and coral structures.

Figure 3. Still image of Bonnethead shark target training session with hammock.

areas. Separating swimming activity by zone and salinity showed the perimeter zones consistently having higher activity regardless of treatment status. However, this analysis highlighted that both sharks decreased their usage of zone D (top/center of the exhibit) by roughly half during the hyposalinity treatment (Figure 4). Under normal salinity parameters (22 ppt), Palmetto was observed over the top of the reef, in zone D, 10.2% of the time. While at 15 ppt, her activity in

zone D dropped to 3.9%. Pigeon showed a similar trend, decreasing her usage of zone D from 12.1% to 5.9% during the hyposalinity treatment. We found that both sharks used zone D significantly less during 15ppt (Palmetto, Wilcoxon $p<0.001$; Pigeon, Wilcoxon $p<0.001$). Usage of the three exhibit areas around the perimeter also reflected the relative areas of each region at stable salinities (22 ppt and 15 ppt) with the highest usage occurring in the largest perimeter region (zone A) and lowest perimeter usage occurring in the smallest perimeter region (zone B).

Table 1. Cumulative activity of each bonnethead shark throughout hyposalinity treatment broken down by activity zone.

	ZONE A		ZONE B		ZONE C		ZONE D	
	COUNT	PERCENT	COUNT	PERCENT	COUNT	PERCENT	COUNT	PERCENT
Palmetto	2022	34.84	1652	28.46	1785	30.78	345	5.94
Pigeon	2329	39.6	1553	26.4	1508	25.64	492	8.36

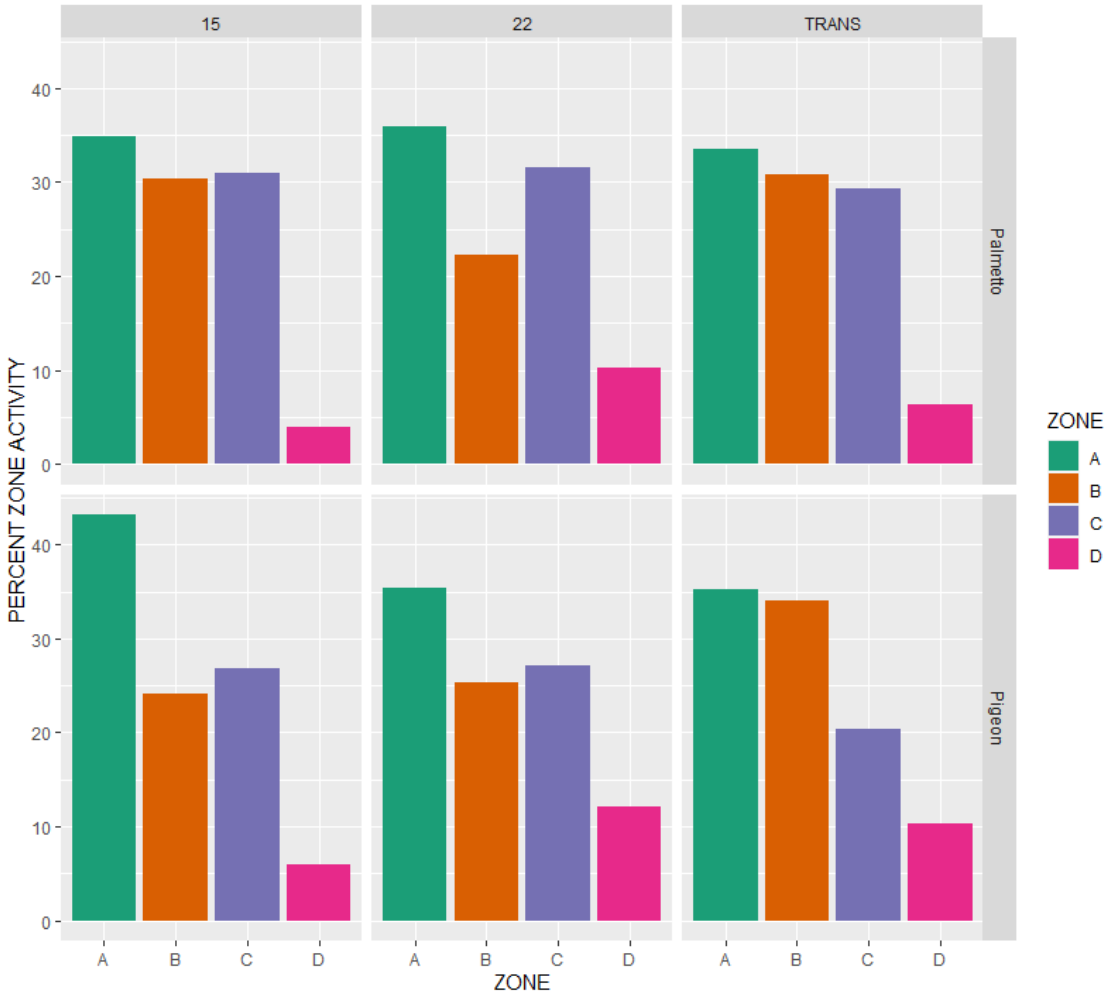

Figure 4. Percentage of activity spent in each zone by each shark during hyposalinity treatment (15 ppt), transitional phase (increasing salinity), and normal salinity (22 ppt).

Feeding Behavior

Feeding behavior, expressed as percent of daily diet consumed, varied for both sharks throughout the treatment and was generally lower when compared to consumption percentages at 22 ppt (Figure 5). On average, both sharks received 100% of their daily diet while at 22 ppt. Food consumption was lowest for both sharks during the transition to 15 ppt, with Palmetto and Pigeon consuming an average of 63% and 33% of their offered diet respectively. Feeding behavior improved once salinity stabilized at 15 ppt and during the return to 22 ppt. On average, Palmetto consumed 75% of her diet and Pigeon consumed 66% during these times.

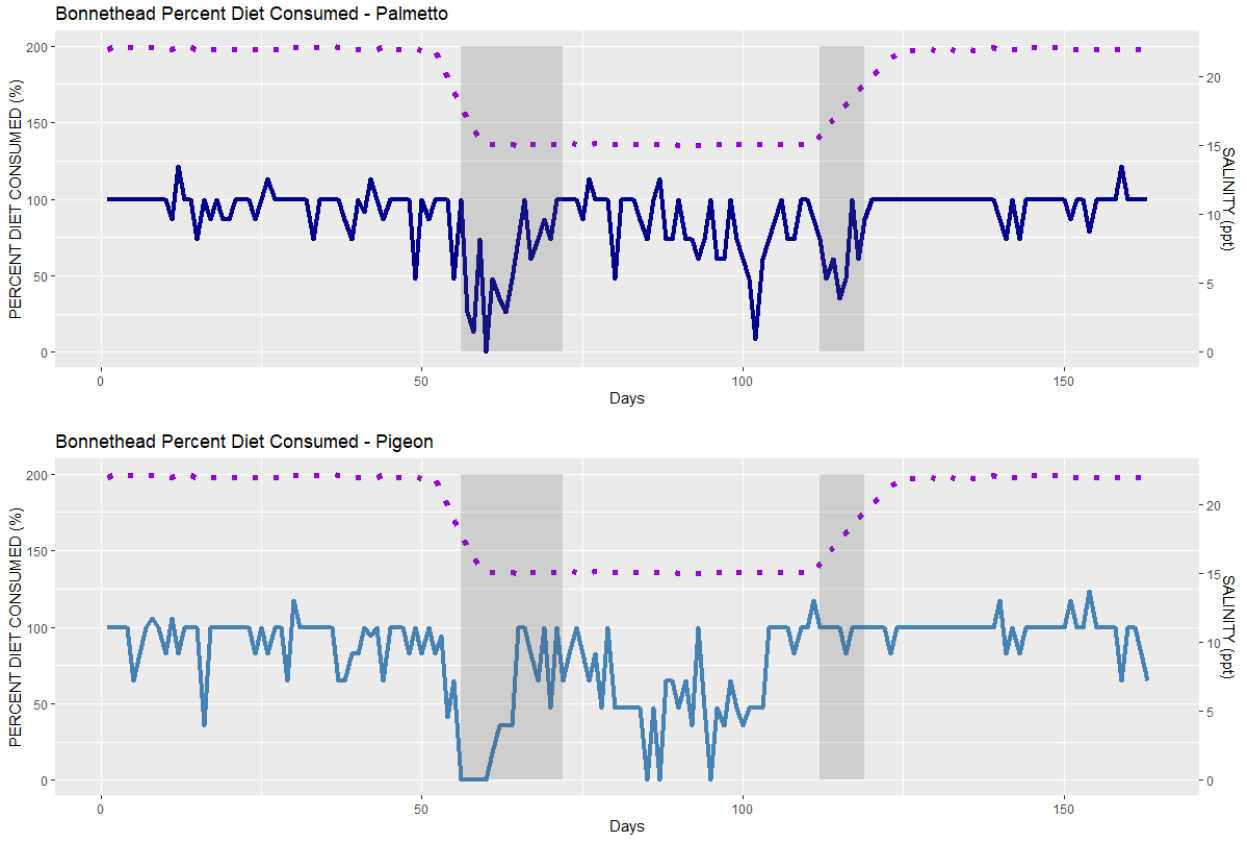

Figure 5. Percent of diet consumed by Palmetto (solid dark blue line) and Pigeon (solid light blue line) throughout hyposalinity treatment (15 ppt), transitional phase (increasing/decreasing salinity), and baseline salinity (22 ppt) represented by dotted line. Shaded regions (dark grey) denote days where feeding effort was increased. Each shark receives a daily offering based on body weight – Palmetto is offered 8 oz and Pigeon 6 oz.

Discussion

Though there is evidence that numerous marine cartilaginous fishes can tolerate some degree of salinity fluctuation (Heupel & Simpfendorfer, 2008; Froeschke et al., 2010), there remain many unknowns regarding the duration and severity of salinity change tolerable by different species including bonnethead sharks. The primary concern in exposing these sharks to prolonged reduced salinity is physiological stress due to challenges with osmoregulation, buoyancy, appetite, etc. Animal care staff hypothesized that the bonnethead sharks would express a preference for stable salinity, and that any alteration to exhibit salinity would elicit an increase in swimming or searching behavior by the sharks in order to move to an area of stable salinity. We expected this

behavior change to manifest itself in elevated swimming activity around the perimeter of the exhibit, which has been observed at NEAQ during known periods of elevated stress following transport and capture. Swimming activity data collected during this treatment appears to support this hypothesis with the percentage of activity around the perimeter increasing from 90% to 96% for Palmetto and from 88% to 94% for Pigeon. Elevated swimming or searching activity may have also been revealed in a comparison of total tally marks logged during periods of transitioning salinity, though the collected data did not reveal such differences. This may be due to limited sample size and a lack of precision in the technique. Enhanced precision could be achieved through alternative data collection methods including video surveillance and accelerometer use. The GOT presents challenges to these methods as the cylindrical shape of the exhibit and reef structure make comprehensive video surveillance unattainable and the use of a more invasive accelerometer method was beyond the scope of this study.

While we believe that elevated searching behavior by the sharks during the treatment lead to a decrease in activity over the top of the reef, shark activity in this region (zone D) may also point to implications of buoyancy compensation during treatment. Zone D represents the center, and most shallow region of the exhibit which is generally used by both sharks approximately 10-12% of the time during baseline exhibit salinity of 22 ppt. While both sharks decreased their activity in this area during the treatment, the larger shark, Palmetto, was also observed spending more time lower in the water column while at reduced salinity. As bonnethead sharks, and cartilaginous fish in general, operate without swim bladders, perhaps this change in behavior is part of a buoyancy maintenance strategy by Palmetto to minimize energy usage during this period. The lack of a swim bladder is thought to be partially responsible for the lack of evolutionary proliferation of cartilaginous fishes into freshwater ecosystems (Gleiss et al., 2015). Due to their regulatory mechanisms, Gleiss et al. found that sharks would need increased liver volume 3- to 8-fold depending on their liver density to compensate for their relative buoyancy change in freshwater. Perhaps swimming at a lower depth allowed Palmetto to conserve energy required for forward locomotion to generate the lift to maintain her position in the water column. Swimming continuously in the upper third of the exhibit, as is observed at baseline salinity, may be too energetically taxing during a hyposalinity exposure, and maintaining a mid-water depth allows for more efficient buoyancy control. This response would also contribute to observed reduction in swimming activity in zone D where exhibit depth is the shallowest. Palmetto may have resorted to this strategy due to her larger size compared to Pigeon, making buoyancy control more impactful to behavior.

Shark appetite served as the most obvious indicator of environmental stress during the hyposalinity treatment. While each shark maintained unique food preferences and feeding behavior during each phase of the treatment (decreasing, 15 ppt, and increasing), both sharks clearly presented higher incidence of erratic appetites. The transition phases between 15 ppt and 18 ppt appeared to cause the most significant swing in appetite even with increased feeding effort by staff, though Pigeon's appetite during the recovery back to 22 ppt was largely unremarkable. Both sharks appeared to have improved appetites during the increasing salinity compared to the response to decreasing salinity. This could be due to increased motivation following less-consistent appetites throughout the 15 ppt duration, and/or due to the slower rate of salinity change during the increase (0.5 ppt/day vs. 1.0 ppt/day). Even when the majority of their diets were consumed, both sharks were far less likely to target feed through the hammock during the treatment.

Another variable to consider in the physiological experiences of bonnethead sharks to reduced and transitioning salinity is the effect on osmoregulation. Periodic blood sampling has shown that sodium levels in sharks decrease with decreasing environmental salinity (Urist, 1962; Piermarini and Evans, 1998; Pillans et al., 2004) though the impact of these alterations in osmoregulation and its behavioral implications in sharks is not fully understood. Piermarini and Evans (2000) also demonstrated that changes in NA(+)/K(+)-ATPase activity and abundance in the gills and rectal glands of Atlantic Stingrays (*Dasyatis sabina*) varied with acclimation to fresh or marine environments. Perhaps bonnethead sharks are capable of similar osomoregulatory adjustments and repeated exposure to hyposaline environments produces sustained physiological changes. Throughout this treatment, both bonnethead sharks received oral salt supplementation, though consistent administration of this supplement was made more difficult by their erratic appetite during treatment. This supplementation may have aided osmoregulation during the 2020 treatment by providing an alternative source of biologically available sodium while at reduced environmental salinity. The impact of the oral supplementation of salt on the osmoregulation of bonnetheads and resulting behavioral changes remains to be explored further. It is possible that smaller sharks receive energetic benefits in reduced salinity as less energy is required by osmoregulatory mechanisms and could explain observed congregations in other shark species correlated with environmental salinity gradients (Ballantyne, 1997; Wingar, 2019). Differentiation by size may point to another influencing factor regarding the variation in swimming activity and depth observed between Pigeon and Palmetto. Regardless, the physiological impacts of reduced salinity on bonnethead sharks and the observed variation in behavior response requires further investigation.

This study provides valuable information regarding the appetite and behavior of bonnethead sharks in response to changing salinity and continues to expand our knowledge of the species and associated husbandry. The Giant Ocean Tank has maintained an operational salinity of 22 ppt and the results of this study have continued to shape husbandry decisions. The data collected in early 2020 affected how salinity adjustments were implemented for another outbreak of *C. irritans* in October 2020. Initial findings indicate that a slower salinity adjustment (~0.5 ppt/day) while decreasing and increasing salinities resulted in a less significant behavior response from the bonnethead sharks. Swimming activity and feeding behavior were monitored throughout the second treatment as well and will be compared to the dataset of treatment one. While Palmetto and Pigeon continue to fare well and adjust to these treatments, we hope to understand more regarding the physiological impacts of transitioning environmental salinities on bonnethead sharks. Variations from one treatment to another and among individual sharks indicate that there are several influencing factors shaping observed behavioral phenomena.

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References

Ballantyne, J. S. 1997. Jaws: the inside story. The metabolism of elasmobranch fishes. Comparative Biochemistry and Physiology Part B: Biochemistry and Molecular Biology. 118(4): 703 - 742.

Cortés, E., and G. R. Parsons. 1996. Comparative demography of two populations of the bonnethead shark *(Sphyrna tiburo)*. Canadian Journal of Fisheries and Aquatic Sciences. 53(4): 709-718.

Cortes, E., C. A. Manire, and R. E. Hueter. 1996. Diet, feeding habits, and diel feeding chronology of the bonnethead shark, *Sphyrna tiburo*, in southwest Florida. Bulletin of Marine Science. 58(2): 353-367.

Froeschke, J., G. W. Stunz, and M. L. Wildhaber. 2010. Environmental influences on the occurrence of coastal sharks in estuarine waters. Marine Ecology Progress Series. 407: 279-292.

Gleiss, A. C., J. Potvin, J. J. Keleher, J. M. Whitty, D. L. Morgan, and J. A. Goldenbogen. 2015. Mechanical challenges to freshwater residency in sharks and rays. The Journal of Experimental Biology. 218: 1,099-1,110.

Grosell, M., C. M. Wood, and P. J. Walsh. 2003. Copper homeostasis and toxicity in the elasmobranch *Raja erinacea* and the teleost *Myoxocephalus octodecemspinosus* during exposure to elevated water-borne copper. Comparative Biochemistry and Physiology Part C: Toxicology and Pharmacology. 135(2): 179-190.

Hadfield, C. A., and L. A. Clayton. 2017. Elasmobranch quarantine. *In:* The Elasmobranch Husbandry Manual II - Recent Advances in the Care of Sharks, Rays and their Relatives. Smith, M., D. Warmolts, D.Thoney, R. Hueter, M. Murray, and J. Ezcurra (Eds.). Ohio Biological Survey. p.113-133.

Heupel, M. R., and C. A. Simpfendorfer. 2008. Movement and distribution of young bull sharks *Carcharhinus leucas* in a variable estuarine environment. Aquatic Biology. 1(3): 277-289.

Hyatt, M. W., P. A. Anderson, and P. M. O'Donnell. 2018. Influence of temperature, salinity, and dissolved oxygen on the stress response of bull *(Carcharhinus leucas)* and bonnethead *(Sphyrna tiburo)* sharks after capture and handling. Journal of Coastal Research. 34(4): 818-827.

Leigh, S. C., Y. P. Papastamatiou, and D. P. German. 2018) Seagrass digestion by a notorious 'carnivore'. Proceedings of the Royal Society B: Biological Sciences. 285(1,886), 20181583.

Piermarini, P. M., and D. H. Evans. 1998. Osmoregulation of the Atlantic stingray *(Dasyatis sabina)* from the freshwater Lake Jesup of the St. Johns River, Florida. Physiological Zoology. 71: 553 - 560.

Piermarini, P. M., and D. H. Evans. 2000. Effects of environmental salinity on NA(+)/K(+)-ATPase in the gills and rectal gland of a euryhaline elasmobranch *(Dasyatis Sabina).* Journal of Experimental Biology. 203(19): 2,957-2,966.

Pillans, R. D., and C. E. Franklin. 2004. Plasma osmolyte concentrations and rectal gland mass of bull sharks *Carcharhinus leucas*, captured along a salinity gradient. Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology. 138: 363-371.

R Core Team. 2019. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL: https://www.R-project.org/.

Ubeda, A. J., Simpfendorfer, C. A., & Heupel, M. R. (2009). Movements of bonnetheads, *Sphyrna tiburo*, as a response to salinity change in a Florida estuary. Environmental Biology of Fishes. 84(3): 293-303.

Urist, M. R. 1962. The bone-body fluid continuum: calcium and phosphorus in the skeleton and blood of extinct and living vertebrates. Perspectives in biology and medicine. 6: 75 - 115.

Wingar, J. 2019. Osmoregulation and Salinity Preference in Juvenile Sandbar Sharks *(Carcharhinus plumbeus)* in Winyah Bay, SC, USA. Electronic Theses and Dissertations. 111. https://digitalcommons.coastal.edu/etd/111

Barred Sandbass and Giant Kelp. Bruce Koike.

VISUAL SIGNALS OF THE EAST PACIFIC RED OCTOPUS (*Octopus rubescens*) DURING CONSPECIFIC INTERACTIONS

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Abstract

Multiple species of octopuses have recently demonstrated the use of specific visual signals (such as chromatic, postural, locomotor, and textural indicators) to communicate with conspecifics. This study aimed to identify the visual signals of the East Pacific red octopus, *Octopus rubescens,* during interactions with conspecifics. *Octopus rubescens* were collected from Admiralty Bay, WA – a habitat littered with discarded glass bottles which *O. rubescens* opportunistically use as dens. To identify the visual signals of *O. rubescens*, GoPro cameras recorded videos of octopuses interacting with conspecifics of the same and opposite sex in an observation tank over the course of 15 min. *Octopus rubescens* were predominantly aggressive toward conspecifics, but nonetheless displayed visual signals, such as 'upright', 'attack', 'approach', 'ochre', and 'dark ochre', which were recorded in an ethogram. Due to the unique, bottle-dense habitat of Admiralty Bay, the observed visual signals of *O. rubescens* may be specialized compared to other *O. rubescens* individuals living in different, but more natural habitats. Consequently, the ethogram produced in this study may be used as a source of comparison for future studies documenting the visual signals of this species in other habitats; this could reveal potential variations in visual signals and may suggest that the visual signals used by *O. rubescens* are influenced by their surroundings.

Keywords: cephalopods, communication, conspecifics, *Octopus rubescens*, visual signals

Introduction

Historically, octopuses have been thought to be solitary, asocial individuals (Barbato et al., 2007; Hanlon and Messenger, 2018); however recent studies have suggested that octopuses use a unique and systematic arrangement of visual signals to communicate with conspecifics (Huffard, 2007; Caldwell et al., 2015; Scheel et al., 2016). These visual signals include chromatic and textural changes, postures, different forms of locomotion, and inking, which can be combined or used consecutively to create specific displays (Hanlon and Messenger, 2018). Displays are characterized by being repetitive and discrete, allowing octopuses to portray clear messages to receivers.

Some of the most complex signals octopuses use are chromatic signals. Since octopuses have direct neural control of pigment-containing cells, called chromatophores, octopuses can quickly change chromatic signals, adjust signal strength, and even perform bilateral signaling (Barbato et al., 2007; Hanlon and Messenger, 2018). Hanlon and Messenger (2018) have observed that chromatic signals generally include forming line-stimuli consisting of bands (lines, stripes, bars) or spots that are easily detected by other octopuses. Although colorblind, octopuses have excellent vision – consequently, by using highly contrasting chromatic signals, octopuses can clearly display their intent (e.g., to show dominance or submissiveness) toward a conspecific (Tricarico et al., 2011; Hanlon and Messenger, 2018).

In combination with chromatic signals, textural signals (defined as smooth or papillate skin) can be used to modify the appearance of an octopus. Additionally, postural signals, such as raised arms or flattening of an octopus's body, are often used to adjust an individual's apparent size to demonstrate intimidation or submissiveness (Hanlon and Messenger, 2018). Furthermore, specific movements, labelled 'locomotor' visual signals, may include chasing or fleeing (Hanlon and Messenger, 2018). All of these signals can be combined in a wide variety of patterns and intensities, allowing octopuses to effectively communicate with conspecifics.

Three species of octopuses that have been shown to use visual signals to communicate with conspecifics include the larger Pacific striped octopus (*Octopus* sp.), the Algae octopus (*Abdopus aculeatus*), and the common Sydney octopus (*Octopus tetricus*) (Huffard, 2007; Caldwell et al., 2015; Scheel et al., 2016). Each of the studies described specific visual signals used by octopuses during conspecific interactions that were typically agonistic. Both Huffard (2007) and Caldwell et al. (2015) performed observational studies and recorded octopuses' visual signals in ethograms which act as libraries that describe and identify behaviors displayed by animals.

Many visual signals that octopuses utilize are species-specific, therefore characterizing and documenting visual signals of octopuses via ethograms provides useful supplementary information for validating species identification (Barbato et al., 2007; Huffard, 2007). Additionally, both Sinn et al. (2001) and Scheel et al. (2016) suggest that ethograms can act as resources for scientists studying how ecological influences, such as conspecific interactions or habitat availability, may affect the evolution of signal development or communication. For example, a population of octopuses living in one type of habitat may utilize a slightly different or more specialized set of visual signals to communicate with each other compared to a population of the same species living in a different type of habitat.

Although 19 common visual signals were identified for the East Pacific red octopus (*Octopus rubescens)* by Mather and Anderson (1993), these signals were in response to human stimuli during three different situational laboratory tests. No ethogram has been created to describe the visual signals used by *O. rubescens* while interacting with conspecifics. *Octopus rubescens* is a subtidal species found along the west coast of North America (from Alaska to California), sheltering in kelp beds and rocky areas, and are commonly found in Admiralty Bay, WA (Cowles, 2005). The benthic habitat of this bay is generally barren and flat, characterized by mud, sand, and small rocks, with few hiding places for this non-burrowing octopus species. However, the bay is littered with discarded glass bottles which *O. rubescens* opportunistically use as dens (Anderson et al., 1999). *Octopus rubescens* have capitalized on this new habitat source which may have inadvertently concentrated individuals of this species within the bay (Chase and Verde, 2011).

Consequently, *O. rubescens* may interact with conspecifics more frequently within this "artificial" environment and these interactions may be characterized by visual signals used by octopuses to communicate with each other.

Given that octopuses use visual signals to interact, the purpose of this project was to determine the frequency of such signals used by *O. rubescens* individuals to communicate with conspecifics, and to document those visual signals in an ethogram. As such, this study addressed the following questions:

1) What are the visual signals that *O. rubescens* use during interactions with conspecifics?

2) Is the frequency of interactions influenced by the sex of octopuses?

3) Do the type or frequency of visual signals differ between initiators and reactors of an interaction?

Methods

Overview

To identify the visual communication signals of *O. rubescens*, cameras recorded videos of octopuses interacting with conspecifics of same and opposite sex in an observation tank. Videos were analyzed for any visual signals used by the octopuses during interactions and these visual signals were defined and categorized in order to assemble an ethogram for *O. rubescens*.

Octopus Collection and Care

Octopus rubescens individuals were collected via SCUBA from Admiralty Bay, WA (48°9'43.84" N, 122°38'4.67" W) and housed at the Rosario Beach Marine Laboratory (RBML), Anacortes, WA. Because this species is often found inhabiting bottles in this bay, all bottles found were checked for the presence of *O. rubescens* by scraping away any biofouling on the bottle. If an octopus without any eggs was present, the bottle was collected and placed into a Ziploc® (3.8 L) bag and sealed. Upon completion of each dive, collected octopuses were removed from their resident bottles and transferred to red Nalgene© (1 L) bottles. The Nalgene© bottle openings were covered with plastic window screen mesh and secured to the bottle by an elastic rubber band. Bottles were placed in a cooler containing aerated seawater and transported back to RBML; all 'home' glass bottles, or dens, were returned to the ocean prior to leaving the collection site.

Upon arrival to RBML, all octopuses were weighed (g) and their sex determined. Weight was measured by placing a tared jar partially filled with seawater onto a Mettler Toledo™ balance (Model: PL601-S). Individual octopuses were persuaded into the tared jar from their Nalgene© bottle by emptying all seawater from the Nalgene© bottle and holding it above the jar on the scale until octopuses transferred themselves. Octopus mass ranged from 18.8 - 68.0 g, with the average mass being 42.0 g. Octopus sex was determined by looking for the presence of a hectocotylus, the third right arm on male octopuses modified to carry spermatophores; this arm is enlarged and lacks suckers at its tip (Cowles, 2005). Mass, sex, date collected, and the octopus's location in the lab were recorded in a Google spreadsheet.

Individual octopuses were housed in enclosed, opaque plastic containers (36 cm x 23 cm x 28 cm; Chase and Verde, 2011) with constant flowing ambient seawater via a manifold system

(Figure 1). Rocks were placed on top of the containers as additional measures to prevent octopuses from escaping. The enclosed containers were held in seawater raceways (231 cm x 29 cm x 24 cm) to maintain a constant temperature of 12°C (Perron and Verde, 2015). *Octopus rubescens* have been noted to adapt well to captivity and most octopuses are known for being exploratory and responsive to laboratory conditions (Mather, 2006). Each octopus was given a minimum of 48 h to acclimate to the containers and sea water system and were fed purple shore crabs (*Hemigrapsus nudus*). Octopuses were fed once per day at night, after all tests were concluded for the day, to avoid the potential influence of increased metabolism (due to specific dynamic action) on social behaviors (Katsanevakis et al., 2005; Hill et al., 2016).

The total sample size (N) for this experiment was 20 octopuses (10 males & 10 females). The seawater system at RBML dedicated for this study could house a maximum of 10 octopuses at a time, so the study was divided into halves. One set of 10 octopuses was run through all tests while the second set of 10 octopuses was collected. Upon completing all tests, octopuses were released back into Admiralty Bay; release locations were separate from new octopus collection sites within the bay to prevent recollection. Sets of octopuses were assigned letters, to identify the respective sets that octopuses were from (A = set 1, B = set 2). Each set of octopuses participated in the 'Conspecifics treatments' (see below). The sex ratio for this study was 50/50 female to male octopuses, to represent the typical sex ratio found in the local area for this species (Chase and Verde, 2011). Octopuses were identified by their respective locations in the seawater table (e.g. a female octopus in seawater table "H" in container "2" was identified as "H2").

Figure 1. Individual octopuses were housed in enclosed, opaque plastic containers with constant flowing ambient seawater via a manifold system. Containers were maintained in seawater raceways to maintain a constant temperature of 12 °C.

Observation Tank

A flow-through seawater observation tank (80 cm x 50 cm x 24 cm) was outfitted with three GoPro cameras (Figure 2) and placed in a closed room to avoid unnecessary human–octopus interaction while tests were conducted. The tank was divided by a piece of plexiglass with holes drilled into it to buffer the rippling effect of the seawater in/outflow (Figure 2). Consequently, only half of the tank (46 cm x 50 cm x 24 cm) was used as the testing area for the octopuses. Overhead

fluorescent lights provided illumination for the cameras and octopuses were given a minimum of 48 h to acclimate to the laboratory lighting conditions. This highly illuminated environment was necessary to capture clear videos of the octopuses and camera settings were adjusted (see Appendix) to accommodate for the lighting. These settings ensured that the highest-quality videos were recorded. To reduce glare for the overhead camera, some fluorescent light bulbs were removed and white sheets were hung under the lights to filter the light above the tank. The observation tank was white, which provided sufficient contrast between the octopuses and the tank for the cameras to successfully record images. To improve water clarity, two seawater filters were attached to the seawater input lines of the tank. Cotton balls were used as the filtering material in the seawater filters and were changed as needed, typically every two to three days. The observation tank was cleaned, drained, and refilled every morning before any tests commenced.

Cameras were placed at different locations in the tank (Figure 2), one directly above and two submerged at the octopuses' level in opposite corners of the tank. Plexiglass stands (Figure 3A) were made to hold the cameras in place (Figure 3B). To eliminate blind spots for the corner cameras in the tank, two plexiglass dividers were cut and angled width-wise along the tank walls to narrow the space (Figure 2). GoPro cameras were left on and recording independently for the duration of each 15-min trial and videos were downloaded to a personal computer after each trial. The tank was drained and completely flushed at the end of every trial to ensure chemicals released by octopuses (e.g. ink, pheromones, nitrogenous waste) during interactions did not influence subsequent trials.

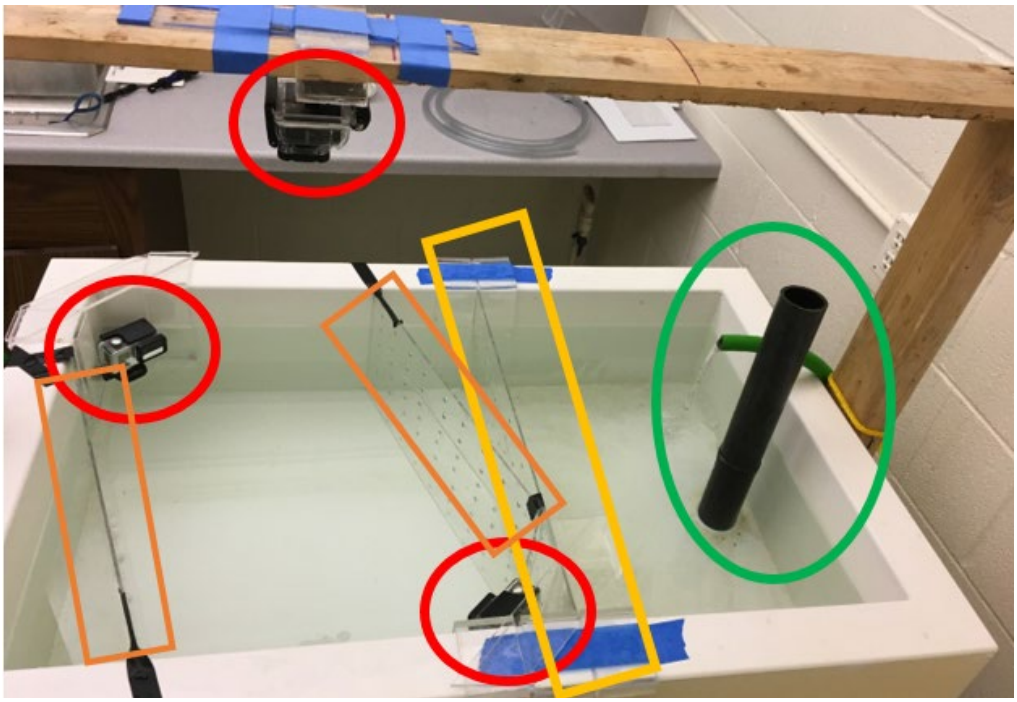

Figure 2. Experimental set-up for the study of visual signals used by *Octopus rubescens*. A flow-through seawater observation tank was outfitted with three GoPro cameras (red circles). The tank was divided by a piece of plexiglass (larger, yellow rectangle) with holes drilled in it to buffer the rippling effect of the seawater in/outflow (green oval). Only half of the tank was used as the testing area for the octopuses. To eliminate blind spots for the corner cameras in the tank, two plexiglass dividers were cut and angled width-wise along the tank walls to narrow the space (smaller, orange rectangles).

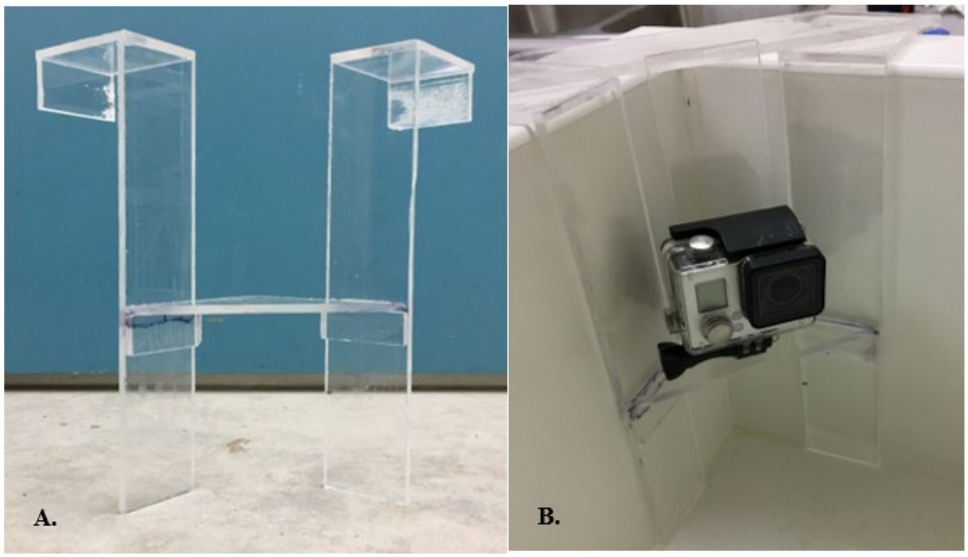

Figure 3. Fabricated plexiglass frames (A) that secured cameras to the corners of observation tank (B).

Since *O. rubescens* have been noted to display aggressiveness toward conspecifics (Mather and Anderson, 1993; Scheel et al., 2016), octopuses were separated in the observation tank space with a piece of plexiglass as a precautionary step while conducting preliminary trials (see 'Ethogram' section below). Most octopuses were aggressive toward one another, but not cannibalistic or noticeably harmful, therefore the plexiglass divider was not used for all other trials following the preliminary trials.

Ethogram

To observe and gather baseline visual communication signs displayed by *O. rubescens*, multiple trial runs of the 'Conspecifics treatment*'* (see below) were made with octopuses previously caught and already at RBML. These visual signals observed were compiled into a basic ethogram and used to categorize additional signs observed during the rest of the study. Visual signals (Table 1) were described utilizing the terminology compiled by Hanlon and Messenger (2018). Ethogram terminology was also adapted from Huffard (2007), Caldwell et al. (2015), and Scheel et al. (2016). These signs included: chromatic (e.g. banding, spots, darkening) and papillae change (e.g. papillate or smooth), forms of locomotion (e.g. chasing, fleeing), and postures (e.g. spreading or flattening of body, raised arms). As additional visual signals were observed, they were added to the basic ethogram library to create the final ethogram for this species.

Conspecifics Treatment

Each octopus (in a set of 10 octopuses) was allowed to interact with all other octopuses of the same and opposite sex within each set (Figure 4). Treatments were as follows: male and male (M/M), male and female (M/F), and female and female (F/F). The order of the octopus combinations was chosen via simple random sampling and random numbers were assigned to each possible octopus combination. To ensure no octopus individual was tested consecutively, selected numbers could be ignored and re-entered into the random numbers table. Tests were performed each day (Sunday to Friday) until all octopus combinations were completed.

Table 1. Visual signals cephalopods may utilize during conspecific interactions. These terms were used to describe recorded visual signals of interacting *O. rubescens* conspecifics in an observation tank. Adapted from Hanlon and Messenger (2018).

Chromatic Signals	**Textural Signals**	**Locomotor Signals**	**Postural Signals**	**Inking Signals**
Whole body	Papillate	Chase	***Whole body***	Pseudomorphs
General paling	Smooth	Flee	Orientation to receiver	
Intense whitening		Forward rush	Up/downward pointing	
General darkening		(Anti)parallel position	Spreading	
Flashing (pulsating)			Flattening	
Passing cloud				
Conflict mottle			***Arms only***	
			Singly	
Partial (often unilateral)			In pairs or all together	
False eyespots			Raised or lowered	
Dark arms			Splayed	
Dark spots (large or small)			Split	
Suckers (white or dark-edged)			V-curled	
			Contorted	
Dark eye rings			Male ligula presentation	
Dilated pupil				
Dark stripes or streaks (longitudinal)				
Dark bars, bands or rings (transverse)				
Bright white spots (large or small)				
Zebra bands or flame markings				
Lateral mantle blush				
Fin lines (dark or light)				
Accentuated white gonad				
Red nidamental glands				
Iridescent rings or stripes				
Polarized light from arms				

Two octopuses were placed in the observation tank as far away from each other as possible. Octopuses were placed in the tank one at a time, therefore the first octopus to enter the tank was always the octopus that was listed first in the written combination name (e.g. combination "H3 and H7"; H3 would be placed in the tank first). Once recording commenced, the octopuses were left to interact for 15 min, as interactions were likely to occur within the first 15 min. Since these organisms are exploratory (Onthank, pers. obs.), this interaction time was chosen to avoid leaving the octopuses in the observation tank for an extended period of time. Octopuses were observed from a distance of at least 2 m to keep track of individuals with no unique identifying characteristics (e.g. unique scars, missing arms) and to intervene if necessary when interactions became too aggressive.

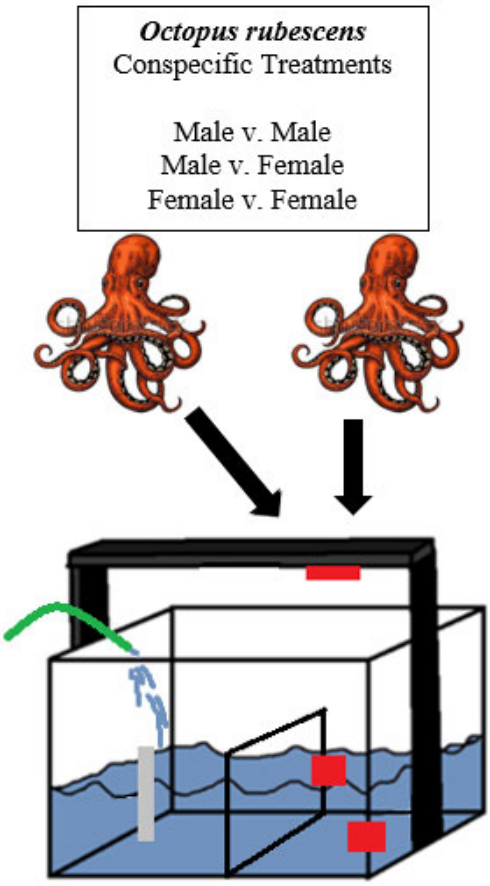

Figure 4. Interaction treatments used in study of visual signals by *Octopus rubescens*. Each octopus (in a set of 10 octopuses) was allowed to interact with all other octopuses of the same and opposite sex within each set (Male/Male, Male/Female, and Female/Female). Octopuses were observed via GoPro cameras (red boxes) in an observation tank. The tank was divided by a piece of plexiglass (black rectangle in tank) to buffer the rippling effect of the seawater in/outflow (green hose, gray pipe).

Collecting and Analyzing Data

VLC Media Player was utilized to observe videos recorded by the GoPro cameras. Snapshots from the videos were added to the basic ethogram created at the beginning of this project

and subsequent videos were analyzed using the basic ethogram. The ethogram was used to describe any octopus interaction that lasted at least 5 s and any new visual signal observed during video analysis was added to the basic ethogram. The approach of one octopus toward the other marked the beginning of an interaction with the approaching octopus deemed the 'Initiator' and the other octopus the 'Reactor', as defined by Scheel et al. (2016). When an interaction began, the time was noted and the visual signals (chromatic, textural, postural, locomotor, inking) of the octopuses were recorded. When there was any change in a given signal during an interaction, the new signal was recorded and the time was noted. A new interaction was recorded only if there was more than a 5 s interval since the end of the last interaction (Sinn et al., 2001). The total number and types of recorded signals displayed by the octopuses in the observation tank were compiled into clustered bar graphs which showed proportions of signals observed within certain categories (e.g. signal or sex categories); this demonstrated how frequently each of the signals was used by octopuses. Due to some behavioral interactions having small sample sizes, comparative statistical analysis could not be performed.

Results

Octopus rubescens used a variety of visual signals to communicate and interact with conspecifics during the 15-min trials. These visual signs included chromatic (Figures 5 & 6), textural (Figure 7), inking (Figure 8), locomotor (Figure 9), and postural cues (Figure 10). Additionally, signal names and descriptions from the final ethogram were summarized and compiled (Table 2). The frequency of interactions differed by 1 interaction per test between the M/M octopus combinations (5.2 interactions per test) and M/F and F/F octopus combinations (4.2 interactions per test).

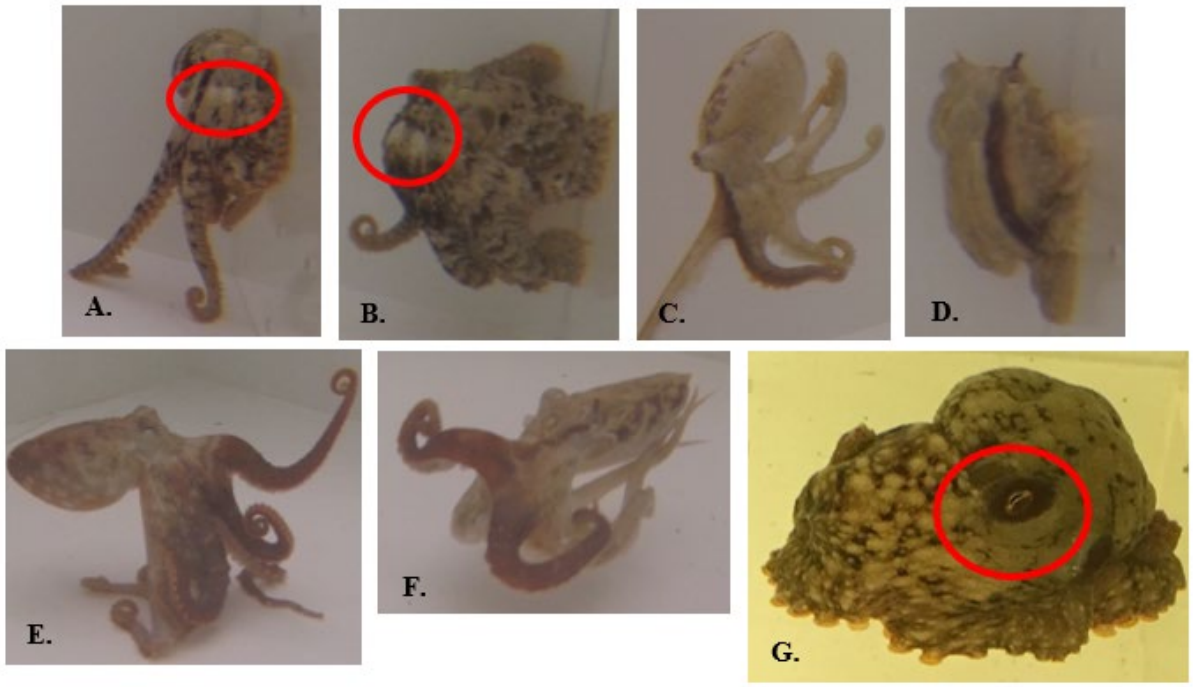

Figure 5. Partial-body chromatic signals used by *O. rubescens*. A.-B. False frontal white eye spots: two adjacent white spots centered below eyes; C.-D. Dark longitudinal stripe(s): typically run(s) from eye down first left and/or right arm(s); does not always run length of arm; E.-F. Darkened arms: typically first left or right (or both) arms of octopus; all arms can be darkened; G. Dark eye rings: darkened patch encircling eyes.

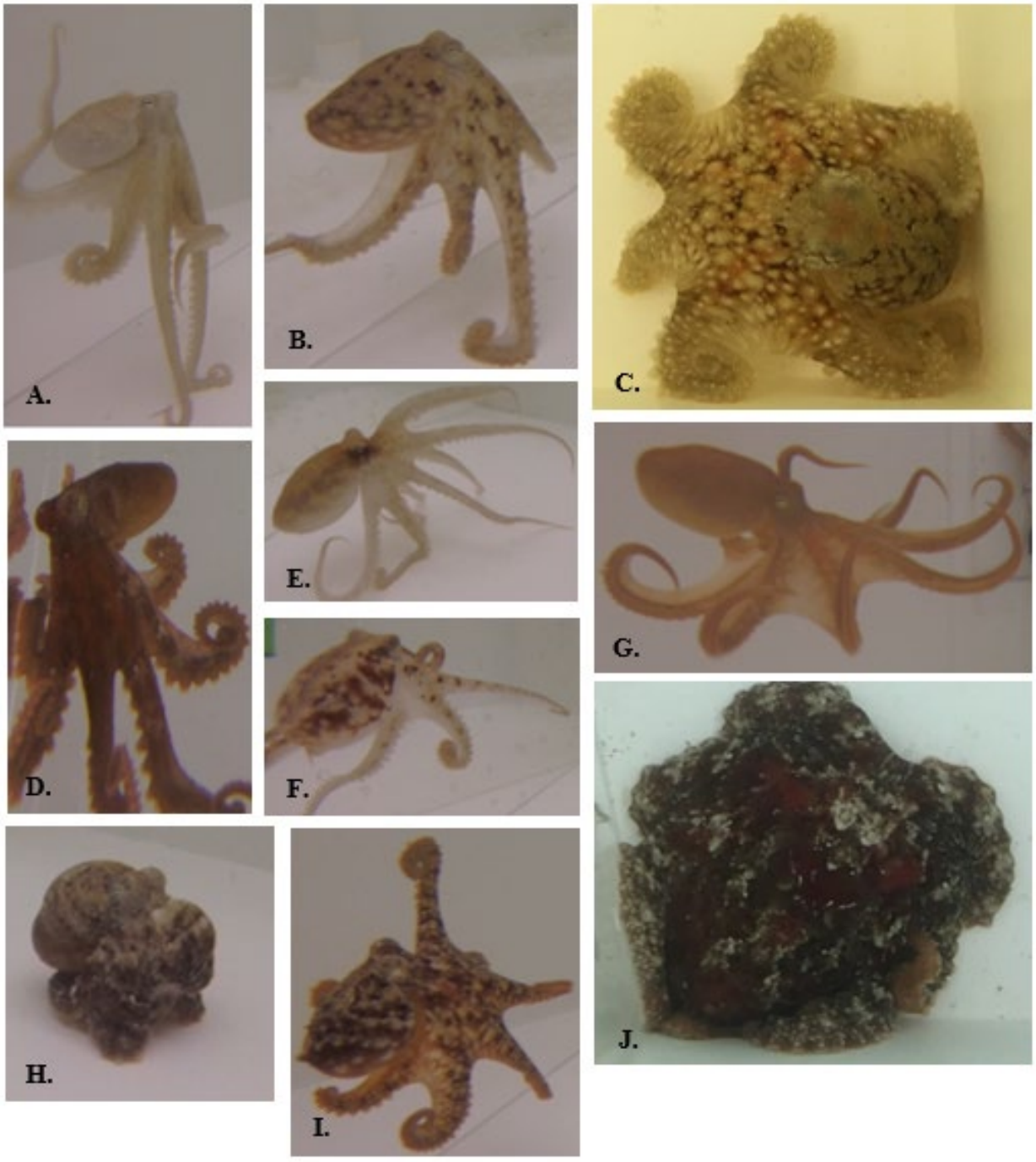

Figure 6. Full-body chromatic signals used by *O. rubescens*. A. Pale: body is light ochre to gray or white; B.-C. Mottled ochre: ochre/sandy-colored body; white and/or brown/black spots (spot density and color varies) across entire body; D. Dark ochre: completely darkened body, red to brown/dark ochre; E.-F. Deimatic: dark spots/patches on mantle, pale arms; G. Ochre: ochre/sand-colored body (some variation in darkness); H.-J. Intense mottle: high contrast between dark and pale markings on body, bars/bands of dark along arms may be present; often papillate.

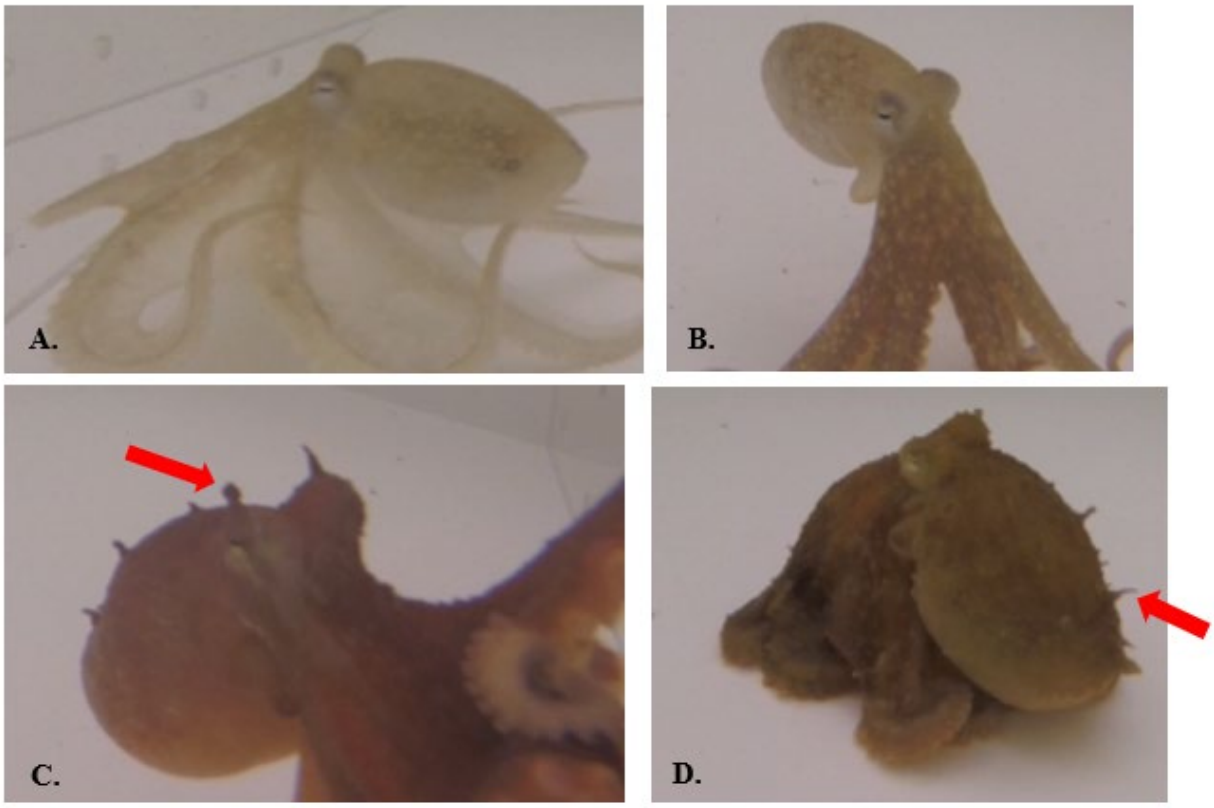

Figure 7. Textural signals used by *O. rubescens*. A.-B. Smooth: no papillae; C.-D. Papillate: papillae visibly raised.

Figure 8. Inking signal used by *O. rubescens*. Inking was recorded as either present or absent.

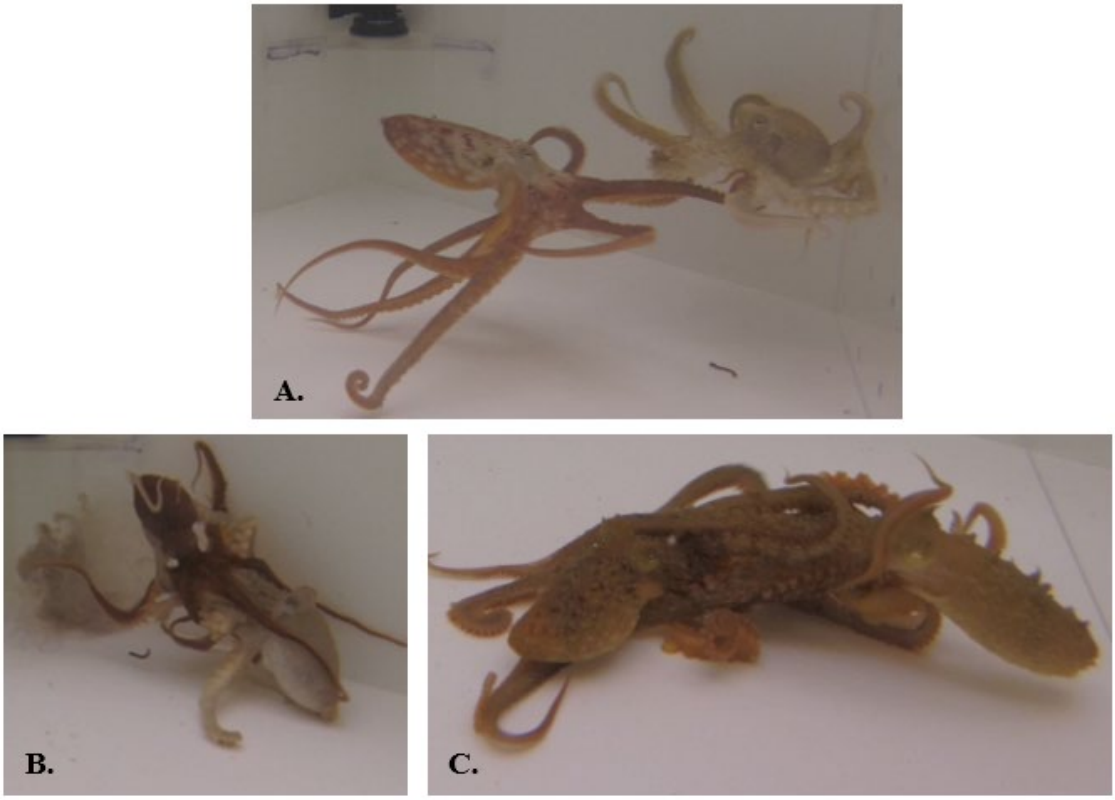

Figure 9. Locomotor signals used by *O. rubescens*. A. Attack: octopus launches self at conspecific; forward rush; jet propulsion commonly utilized; B.-C. Grappling: octopuses entangled in each other's arms, reaching, biting, grabbing. Signals not visualized: Stationary, Threaten, Flee, Chase, Approach.

Octopus interactions (Figure 11) were typically characterized by the locomotor signals 'stationary' (45.9%), 'approach' (21.7%), and 'flee' (24.6%) by one or both octopuses for all sex combinations (M/M, M/F, F/F). The most common chromatic signals included 'ochre' (44.3%), 'dark ochre' (21.9%), and 'pale' (16.4%); octopuses appeared to primarily use 'ochre' as a resting pattern of pigmentation. The most common postures included 'upright' (33.6%) and 'curled arms' (23.9%). Textural signals were predominantly 'smooth' (77.2%) and octopuses rarely inked.

When interactions between octopuses were divided between the initiator and reactor within each sex combination (M/M, M/F, F/F), locomotor signals (Figure 12) used by initiators of an interaction were mostly characterized by 'approach' (36.6%), 'flee' (22.6%) or 'stationary' (32.8%), while reactors predominantly expressed the signals 'stationary' (58.8%) or 'flee' (26.6%). Regarding chromatic signals (Figure 13), initiators and reactors were most often 'ochre' (43.2% & 44.6%, respectively) or 'dark' (23.4% & 21.7%, respectively). The postural signals of both initiators and reactors (Figure 14) were characterized by 'upright' (30.0% & 27.0%, respectively) and/or 'curled arms' (15.5% & 31.7%, respectively) and many interactions were characterized by 'reaching' (12.1%) from initiators (Figure 14). The M/M paired octopuses grappled the most out of the three sex combinations (Figure 12 & 14); however, grappling made up only 5.4% and 3.9% of locomotor and postural signals, respectively (Figure 11).

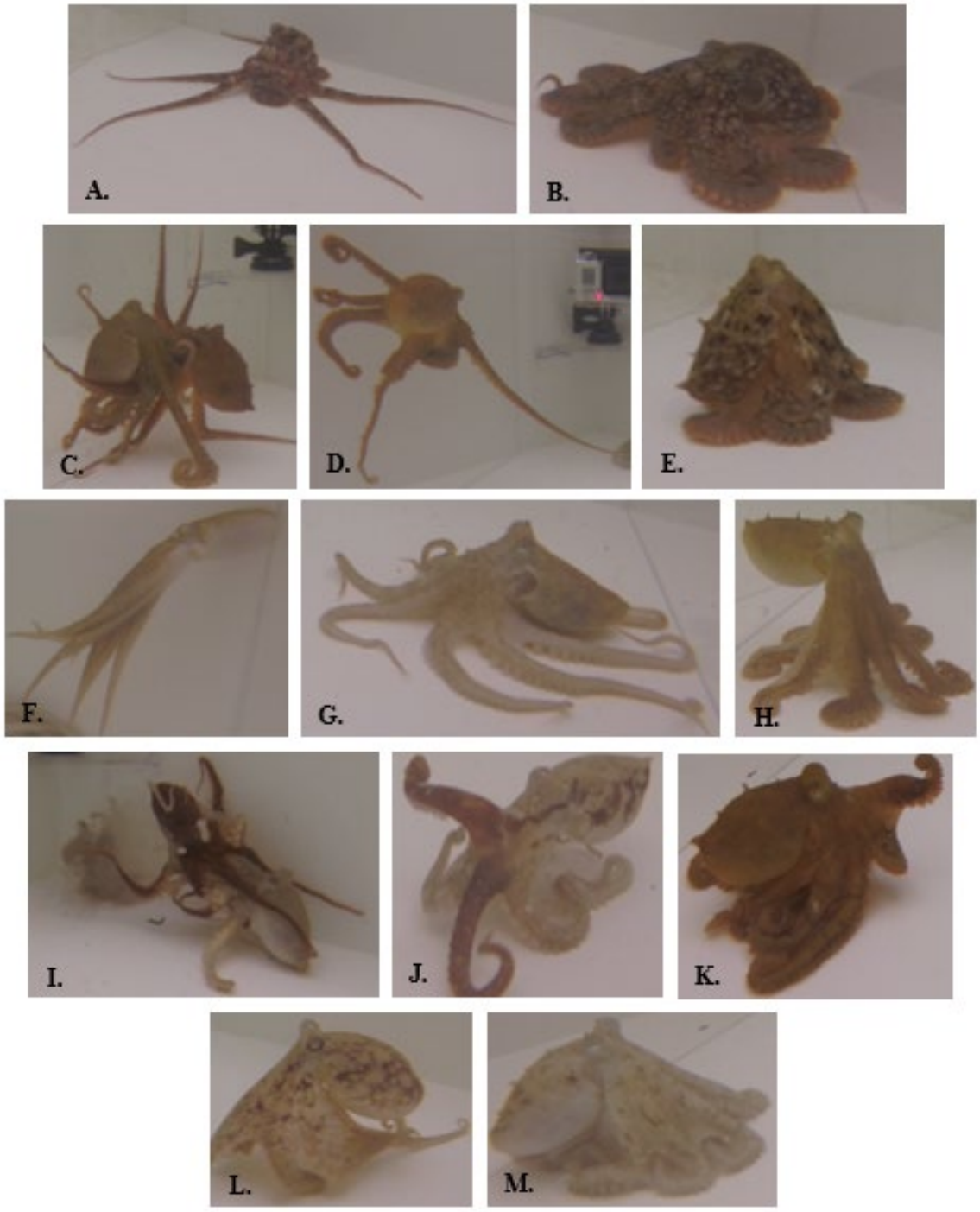

Figure 10. Postural signals used by *O. rubescens*. A. Spreading arms: arms stretched out; B. Flattened: low to bottom, mantle lowered; C. Beak-to-beak: octopuses facing each other, touching close to beaks; D. Reaching: one/multiple arms reaching for conspecific; E. Upright: alert toward conspecific; F. Jetting: arms together, typically, but can be curled; G: Loose arms: arms hanging loosely around/below body, can be slightly curled; H. Stand tall: upright, arms straightened to make self taller/larger; I. Grappling: octopuses fighting; J. Attack: arms poised to attack conspecific (two front arms typically curled and held up); often combined with chromatic signal 'Darkened arms'; K. Raised arms: arms raised, often curled; typically front arms; L. Crawling: arms out, loose or curled, propelling octopus; M. Curled arms: arms curled tightly against body.

Table 2. Summary of visual signals (chromatic, textural, postural, and locomotor signals and inking) used by *O. rubescens* during conspecific interactions observed during 15-min trials in an observation tank. Terminology adapted from Huffard (2007), Caldwell et al. (2015), Scheel et al. (2016), and Hanlon and Messenger (2018).

Visual Signals	
Chromatic	**Description**
Pale	Pale body – light ochre to gray or white.
Deimatic	Dark spots/patches on mantle, pale arms.
Ochre	Ochre/sand-colored body (some variation in darkness).
Mottled ochre	Ochre/sandy-colored body; white and/or brown/black spots (spot density and color varies) across entire body.
Intense mottle	High contrast between dark and pale markings on body, bars/bands of dark along arms may be present; often papillate.
Dark	Completely darkened body, red to brown/dark ochre.
Chromatic: Partial Body (Arms/eyes/mantle)	
False frontal white eye spots	Two adjacent white spots centered below eyes, on front part of octopus body.
Dark longitudinal stripe(s)	Typically run(s) from eye down first left and/or right arm(s), can be symmetrical on other side of octopus; does not always run length of arm.
Dark eye rings	Darkened patch encircling eyes.
Darkened arms	Typically first left or right (or both) arms of octopus. All arms can be darkened.
Textural	
Smooth	No papillae.
Papillate	Papillae visibly raised.
Postural	
Spreading arms	Arms stretched out, feeling bottom.
Flattened	Octopus low to bottom, mantle lowered.
Beak-to-beak	Octopuses facing each other, arms spreading around each other. Touching close to beaks.
Reaching	One or multiple arms reaching for conspecific.
Curled arms	Arms curled tightly against body.
Loose arms	Arms hanging loosely around or below body, can be slightly curled.
Upright	Octopus alert toward conspecific.
Jetting	Arms together, typically, but can be curled.
Grappling	Octopuses entangled in each other's arms, fighting.
Stand tall	Upright, arms straightened to make self taller/larger.
Crawling	Arms out, loose or curled, but clearly being used to propel the octopus.
Attack	Arms poised to attack conspecific (two front arms typically curled and raised).
Raised arm(s)	Arms raised, often curled. Typically first front arms (left and/or right).
Locomotor	
Stationary	Octopus not moving.
Threaten	Octopus lunges at conspecific but does not attack.
Flee	Octopus moves away from conspecific via crawling or jetting.
Attack	Octopus launches self at conspecific, forward rush. Typically jet propulsion.
Grappling	Octopuses entangled in each other's arms, reaching/biting/grabbing.
Chase	Octopus pursues conspecific via crawling or jetting.
Approach	Octopus approaches conspecific via crawling or jetting.
Inking	Ink.
Present/Absent	

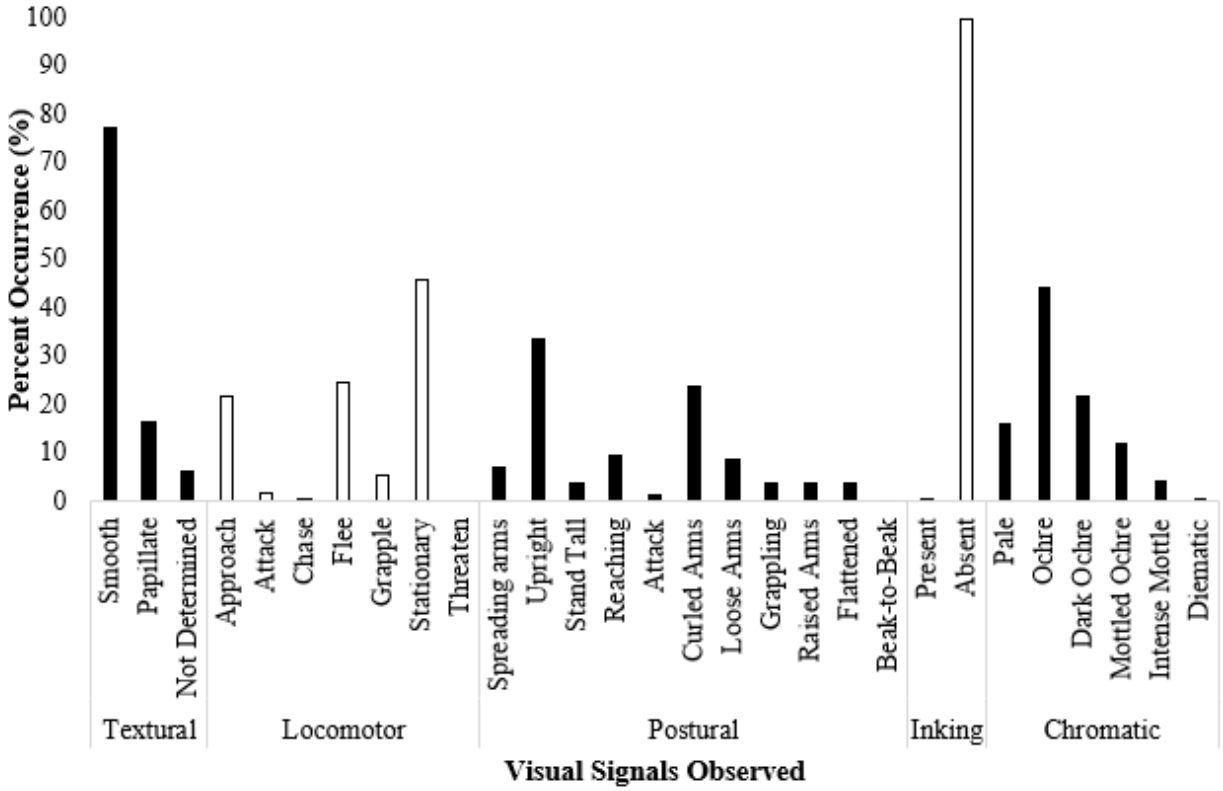

Figure 11. The percent occurrence of the most common visual signals displayed by *O. rubescens* during interactions with conspecifics in an observation tank ($N_{male} = N_{female} = 10$). The five categories of signals include textural, locomotor, postural, inking, and chromatic which all have a variety of subcategories.

Discussion

A variety of visual signals used by *O. rubescens* during conspecific interactions were identified and catalogued in an ethogram. The number of interactions per test for all sex combinations (M/M, M/F, F/F) of octopus was similar which suggests that sex had little influence on the frequency of interactions between octopuses. Both initiators and reactors typically used the same set of visual signals during interactions; however, the sequence in which these visual signals were used was not analyzed. Consequently, it cannot be concluded that a certain visual signal was correlated with initiating or ending an interaction. While *O. rubescens* may gather in an area for a specific habitat resource, such as the bottles used as dens in Admiralty Bay, the species demonstrated predominantly aggressive behavior toward conspecifics during this study, suggesting that they are not a social species even if they are not solitary. When interactions did occur, they were characterized by an approach, which was either aggressive or exploratory (which often led to aggression), and ended with one or both octopuses attempting to escape. Alternatively, octopuses would simply avoid each other which can be interpreted as another indication that this species is asocial. Nonetheless, *O. rubescens* still demonstrated the utilization of multiple visual signals during interactions which suggests communication was occurring between individuals.

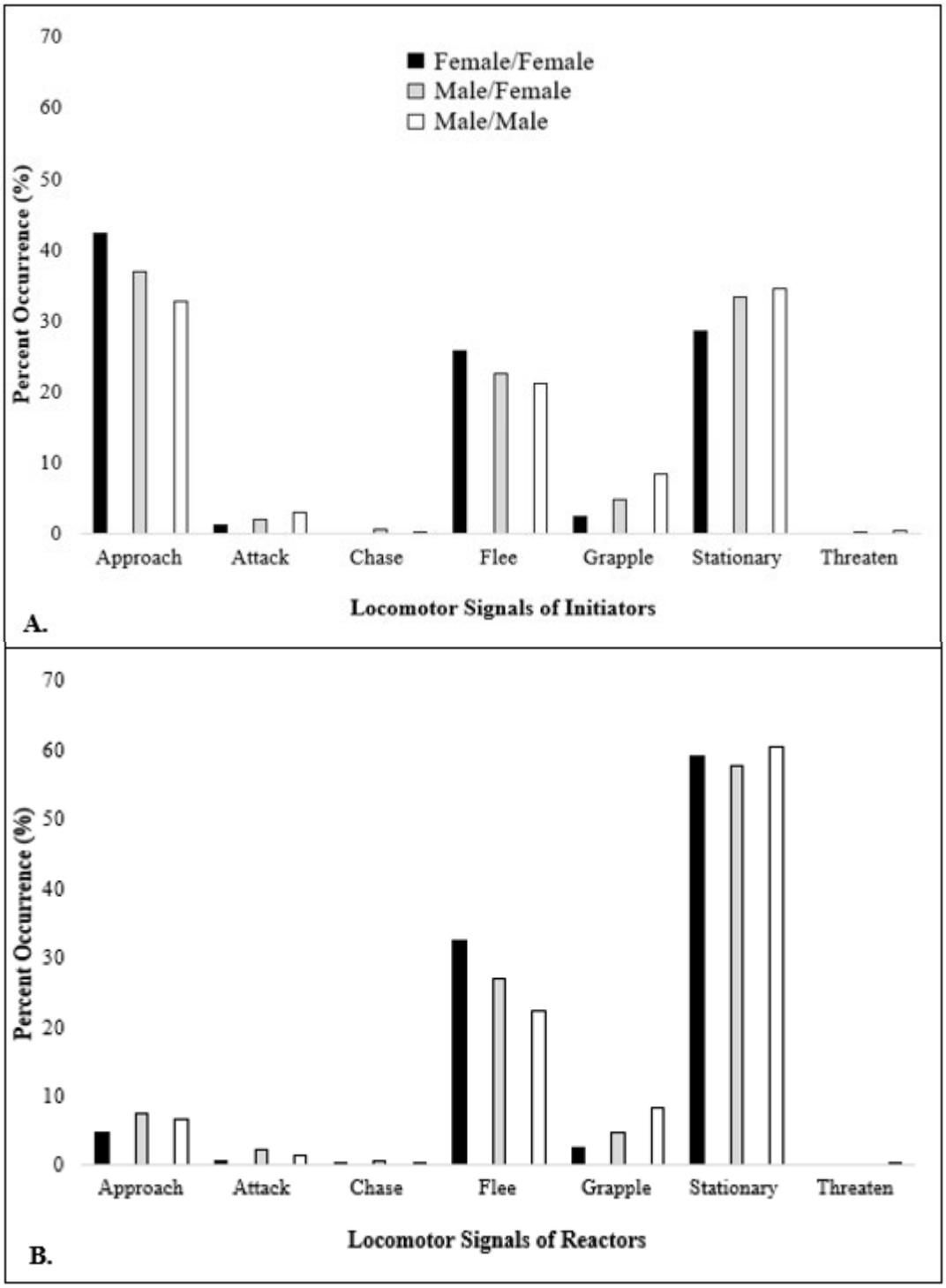

Figure 12. Locomotor signals commonly used by initiators (A.) and reactors (B.) of an interaction. *Octopus rubescens* were allowed to interact with conspecifics in Male/Male, Male/Female, and Female/Female pairs in an observation tank ($N_{male} = N_{female} = 10$).

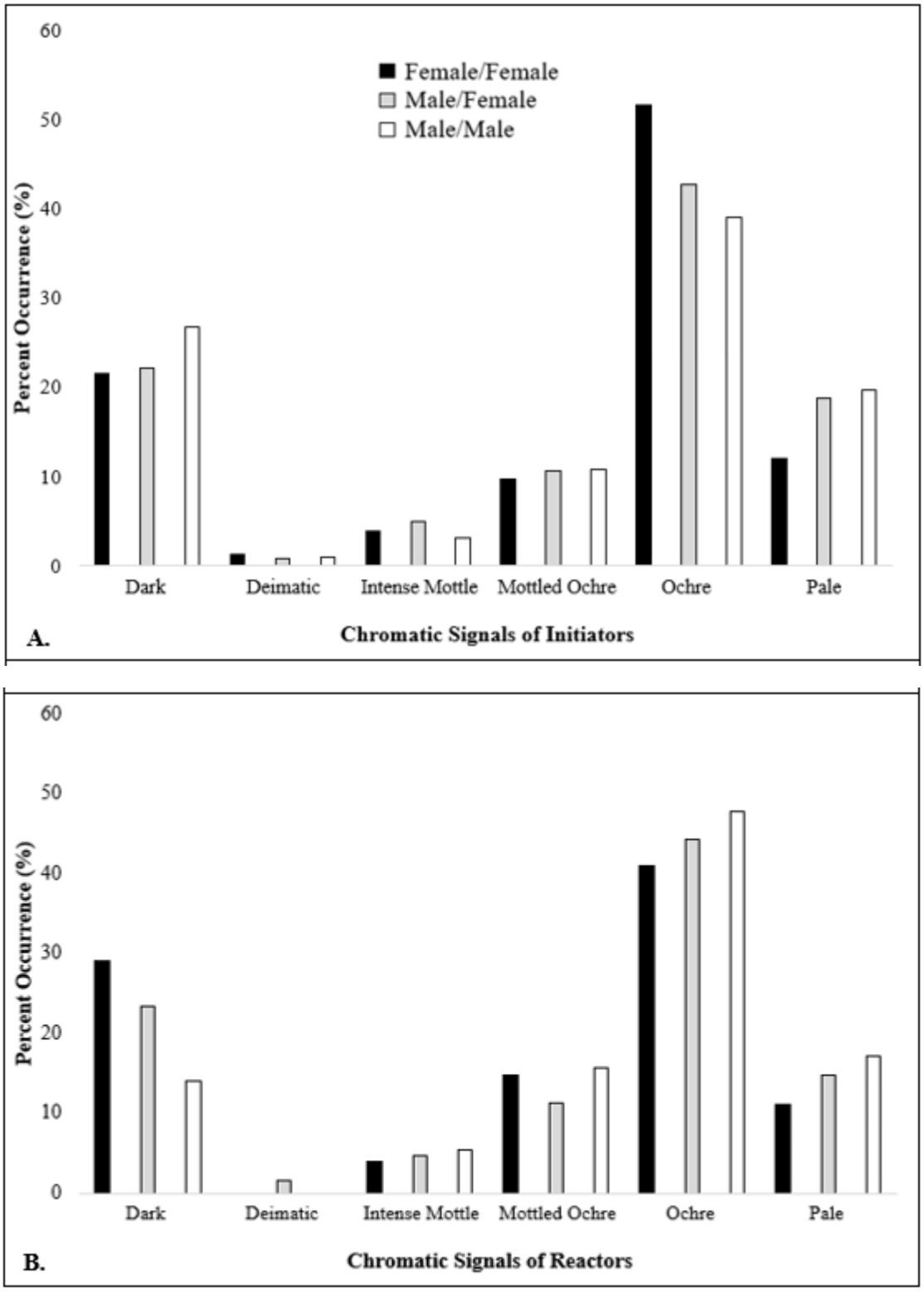

Figure 13. Full-body chromatic signals commonly used by initiators (A.) and reactors (B.) of an interaction. *Octopus rubescens* were allowed to interact with conspecifics in Male/Male, Male/Female, and Female/Female pairs in an observation tank ($N_{male} = N_{female} = 10$).

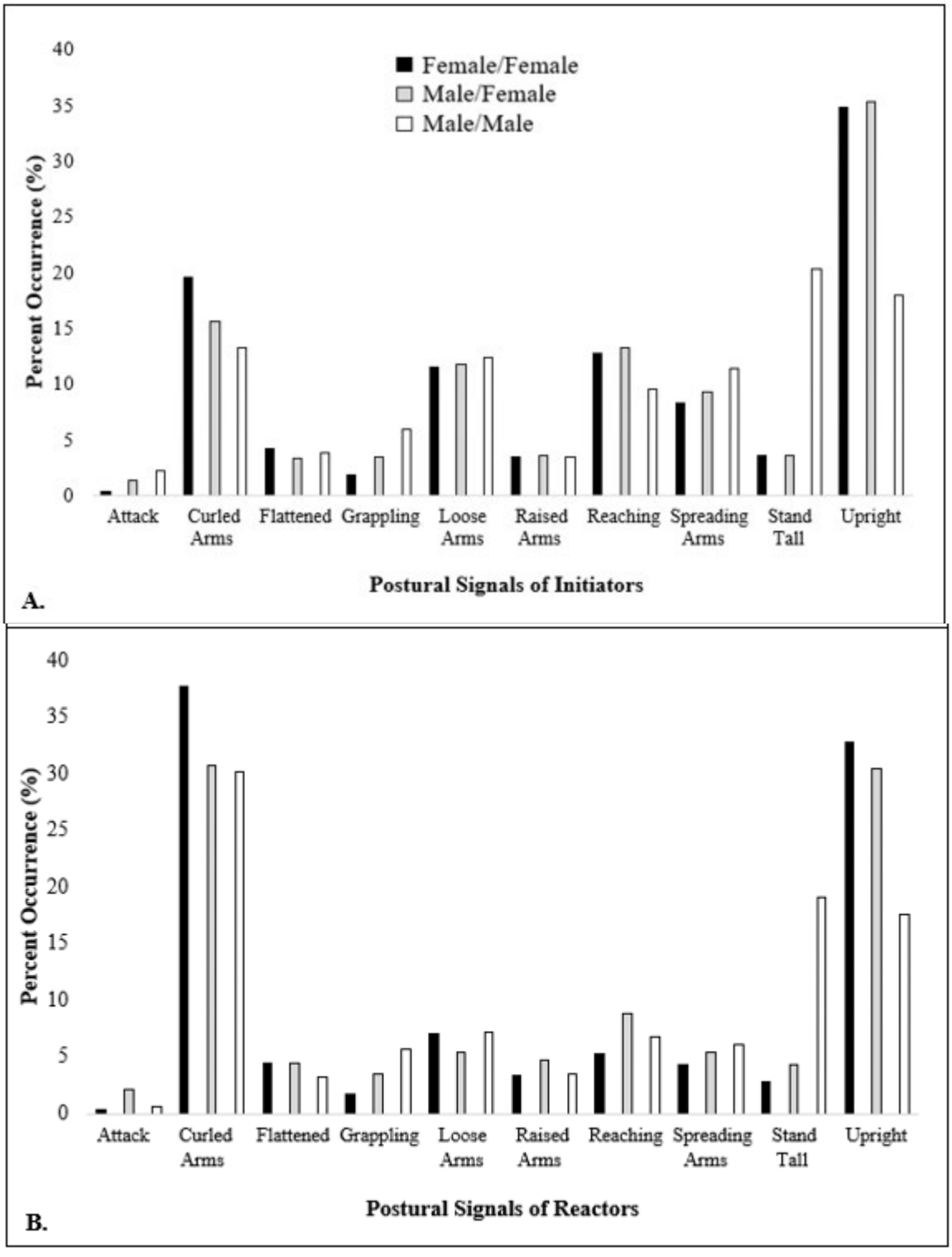

Figure 14. Postural signals commonly used by initiators (A.) and reactors (B.) of an interaction. *Octopus rubescens* were allowed to interact with conspecifics in Male/Male, Male/Female, and Female/Female pairs in an observation tank ($N_{male} = N_{female} = 10$).

Visual signals are especially important for octopuses to use during aggressive interactions because they can clearly display an octopus's intentions to attack or submit, depending on the likelihood of winning or losing a fight (Barbato et al., 2007; Scheel et al., 2016). Having the ability to display such intent helps octopuses avoid unnecessary harm. *Octopus rubescens* used specific and discrete visual signs (the 'attack' posture and chromatic signals 'deimatic' and 'dark longitudinal stripes') to warn a conspecific before attacking. The 'attack' posture, although not used as frequently as other postures, such as 'upright' and 'curled arms', is an important example of a warning system that this species used before attacking a conspecific. The chromatic signal 'deimatic' was also used to warn or threaten a conspecific and is a commonly used threatening display posture among other cephalopods (Scheel et al., 2016; Hanlon and Messenger, 2018). Furthermore, *O. rubescens* displayed 'dark longitudinal stripes', similar to *Abdopus aculeatus* (Huffard, 2007), prior to or while reaching for a conspecific or before attacking.

Another noteworthy chromatic signal *O. rubescens* used was 'false frontal white eye spots' which appeared quite frequently and was interpreted as another warning sign toward conspecifics. This signal, along with 'dark longitudinal stripes', was included in Hanlon and Messenger's (2018) descriptive table of visual signals commonly used by cephalopods. Both forms of communication are examples of how octopuses produce high-contrasting chromatic patterns that are easily visible to an observer. Lastly, *O. rubescens* displayed the posture 'stand tall', similar to *Octopus tetricus* and *Abdopus aculeatus* (Huffard, 2007; Scheel et al., 2016). While *O. rubescens* did not use this posture as frequently as 'upright' or 'curled arms', it is an important posture that should enable individuals to get a better view of a conspecific or to increase apparent size of an individual (Huffard, 2007; Scheel et al., 2016).

One aggressive signal, 'grappling', was not as frequent as other postural or locomotor signals, but nonetheless occurred during interactions and most frequently between males. Huffard (2007) observed similar agonistic interactions primarily between males compared to M/F interactions; no F/F interactions were observed by Huffard (2007) to serve as a comparison with the behaviors documented for *O. rubescens*. Increased aggression between males perhaps could be attributed to their need to compete for females in their natural habitat. Huffard et al. (2010) suggest that M/M aggression is influenced by the value of a resource being competed for (a female) and the likelihood that a male can successfully acquire that resource. Therefore, an aggressive interaction between males may determine whether a male copulates with a preferred female or not which may explain why males are more likely to be aggressive toward one another.

Since the sample of octopuses used in this study was from a population in Admiralty Bay, where they congregate to use bottles as dens, these octopuses may use a specialized system of visual signaling during interactions to communicate with conspecifics, as opposed to more solitary octopuses. Caldwell et al. (2015) suggest octopus populations with higher local densities interact with conspecifics more frequently and often display more aggression toward conspecifics compared to solitary octopus species. Consequently, the observed agonistic interactions of *O. rubescens* may be due to the denser population of this species in Admiralty Bay. As a result, this population of octopuses may experience increased competition for dens, mates or food which may lead to increased aggression (Huffard et al., 2010).

Although the behaviors documented were in a laboratory setting, the ethogram produced in this study may still serve as a useful reference. Future studies can document the visual signals

of *O. rubescens* collected from other habitats and reveal potential variations in visual signals used by this species. This may allow scientists to hypothesize that the visual signals used by *O. rubescens* are influenced by surrounding habitats, like Admiralty Bay, or population density. The basic ethogram created in this study can also act as an additional resource of comparison between octopus species, regardless of the fact that the visual cues identified were during conspecific interactions. Ethograms can be helpful resources that demonstrate evolutionary convergence of signal use (e.g. two distantly related species using similar signals to communicate with conspecifics) or verify a taxonomic similarity between species (Huffard, 2007).

Acknowledgements

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Literature Cited

Anderson, R., P. Hughes, J. Mather, and C. Steele. 1999. Determination of the diet of *Octopus rubescens* Berry, 1953 (Cephalopoda: Octopodidae), through examination of its beer bottle dens in Puget Sound. Malacologia. 41: 455-460.

Barbato, M., M. Bernard, L. Borrelli, and G. Fiorito. 2007. Body patterns in cephalopods: ‘‘polyphenism’’ as a way of information exchange. Pattern Recognition Letters. 28: 1854-1864.

Caldwell, R., R. Ross, A. Rodaniche, and C. Huffard. 2015. Behavior and body patterns of the larger pacific striped octopus. PLoS One. 10: e0134152.

Chase, E, and A. Verde. 2011. Population density and choice of den and food made by *Octopus rubescens* collected from Admiralty Bay, Washington, in July 2011. American Academy of Underwater Sciences. 30: 110-116.

Cowles, D. 2005. *Octopus rubescens* Berry, 1953 [Internet]. College Place (WA): Walla Walla University; [cited 2018 Feb 6]; https://inverts.wallawalla.edu/Mollusca/Cephalopoda/Octopus_rubescens.html

Hanlon, R. and J. Messenger. 2018. Cephalopod Behaviour. Cambridge University Press, Cambridge, England.

Hill, R., G. Wyse, and M. Anderson. 2016. Animal Physiology. 4th Ed. Sinauer Associates, Sunderland, MA.

Huffard, C. 2007. Ethogram of *Abdopus aculeatus* (D’Orbigny, 1834) (Cephalopoda: Octopodidae): can behavioral characteristics inform octopodid taxonomy and systematics? Journal of Molluscan Studies. 73: 185-193.

Huffard, C., R. Caldwell, and F. Boneka. 2010. Male-male and male-female aggression may influence mating associations in wild octopuses (*Abdopus aculeatus*). Journal of Comparative Psychology. 124: 38-46.

Katsanevakis, S., N. Protopapas, H. Miliou, and G. Verriopoulos. 2005. Effect of temperature on specific dynamic action in the common octopus, *Octopus vulgaris* (Cephalopoda). Marine Biology. 146: 733-738.

Mather, J., and R. Anderson. 1993. Personalities of octopuses (*Octopus rubescens*). Journal of Comparative Psychology. 107: 336-340.

Mather, J. 2006. Behaviour development: a cephalopod perspective. International Journal of Comparative Psychology. 19: 98-115.

Perron, J. and A. Verde. 2015. *Octopus rubescens'* prey handling procedures are influenced by bivalve shell thickness and adductor muscle strength. American Academy of Underwater Sciences. 34: 78-94.

Scheel, D., P. Godfrey-Smith, and M. Lawrence. 2016. Signal use by octopus in agonistic interactions. Current Biology. 26: 1-6.

Sinn, D., N. Perrin, J. Mather, and R. Anderson. 2001. Early temperamental traits in an octopus (*Octopus bimaculoides*). Journal of Comparative Psychology. 115: 351-364.

Tricarico, E., L. Borrelli, F. Gherardi, and G. Fiorito. 2011. I know my neighbour: individual recognition in *Octopus vulgaris*. *PLoS One*, 6: e18710.

Appendix

Settings used on the GoPro cameras to capture clear videos of octopuses in high-light situations (GoPro Hero 3 Black Edition User Manual; GoPro Hero 3+ Silver Edition User Manual). Cameras were placed at different locations in an observation tank to record visual signals displayed by octopuses during conspecific interactions.

	GoPro Hero 3	GoPro Hero 3+
Pixels	1080	1440
Frames per Second	30	30
Protune	On	On
White Balance	Auto	Auto
Field of View	Wide	Wide

GoPro Hero 3 Black Edition User Manual [Internet]. 2018. San Mateo (CA): GoPro; [cited 2018 Feb 19]. https://gopro.com/content/dam/help/hero3-black-edition/manuals/HERO3

GoPro Hero 3+ Silver Edition User Manual [Internet]. 2018. San Mateo (CA): GoPro; [cited 2018 April 10]. https://cbcdn2.gp-static.com/uploads/product_manual/file

THE USE OF VISIBLE IMPLANT ELASTOMER TAGS IN JELLYFISH

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Abstract

As recordkeeping practices improve amongst zoos and aquariums, it is becoming increasingly important to develop reliable methods to identify and track animals across all taxa. Gelatinous zooplankton are particularly difficult to tag due to the aqueous composition of their tissues. Although methods have been developed for tagging larger jellies with radio tags, amongst AZA accredited zoos and aquariums, there are no known methods for identifying smaller, individual jellies. We tested visible implant elastomer (VIE) tags on moon jellies varying in size from 2.5 cm to 16 cm bell diameter. Tags were retained for 1 year, and did not cause significant deformities in the animals. This paper is the first documentation of VIE tags being used on jellies at an AZA-accredited zoo or aquarium, and reveals a new tool for record keeping, research, and monitoring the success of jelly culture and care.

Introduction

The Maritime Aquarium (TMA) at Norwalk, CT is considered a moderately-sized aquarium, with over 250,000 gallons of water and over 6,000 species of fish. Despite its moderate size, TMA has a very robust jelly program, which started as a small temporary exhibit in 1995 and has grown tremendously since then. Currently the facility has 6 display tanks in its main jelly gallery, ranging in size from 80 to 1,600 gallons, including a zero-edge globe tank and a zero-edge dome tank. In addition, the jelly culture lab has 7 additional tanks, including a moon jelly touch tank that was opened in 2014. Behind the scenes of the jelly lab are 6 holding tanks, and 3 jelly culture racks. Among all of the jelly tanks at TMA, there are 9 different species in culture, 8 species on display, and approximately 1,630 animals in total. Since jellies have a relatively short life span, new animals are constantly being grown out and moved to display, and any surplus animals are sent to other facilities when possible.

One of the challenges involved in keeping such a large number of soft-bodied invertebrates is monitoring and tracking them for recordkeeping purposes. The AZA requires its members to maintain a records management program for their animal records and veterinary records, as well as any other relevant information (AZA accreditation standard 1.4.0). For jellies, facilities must keep records on the species, animal group number for medusae/ctenophores/polyp colonies, any acquisition, disposition, transfer and mortality information, and facilities must inventory their jellies at least annually (AZA, 2013). Identifying individual and groups of jellies so that they can be recorded accurately is a challenge, and most facilities track their jellies as groups or colonies based on their location, or the exhibit name. This means that if a few individuals are moved from one tank to another, they merge into the new group and assume that group's accession number, and their original records can no longer be distinguished from the records for the rest of the group. Without being able to connect historical records to individual jellies or batches of jellies from the

same culture event, it is nearly impossible to link culture techniques to success rates in large displays.

The 2013 edition of the AZA jelly husbandry manual states there are no reliable methods for identifying individual jellyfish medusa or polyps (AZA, 2013). Coming up with a method for tracking individual jellies, or even groups of jellies, would be useful for monitoring them as they move from one tank to another. Additionally, aquarists could deduce what culture techniques create the most robust medusae, and even predict when their jellies might start to undergo senescence. Knowing when exhibit animals are nearing the end of their natural life would help with exhibit planning and maintenance, by allowing aquarists to begin culturing new batches of animals in advance. Researchers could also run experiments with multiple treatment groups in a single tank, by tagging each treatment group with a different unique identifier. This would not only save room and reduce the workload of maintaining multiple tanks, it would also make such research experiments more cost effective by saving money on multiple costly kreisel systems.

Several methods have been used to tag soft-bodied invertebrates (including jellies) in both field and laboratory studies. In the field, large tags, such as acoustic transmitters, time/depth recorders, and a technology called the ITAG, have been used to tag and track larger jellies (Fossette, 2016; Hays et al., 2008; Mooney et al., 2015). However, these tags only work on very large jellies, and would inhibit normal behavior and swimming in an aquarium. Vital staining has been used to mark the tissues of soft-bodied animals for a period of time (Wells and Sebens, 2017), but in an aquarium this technique would alter the appearance of the animals to such a degree that they may be unsuitable for display. Both of these issues could be avoided with genetic markers and elemental tags, which are a discrete way to tag or identify smaller animals (Hagler and Jackson, 2001; Thorrold et al., 2002), but these technologies are expensive, and the cost is too prohibitive.

The discovery of an unused visible implant elastomer (VIE) tag kit at TMA inspired staff to look into whether or not these tags could be used on jellies. Several studies have been published that used VIE tags to track soft-bodied invertebrates, such as earthworms and octopuses (Butt et al., 2009; Brewer and Norcross, 2012). A couple of studies have also used VIE tags in *Aurelia aurita* (Xu and Dabiri, 2017; Suzuki et al., 2018). In these last two studies, *A. aurita* were marked with VIE tags, and a camera identified the tags and tracked the swimming pattern of the jellies as they moved through the water column in a tank. However, VIE tags were only used for a short amount of time in these studies, and there was no indication as to whether the tags had any lasting negative effect on the animals. Lastly, a query was sent to the Aquatic Info listserv asking if anyone had a reliable method for tagging small jellies. Interestingly, several people responded that they had used VIE tags in fish, and suggested trying them in jellies. However, no one had attempted it themselves.

Visible implant elastomer tags are a bio-compatible elastomer solution made by Northwest Marine Technologies. The tags consist of a two-part mixture that, when combined, forms a viscous liquid that hardens into a semi-pliable solid. VIE tags are available in a variety of colors, some of which fluoresce under deep violet (405nm) light. When these fluorescent tags are injected under a translucent section of skin and illuminated with a deep violet light, they visibly fluoresce through the tissue. Though VIE tags are most often used to mark fish, they have also been used on a variety of other organisms (Retrieved from https://www.nmt.us/visible-implant-elastomer/).

In order to see if VIE tags could be used to permanently mark gelatinous zooplankton, a study was designed to see how long the tags would stay embedded in the mesoglea of *A. aurita*, as well as whether tagging would cause any health problems for the animal, or noticeably alter their appearance on display. All of these topics were examined in a three-part study: The first part of the study looked at how long tags would stay in the bell of *A. aurita*. The second investigated whether VIE tags had any physical effects on the growth and development of *A. aurita* over the course of 2 months. Lastly, the third part looked into whether other species of jellies had the ability to retain VIE tags.

Materials and Methods

Long-term Tag Retention:

The long-term tag retention study morphed from a quick experiment to see if *A. aurita* could retain tags. On September 9, 2018, neon-pink VIE tags were injected into 4 large (~16 cm bell dia.) *A. aurita* from a 1,600-gallon cylinder tank, as well as into 6 medium-sized (~12 cm bell dia.) *A. aurita* from a 300-gallon reserve tank, and into one small (~2.5 cm bell dia.) *A. aurita* from a 10-gallon grow-out tank. Animals from each tank were checked weekly, and tagged jellies were identified and counted. On October 12, jellies were tagged again with orange tags. On November 20, jellies were tagged once more with green tags, this time varying the number of tags in each animal from 1 to 4 (Figure 1).

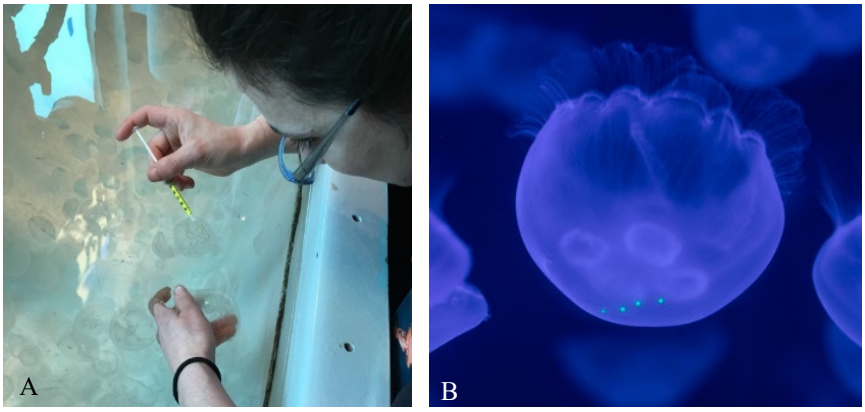

Figure 1. (A) Injecting *A. aurita* with VIE tags in our jelly reserve tank. (B) *A. aurita* that was tagged with 4 green tags.

Since the long-term tag retention study only focused on how long the tags were retained, detailed notes were not taken on which size jelly, color tag, number of tags, or tag location worked the best. Animals from each exhibit were only checked for the presence/absence of the tags, and notes were taken on any useful preliminary data that was noticed at the time.

Jellies were injected with VIE tags according to the protocol listed in the Northwest Marine Technologies VIE implant tag manual (https://www.nmt.us/visible-implant-elastomer/). Animals were secured by hand and gently lifted to the surface of the water, bell up, until the top of the bell was just above the water line. A 0.3 cc insulin syringe with a 29 gauge needle was loaded with pre-mixed elastomer solution and placed in the provided manual injector. VIE tags were embedded into the jelly by inserting the needle about half-way into the mesoglea at the center of the bell, and gently applying pressure to the syringe, while slowly pulling the needle out of the mesoglea. This

left a 1 mm segment of elastomer behind. A few jellies were also tagged in locations other than the center of the mesoglea, given that the tissue was thick enough to accept the tag. Other locations included the bell margin or on the oral arm.

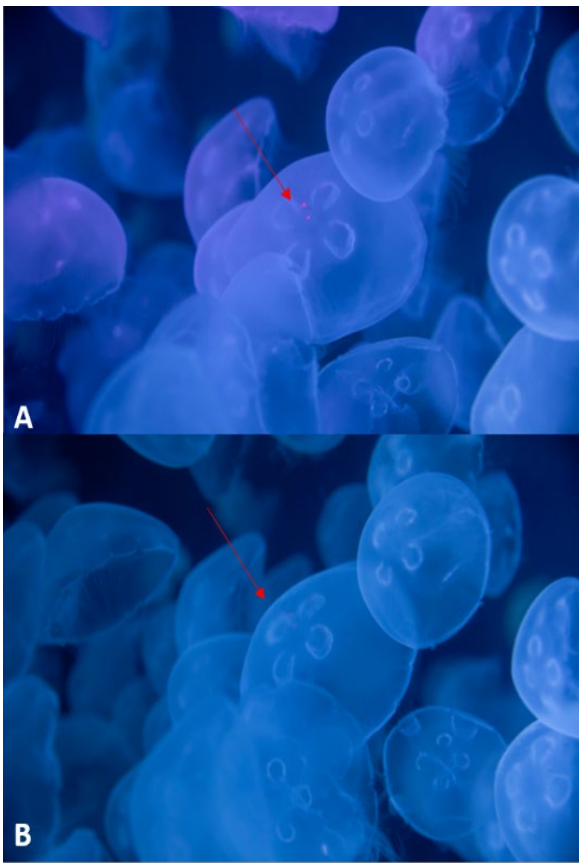

Figure 2. VIE tags were discrete under normal exhibit lighting, but were highly fluorescent under deep blue light. (A) *A. aurita* with pink VIE tags under deep violet light. (B) Same *A. aurita* under normal exhibit lighting.

Several different lights were used to check the tanks for elastomer tags, including the VI 405 nm flashlight that came with the VIE tagging kit, a Kessil A160 Tuna Blue tuned to the bluest actinic setting (listed as "actinic" wavelength on the Kessil website), and an Onforu 24W LED Black Light Bar, model IP66 (365 nm) (Figure 2). Occasionally, patrons were asked if they could find any of the tagged jellies on their own. This was done to see if the tags were obvious to the general public.

Effect of VIE Tags on Growth and Behavior of *A. aurita*:

The second part of the study looked at whether tagging *A. aurita* with VIE tags had any effect on growth, behavior, and mortality. On March 4, 2019, fifteen *A. aurita* with bell diameters of about 10 cm were selected, and were divided into 3 groups of 5. Each group was tagged with either 1, 5, or no tags, and moved into a 90-gallon Envision Acrylic pseudokreisel kept at about 13.5˚C. The jellies with no tags were the control. The other two groups were injected with either 1 or 5 tags in order to look at the difference between injecting jellies with a single tag and a high

number of tags. All jellies were tagged in the center of the bell, and jellies that received five tags were injected in a straight line with tags spaced about 5mm apart.

Animals were observed weekly for deformities and abnormal swimming patterns. Possible deformities included balling up ("canon-ball"), everting, developing lesions on or around the bell, loss of oral arms or tentacles, or disproportionately sized gonads. Possible abnormal swimming included uncoordinated pulsing, entrapped-wave type pulsation, or a general lack of pulsation, which might cause the animal to sit on the bottom of the tank.

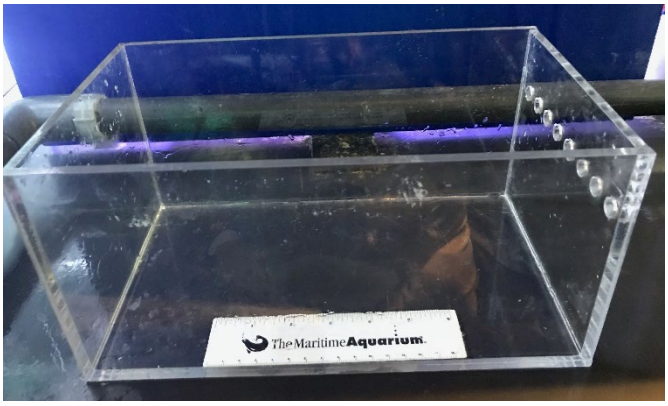

Figure 3. Acrylic box used to measure jellies.

Once a week, animals were measured for bell diameter and thickness. Using a 1 L plastic pitcher, animals were transferred to an 8.25 L acrylic box (30 cm long, 18 cm wide, 15 cm tall) that was filled 1/3 of the way with tank water (Figure 3). Jellies were gently pressed against the bottom of the box to flatten them out. A small ruler was placed under and behind the box, and the jellies were photographed from above, as well as in profile (Figure 4). To help illuminate the elastomer tags, a Kessil Tuna Blue A160 LED pendant was hung above the work station, and was

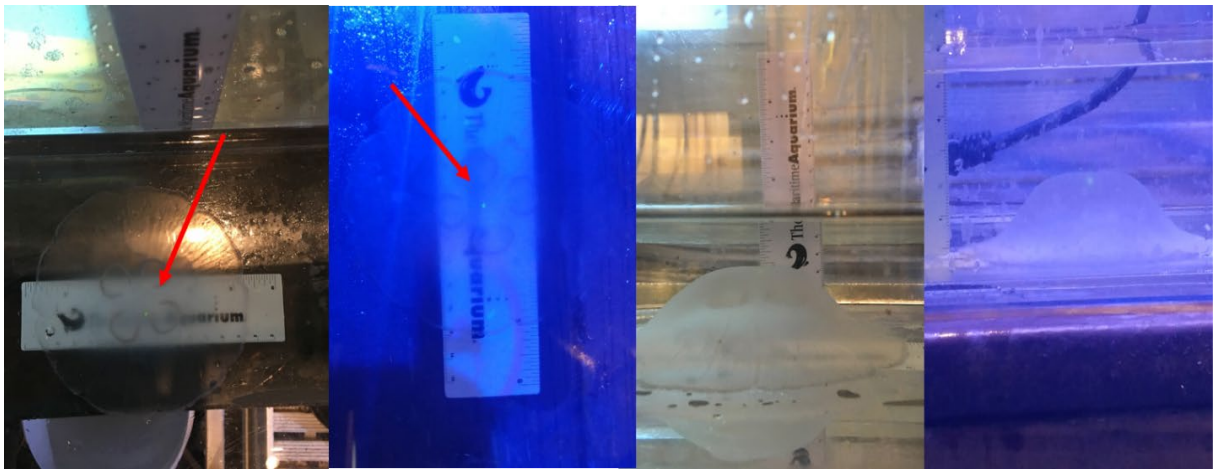

Figure 4. Jellies being measured in an acrylic box. Red arrows point to the single elastomer tag in this animal. Blue images are being illuminated with the Kessil light, and show how the elastomer tag is more visible under deep violet lighting.

tuned to the bluest actinic setting. After photographs were taken, the jellies were moved to a 5-gallon bucket, so they could be kept separate from the jellies that had not been photographed yet.

Photographs were uploaded into ImageJ (Rasband, 1997-2018), and bell diameter and thickness were extracted using the measuring tool and entered into MS Excel, along with the number of tags in each jelly. Once in Excel, the height to diameter ratio was calculated. This measurement would be used to assess body condition, and would help us determine if the tags were creating a change in the overall body shape of the jellies. Statistical analysis was performed in MS Excel using 2-factor ANOVA with replication.

Tag Retention in *Cassiopea andromeda, Phyllorhiza punctata,* and *Chrysaora fuscesens*:

In order to see if other types of jellies could retain tags, VIE tags were injected into *C. andromeda, P. punctata,* and *C. fuscescens* (Figure 5). These species were selected because they represented three unique morphologies, and because there were specimens on hand that were large enough to hold tags. Similar to the long-term tag retention study, several jellies of each species were tagged, and then checked periodically for the presence/absence of tags. No detailed notes were taken on which size jelly, color tag, number of tags, or tag location worked the best. Data was only recorded on the presence/absence of the tags from each exhibit over time, along with any anecdotal information that was relevant to the study.

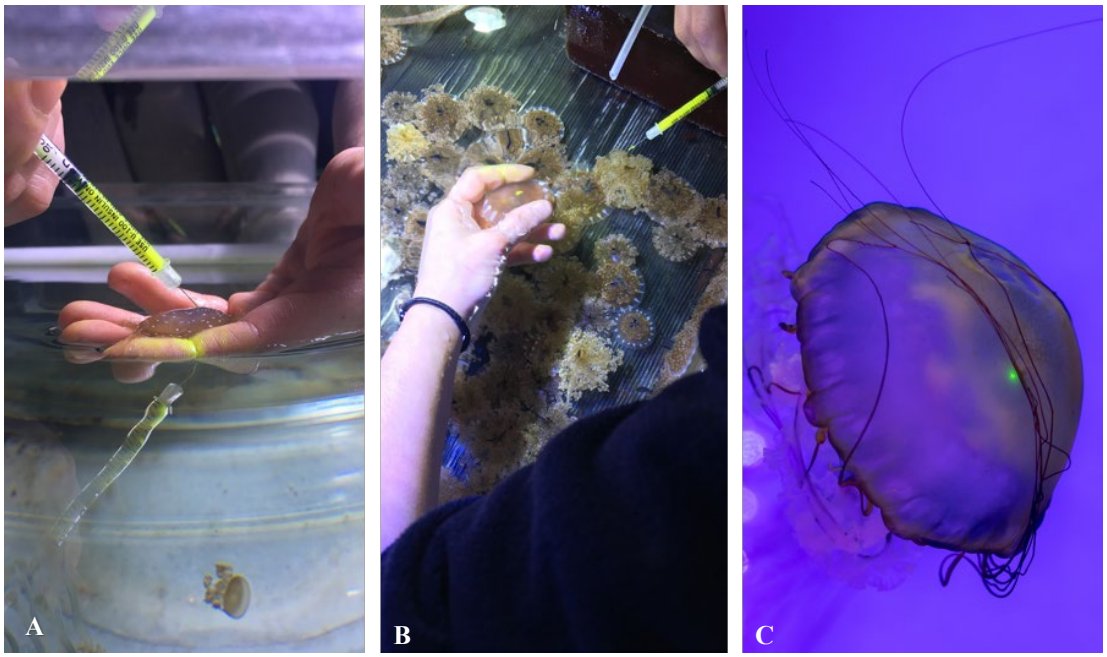

Figure 5. VIE tags being injected into 3 different species of jellies: (A) *Phyllorhiza punctata*, (B) *Cassiopea andromeda,* (C) *Chrysaora fuscescens.*

Jellies were tagged in the beginning of April, and at the end of the month they were checked for the presence/absence of tags using a Kessil Tuna Blue A160W, tuned to the bluest actinic setting. Unfortunately, by this time the entire collection of *P. punctata* had passed due to a complication in their tank, so tag data was only collected on *C. andromeda* and *C. fuscescens.* It should also be noted that while tagging *C. andromeda*, a handful of jellies were selected to receive

their VIE tags at the base of the oral arm. All other jellies were tagged following the same methods used for the growth study.

Results

Long-Term Tag Retention:

Jellies for this study were initially tagged on September 6th 2018. Although tag counts for the presence/absence study ended in April of 2019, the tank was briefly examined one last time at the end of August. At that last census (not recorded), there were still some jellies tagged with pink, orange, green, and blue tags (Table 1).

Table 1. Dates and number of tagged *A. aurita* in the Cylinder tank. Animals were only checked for presence/absence of tags

Date	Pink	Orange	Green
9/6/2018	11	0	0
10/30/2019	9	11	0
11/20/2019	9	11	8
1/8/2019	6	6	5
3/3/2019	4	4	4
4/28/2019	6	5	5

When patrons were asked whether or not they could see any jellies with VIE tags, very few of the questioned patrons could identify a tagged jelly on their own. However, there were a couple of times when a patron noticed a tagged jelly and asked the husbandry staff about it. This is most likely because the light over the cylinder tank cycles through a series of colors. Although most of these colors do not cause the VIE tags to fluoresce, the blue shades cause the tags to fluoresce slightly. Even so, the patrons that asked about the tags were very excited to hear about the research being done, and did not seem put off by the markings.

Following the suggestions from Northwest Marine Technologies, green and yellow tags were not used together in any of the 3 studies, as these colors are very hard to tell apart. However, orange and pink tags turned out to be equally difficult to distinguish. All of the deep violet lights used to locate tags in the long-term retention study worked well, but the Kessil lights seemed to cast a bright blue light on the entire tank, while the black light only lit up the tags.

Lastly, some of the tags in *A. aurita* seemed to migrate through the bell tissue during the study. Tags that were initially injected into the center of the mesoglea at the top of the bell dome seemed to slowly migrate towards the gastric pouch. Visually, these jellies looked like the tag was almost sitting in the gastric pouch. Jellies with tags that were injected in locations other than the center of the bell (e.g. closer to the bell margin, oral arms) were not observed as frequently towards the end of the study. Similarly, smaller jellies with tags became harder and harder to locate as the study progressed.

Effect of VIE Tags on Growth and Behavior of *A. aurita*:

A. aurita medusae were monitored and measured for 8 weeks to see if elastomer tags had any effect on growth, development and swimming behavior. During this time, all the jellies in the growth study were observed eating and pulsing. The swimming behavior of the jellies was

monitored weekly. None of the animals were seen exhibiting abnormal behaviors, such as balling up ("cannon-ball"), everting, or pulsing with an entrapped wave ("hula-hooping"). Jellies continued to eat during all 8 weeks of data collection, and jellies were not seen resting at the bottom of the tank.

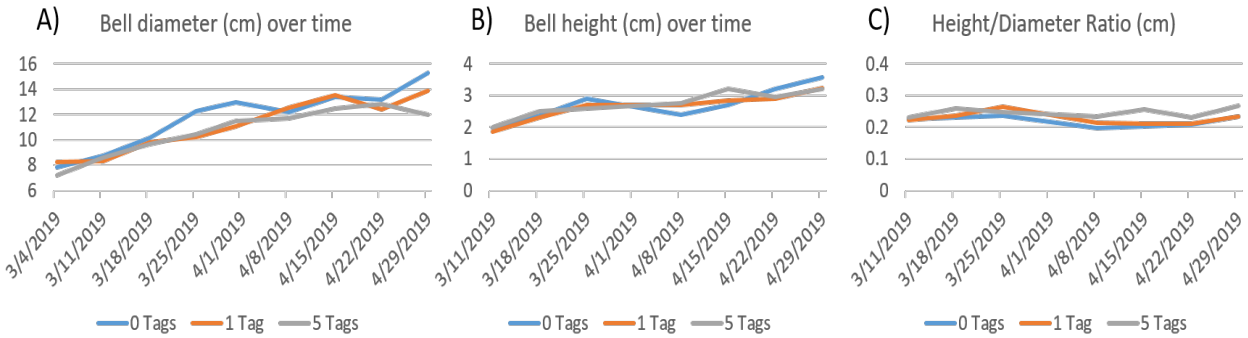

Figure 6. (A) Change in bell diameter over time, p = 0.001; (B) Change in bell height/thickness over time, p = 0.08; (C) Change in the bell height/diameter ratio over time, p = 0.016.

Bell width and height data in the 0, 1 and 5 tag groups were graphed (Figure 6), and was analyzed using two-factor ANOVA with replication. Jellies in all 3 groups continued to grow over time, though the jellies with no tags had a significantly elevated growth rate compared to the jellies with 1 or 5 tags (p = 0.001). The no-tag jellies grew the most, followed by the 1 tag jellies, and then the 5 tag jellies. The bell height (or thickness) was very similar between all tag groups (p = 0.08), though the jellies with no tags showed a higher growth rate trend than the other two groups. Height/diameter ratio was also very similar among all the groups (p = 0.016), and the jellies with no tags also showed a statistically lower ratio than the other two groups.

Tag retention rate was analyzed by counting how many tags were in each jelly during the weekly measurements (Figure 7). All of the animals in the one-tag group kept their tags throughout

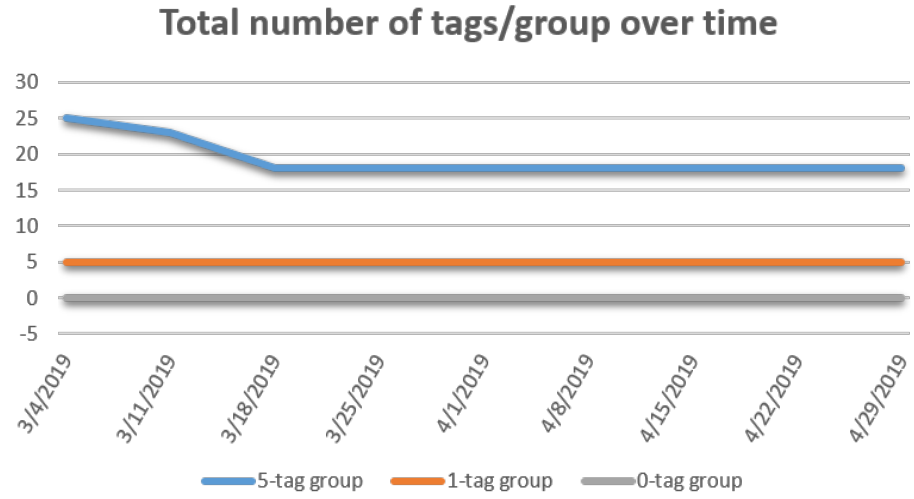

Figure 7. Total number of tags in each group over time.

the study. The jellies that had 5 tags dropped some of their tags, but only during the first 2 weeks of the study. This translated to a 72% tag retention rate in the 5-tag group.

Tag Retention in Other Species of Jellies:

During the last few weeks of our study, other species of jellies were tagged, including *P. punctata* (in the bell), *C. andromeda* (in the bell and oral arm), and *C. fuscescens* (in the bell). Although there was a complication with the *P. punctata* tank that resulted in all of those animals being lost before we could begin collecting data, we did observe that the tag stayed in the animals for at least the day that they were tagged.

By the end of the study, we were only able to locate one of the tagged *C. andromeda* – An individual that had been injected in the oral arm. We were able to locate all of the tagged *C. fuscescens*.

Discussion

This study showed that elastomer tags are an effective and discrete way to mark and differentiate between individual and groups of jellies, without having a negative impact on the animals or their exhibit. Visitors to The Maritime Aquarium were rarely able to identify tagged animals in the display tank, while staff were able to find them quickly and easily when they lit the exhibit with a deep violet or black light.

In all 3 parts of this study there was evidence that tags were being rejected from the jellies. Although data on tag retention was only collected in the growth portion of this project, staff noticed that, over time, there were fewer tagged jellies in all of the study groups. Because data on tag retention was not collected in the long term and multi-species studies, it is hard to say if there were fewer tagged animals towards the end of these studies because tags were dropped, because the jellies underwent senescence, or because they were accidentally added to feeder populations. However, the growth study clearly showed that jellies were rejecting tags. Animals in the 5-tag group lost tags during the first 2 weeks of the study, and after that did not lose any more tags. Also, pieces of tags would occasionally be found on the bottom of some of the holding tanks, which were likely rejected tags. In the growth study, it remains unclear why the jellies with 5 tags showed some tag rejection while the other group did not, but it is most likely related to the location of the additional tags (slightly off-center from the middle of the bell), or because there was a higher error rate when staff were applying multiple tags in one animal.

In the long-term tag retention study, *A. aurita* were able to hold elastomer tags for at least one year. It is possible that *A. aurita* can hold tags for even longer, and this should be investigated further. Although jellies of all sizes did retain tags, staff noticed that as the studies progressed, more tags were seen in the larger jellies (>12 cm dia.) than the smaller jellies. It is possible that the small jellies simply grew large enough to blend in with the larger animals, but based on the size differences and the observation period, this is highly unlikely. Mesoglea is composed of collagen and protein, and is traversed by thick fibers. As jellies age, these fibers thicken and become more tightly wound. Because of this, it is harder for particles to migrate across older, more tightly wound fibers, which could result in a higher tag retention rate in older jellies (Gambini et al., 2012). However, this phenomenon was only noted anecdotally, and should be investigated further to see if there is a statistical correlation to this observation.

Some tag colors were very difficult to distinguish from one another, such as orange and pink, and yellow and green. These colors should not be used together in future studies unless there are no other color combination options. Northwest Marine Technologies describes methods that can be used to create multiple batch marks, or even individual identification, from just a handful of tags. For example, use of a single tag but using four colors in five different body locations immediately gives 20 unique marks (Northwest Marine Technology manual). Northwest Marine Technologies also provides a VIE color code generator on their website that can be used to determine how to make the most unique identifiers out of any tag combination. However, they also point out that because of the potential for tag loss, it is recommended that all study individuals receive the same number of tags. Otherwise, an animal with multiple tags could be mistaken for one with less. This almost happened in the growth study, where the 5-tag group lost some tags during the first couple of weeks. It was extremely fortunate that the 1 tag and 5 tag jellies retained enough tags that they could still be distinguished from each other. Because this part of the study focused on how the number of tags affected growth rates, it was important to use a different number of tags in each group.

Other species, such as *P. punctata, C. andromeda,* and *C. fuscescens* are also able to hold elastomer tags, although these species were not monitored as long as *A. aurita*. Tagging *C. andromeda* presented a unique challenge. Because this species tends to orient itself with the bell against the bottom of the tank, it is not practical to inject them in the mesoglea, since tags will not be visible unless the jelly is flipped over. In order to make it easier to find tagged *C. andromeda,* some individuals were tagged in the oral arm. This technique ended up being helpful, and the only tagged *C. andromeda* that was identified at the end had a tag in the oral arm. Even so, it was still difficult to find the tagged *C. andromeda*, since the secondary mouths on the oral arm obscured our view of the tags.

The growth study showed that using VIE tags on *A. aurita* has either minimal or no impact on their growth and development. Behavior-wise, the jellies continued to eat and grow when injected with either 1 or 5 tags, and did not exhibit erratic pulsing behavior. There was some difference in growth rate across all groups, but the most significant difference seemed to be in the bell diameter growth rate (ANOVA, $p = 0.001$) (Figure 6A). At the end of the study, the jellies with 5 tags were overall the smallest, and the jellies with no tags were overall the largest. Bell height (or thickness) growth data showed similar statistical trends as bell diameter (Figure 6B), with the control group showing the most growth during the study (ANOVA, $p = 0.08$). However, these differences were minimal, and the jelly aquarists were not able to tell the difference between 0, 1, and 5 tag jellies with the naked eye alone. The animals all ended the study in excellent condition, and were all considered suitable for display.

Bell height to diameter ratio showed a statistical difference between the three groups, with the 5-tag group having the highest ratio at the end of the study (ANOVA, $p = 0.016$), although it should be noted that the p value for these measurements was much higher than for bell diameter (Figure 6C). Bell height to diameter ratio was used to assess body condition. Jellies undergoing stress will often develop abnormal body conditions, and will either ball-up, evert, or flatten out. Comparing bell height to diameter would help assess if the tags were creating a change in the overall body shape of the jellies. Because the bell diameter growth rate was lower for the 5-tag group while the height to diameter ratio ended up being comparatively higher, there is a possibility

that the 5 tag jellies were developing abnormally. However, as was the case with diameter measurements, husbandry staff were not able to see any differences between the groups with the naked eye. A longer study would be necessary to determine if this trend would develop into a true, visible difference between tagged and untagged jellies.

Although jellies did not develop major deformities during this study, one of the jellies in the growth study did developed a temporary edema in the bell, which disappeared by the end of the study. The edema appeared as a bulge on top of the bell, and felt soft and fluid-filled when touched. Though it is possible that this edema was in response to the elastomer tag, we cannot be sure without further investigation. Jellies have a very rudimentary immune response system, and although edema of the bell has never been clinically documented, some research supports that it may be part of the immune response in cnidarians (LaDoucer et al., 2013).

This study was the first such study done on the effects of elastomer tags in jellies. Though other studies have used elastomer tags as a means to temporarily mark jellies so they can be tracked by a camera, none have looked at whether the tags stay in jellies over a longer period of time, or whether they have an effect on the growth and behavior of the animals. Based on this research, one can conclude that *A. aurita*, *C. fuscescens,* and *C. andromeda* are able to retain elastomer tags, and *A. aurita* can hold tags for at least one year. However, this study should be seen as a first look at some of the many possible applications of VIE tags in jellies. More research is needed to determine whether or not there is a way to increase tag retention so that it remains in the mesoglea for the duration of the jelly's life, or if there is a way to prevent tags from being rejected. It also seems that larger jellies accept tags better than smaller ones, and that the center of the bell is the best location for tag retention, but this should be confirmed with more trials. This technology also has the potential to be used on other species of jellies. Further studies should be done to determine the best way to tag *Cassiopea spp.*, or to see if some of the more delicate gelatinous zooplankton (like *Mnemiopsis leidyii*) are able to retain tags.

When this study began, it was a surprise that so few other facilities had tried using elastomer tags on their jellies. Hopefully this study helps encourage others to try using elastomer tags on their animals, and share their successes with the aquatic community.

Acknowledgements

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Literature Cited

AZA, Aquatic Invertebrate TAG. 2013. "Jellyfish Care Manual." Association of Zoos and Aquariums. Silver Spring, MD.

Brewer, R. S., and B. L. Norcross. 2012. Long-term retention of internal elastomer tags in a wild population of North Pacific giant octopus (*Enteroctopus dofleini*). Fisheries Research. 134: 17-20.

Butt, K. R., M. J. I. Briones and C. N. Lowe. 2009. Is tagging with visual implant elastomer a reliable technique for marking earthworms? Pesquisa Agropecuária Brasileira. 44(8): 969-974.

Fossette, S., K. Katija, J. A. Goldbogen, S. Bograd, W. Patry, M. J. Howard, T. Knoelws, S. H. D. Haddock, L. Bedell, E. L. Hazen, B. H. Robison, T. Mooney, K. A. Shorter, T. Bastian, and A. C. Gleiss. 2016. How to tag a jellyfish? A methodological review and guidelines to successful jellyfish tagging. Journal of Plankton Research. 38(6): 1,347-1,363.

Gambini, E., B. Abou, A. Ponton, and A. J. M. Cornelissen. 2012. Micro- and macrorheology of jellyfish extracellular matrix. Biophys J. 102(1): 1-9.

Hagler, J. R., and C. G. Jackson. 2001. Methods for marking insects: current techniques and future prospects. Annual Review of Entomology. 46(1): 511-543.

Hays, G. C., T. K. Doyle, J. D. R. Houghton, M. K. S. Lilley, J., D. Metcalfe, and D. Righton. 2008. Diving behaviour of jellyfish equipped with electronic tags. J. of Plank Res. 30(3): 325-331.

Mooney, T. A., K. Katija, K. A. Shorter, T. Hurst, J. Fontes, and P. Afonso. 2015. ITAG: an eco-sensor for fine-scale behavioral measurements of soft-bodied marine invertebrates. Anim. Biotelem. 3: 1-14.

Rasband, W.S., ImageJ, U.S. National Institutes of Health, Bethesda, Maryland, USA, https://imagej.nih.gov/ij/, 1997-2018.

Suzuki, K. S., Y. Niida, T. Tsubono, H. Takimoto, E. Kumakura, H. Ishii, and Y. Nogata. 2018. Mechanisms underlying heterogeneous distribution of moon jellyfish *Aurelia aurita* across a sharp pycnocline. Marine Ecology Progress Series. 591: 229-239.

Thorrold S. R., G. P. Jones, M. E. Hellberg, R. S. Burton, S. E. Swearer….. 2002. Quantifying larval retention and connectivity in marine populations with artificial and natural markers. Bull. Mar. Sci. 70(Suppl.1): 291-308.

Wells, C. D., and K. P. Sebens. 2017. Individual marking of soft-bodied subtidal invertebrates *in situ*–A novel staining technique applied to the giant plumose anemone *Metridium farcimen* (Tilesius, 1809). Plos one. 12(11): e0188263.

Xu, N., and J. Dabiri. 2017. Characterization of jellyfish turning using 3D-PTV. *APS*, Q9-004.

Historical Book Review

A SUMMER CRUISE ON THE COAST OF NEW ENGLAND

Robert Carter

Crosby and Ainsworth, Boston, 1865, 261pp

Reviewed by Pete Mohan

Why review a 155-year-old travel book? Simple - it is the first record of both successful aquarium-keeping, and an aquarium collector, in the United States. Most of the participants in this July 1858 adventure are unnamed, but a main character, "the professor," is confirmed as William Stimpson. He was an extremely precocious scientist, and boldly notes that at the age of 17 (in 1849), he was maintaining a number of what we would now call balanced aquariums. This is confirmed in a suspiciously anonymous publication from the same month as this voyage (Anon. 1858). This effort slightly predates Robert Warrington and Philip Henry Gosse's publications in Europe.

Stimpson studied under Louis Agassiz and as a 22-year-old was the first naturalist to have access to Japan as a member of the United States North Pacific Exploring Expedition (1853-1856). Upon his return he founded the "Megatherium Club" in 1857. This rowdy bunch of young scientists were subsequently banned from partying in the Smithsonian Castle due to their loud after-hours exploits (Vasile, 2018). The author, narrator, and fellow passenger, Robert Carter, would have been acquainted with Stimpson through his role as Washington Correspondent for the New York Tribune at that time.

Early in the book, the passengers meet up with "Tufts" (Samuel Tufts, Jr.), an aquarium maker and stocker from Swampscott, Massachusetts. Tufts was a shoemaker by trade, but also an accomplished conchologist. He was in the aquarium trade in the 1850s and appears to have helped Stimpson set up a large aquarium at the Smithsonian in 1857. He is also the only known aquarium stocker from the U.S from this time (Burchsted and Burchsted, 2007).

The description of the voyage itself is very entertaining. While Stimpson was frequently engaged in collecting specimens, the travelers also got themselves into a fair amount of mischief. The descriptions of the fishing are breathtaking. The abundance of marine life in New England at that time puts the relatively sorry state of the oceans today into sharp perspective.

This book is available in a variety of forms. An annotated print version exists and older, out of print editions can be had on Amazon. However, scans of the original are available as a free PDF via Google Books.
https://books.google.com/books/about/A_Summer_Cruise_on_the_Coast_of_New_Engl.html?id=jL5HAAAAIAAJ

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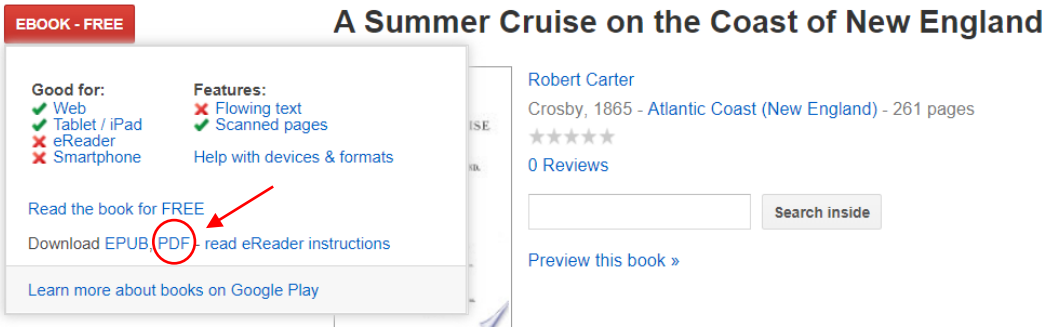
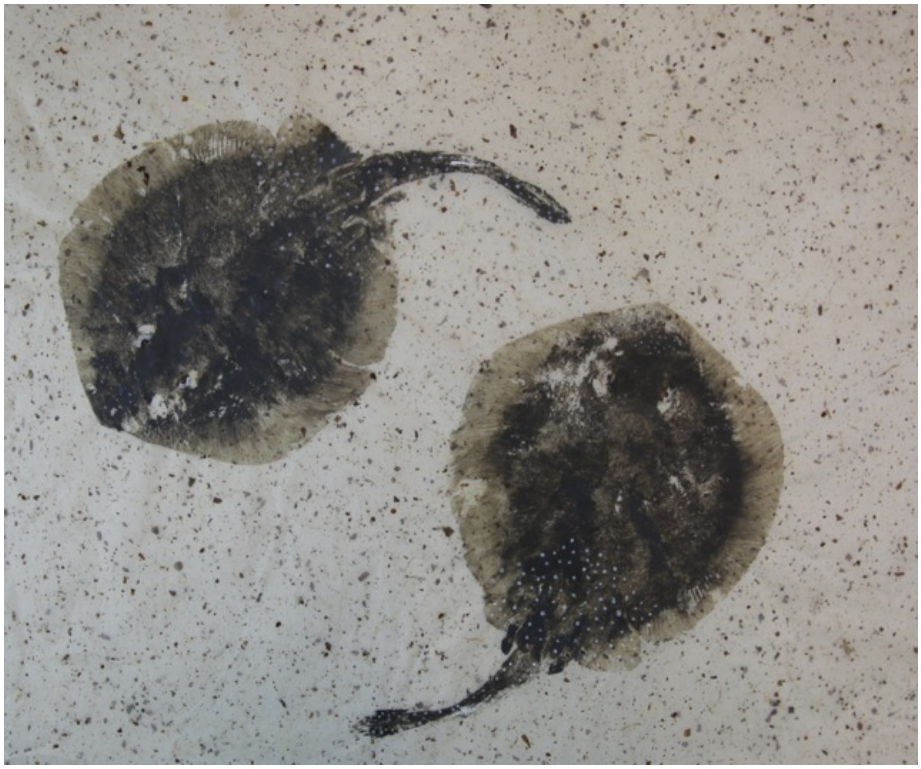


References

Anon. 1858. Book Reviews: The Aquarium, an Unveiling of the Wonders of the Deep Sea by Philip Henry Gosse, and The Book of the Aquarium and Water Cabinet by Shirley Hibberd. The North American Review. 87(180): 143- 157. University of Northern Iowa. https://www.jstor.org/stable/25107302

Burchsted, J., & F. Burchsted 2007. Samuel Tufts, Jr. (1817–1902), a Massachusetts shell collector and aquarium stocker. Archives of Natural History. 34(2): 229–234. 2007.

Vasile, R. S. 2018. William Stimpson and the Golden Age of American Natural History. Northern Illinois University Press, Dekalb 60115. 300p.



Round Stingrays. Bruce Koike.

CASE REPORT OF THE LEECH *Zeylanicobdella arugamensis* IN A PUBLIC AQUARIUM SETTING, WITH A REVIEW OF MANAGEMENT OPTIONS

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Abstract

Zeylanicobdella arugamensis is a leech from the Indo-Pacific and Indian Ocean regions, and a known parasite of marine and estuarine cultured fishes. A brief literature review and history of this species is given, before examining a case of an infestation occurring in a public aquarium setting with a brackish water species, the mudskipper *Periophthalmus argentilineatus*. Currently accepted and potential treatment methods are explored in the context of the aquarium environment. Finally, this paper presents a new host record for *Z. arugamensis* on a member of the family Eleotridae.

Introduction

Zeylanicobdella arugamensis de Silva 1963 is a piscicolid leech known to parasitize numerous species of marine and brackish-water fishes throughout the Indo-Pacific through the coastal Indian Ocean region down to south-western Africa (Hayes et al., 2006; Nagasawa et al. 2012). *Z. arugamensis* is not host-specific, and although it was originally thought to only be a parasite to estuarine fishes, it has since been found to also parasitize several coastal marine species (Nagasawa et al., 2012).

While *Z. arugamensis* is considered an important parasite in aquaculture (Seng et al., 2006), there has been little documented evidence of its occurrence in private or public aquarium environments. As such, it is unknown if the control and treatment measures developed in aquaculture are suitable for application in an aquarium.

This paper reviews the current knowledge of *Z. arugamensis*, the treatment protocols applied in aquaculture, their suitability for aquarium use, and overviews a case study of infestation in a public aquarium display.

External Morphology

Adults bear a classic leech morphology, bearing both an anterior and posterior sucker, a cylindrical and elastic body, and a moderately widened urosome. *Zeylanicobdella arugamensis* can be externally differentiated from other leeches by a pair of pigmented "eye spots" on the anterior sucker, the posterior sucker being much larger than the anterior sucker, and a smooth body lacking papillae, tubercles, or vesicles (Sawyer et al., 1982); internally this species possesses 5 pairs of testes and an ovary, being hermaphroditic (de Silva, 1963).

Adult leeches may reach 19.8 mm in length (Nagasawa and Uyeno, 2009), with the most common size appearing to be approximately 10 to 15 mm (Cruz-Lacierda et al., 2000). Maturity

and reproduction may occur in specimens as small as 10mm (Mahardika et al., 2018). This species bears morphological similarity to another piscicolid *Ottoniobdella stellata* Moore 1958 (syn. *Janusion stellata*) of the east coast of South Africa, and has been suggested to be synonymous with the latter by several authors (E. Burreson, unpublished obs.; Hayward, 1997).

Figure 1. 40x magnification of *Zeylanicobdella arugamensis*, ventral surface presented.

Life Cycle and Occurrences in Nature

Zeylanicobdella arugamensis has been recorded to occur in coastal marine waters and estuaries in 12 countries to date: Australia, Borneo, India, Indonesia, Iran, Japan, Malaysia, Philippines, Singapore, South Africa, Sri Lanka, and Thailand. Little is known of their ecology and host-parasite interactions in nature, although wild fishes discovered hosting *Z. arugamensis* typically display low prevalence (≤ 25%) and low parasite loads (≤ 7 per fish) (Polgar et al., 2009; Rueckert et al., 2009; Hayes et al., 2014). There have been no recorded large mortality events associated with this parasite in nature.

Life cycle

The life cycle of *Z. arugamensis* can be completed in as little as 16 days, although this process may take as long as 21 days in less ideal conditions (Kua et al., 2010; Mahardika et al., 2018). Temperature and salinity appear to be the most important environmental factors. Once mature, individuals may go through several egg-laying periods, although some adults die after cocoon deposition (Cruz-Lacierda and Erazo-Pagador, 2004).

Cocoons containing a single egg are laid; each are approximately 0.5 mm diameter are typically laid on hard attachment sites such as rocks or tank walls (Kua et al., 2010; Cruz-Lacierda and Erazo-Pagador, 2004). Up to 49 eggs may be laid by an individual per day (mean 12 per individual per day), with egg laying taking place continuously for up to 3 days (Kua et al., 2010; Mahardika et al., 2018). Eggs may hatch in 7 to 12 days, emerging as at approximately 0.1 to 0.5 mm in length and transparent (Kua et al., 2010; Murwantoko et al., 2017; Mahardika et al., 2018).

Leeches may begin feeding on fish hosts between 4 and 6 days after hatching, although this may be difficult to see owing to their size and transparency (Mahardika et al., 2018). Reproduction occurs at approximately 7 mm in length and 9 to 11 days post-hatch (Mahardika et al., 2018). *Z. arugamensis* are hermaphroditic, and can couple with any partner; additionally, the life cycle can be completed through self-fertilization (Kua et al., 2010), thereby requiring extensive treatment protocols to completely eliminate the parasite.

History in Captivity

The first verifiable record of this species parasitizing aquacultured species was in the Philippines, hosted by cultured grouper *Epinephelus coioides* (de Silva, 1963), although there had been earlier records of an unknown marine leech in Malaysia that have since been attributed to *Z. arugamensis* infestations (see Leong and Wong, 1988).

This species has become an increasingly important parasite of Asian and Australian cage-cultured species with prevalence being as high as 80 to 100% on some farms (Seng et al., 2006, Kua et al. 2014). Such infestations result in economic losses attributable to fish deaths, poor growth rates, and poor appearance of affected individual fishes (Murwantoko et al., 2017).

The only literature references of *Z. arugamensis* occurring in an aquarium environment are those brought into an aquarium specifically for study purposes (e.g. Polgar et al., 2009; Kua et al., 2014). It is not currently acknowledged as an aquarium problem, typically being associated with wild, wild-caught, or pond raised fishes.

Symptoms and Host Preferences

Attachment sites are usually externally based on the skin and fins, although leeches may be found attached to the gill arches or within the oral cavity (Nagasawa & Uyeno, 2009). The leech is known to parasitize all life stages of fish (Seng et al., 2006). During initial stages of hosting the leech, there are no immediately apparent symptoms displayed by the host. Increased parasite density, especially over a prolonged period, appear to be associated with a decrease in appetite and activity, localized swelling at attachment sites, as well as overall darkened pigmentation (Cruz-Lacierda and Erazo-Pagador, 2004; Seng et al., 2006; Kua et al., 2010). Fraying of the fins, fin rot, and scale drop are known to occur with high parasite loads over long periods (Kua et al., 2010; Kua et al., 2014), with mortality typically being associated with blood loss in heavily infested hosts (Cruz-Lacierda et al., 2000).

Z. arugamensis has been known to be a vector of numerous pathogens in South African waters, included among them *Haemogregarina curvata* and several trypanosoma (Hayes et al., 2006) as well as being a suspected transmitter of *Vibrio algniolyticus* bacteria (Kua et al., 2006). Secondary pathogens may subsequently take hold in heavily parasitized fishes, especially around

the attachment sites of the leeches (Kua, 2008), which in turn may result in mortality (Kua et al., 2014). The mortality rate in an untreated system can be as high as 60% (Kua et al. 2006),

Zeylanicobdella arugamensis has so far proven to be generalist regarding host choice, although papers by Nagasawa and Uyeno (2009) and Nagasawa et al. (2012) summarize the 24 known host fish species from 16 families. The case study detailed in this paper adds one additional host species and family: *Eleotris fusca* (Forster, 1801), Eleotridae, Gobiiformes.

Figure 2. Deceased specimen of *Periophthalmus argentilineatus* 90mm TL, with *Zeylanicobdella arugamensis* adults continuing to feed. Note the frayed fins and abundance of leeches on the ventral surface.

Review of Control and Treatment Methods

A number of control and treatment protocols have been explored for use within the aquaculture sector, however, few have seen application. The majority of treatment protocols focus on bath treatments for individuals or groups of fishes, rather than system-wide treatment. This has arisen due to the extensive use of cage culture in the regions where *Z. arugamensis* is most prolific; whole system treatments are not practicable.

No studies have explored the efficacy of chemical treatments on the leech eggs or cocoons, which are essential to remove to break the life cycle of the parasite in closed and semi-open systems. While many aquatic leech species' eggs are known to be resistant to chemical agents (Burreson, 1995), the negative effect of freshwater treatment on egg hatching rates (Kua et al., 2014) indicates that the egg stage of *Z. arugamensis* may be vulnerable to environmental and chemical treatments.

Manual removal techniques are a quick and cheap method of removal adult and juvenile leeches from the body surface of affected individuals. *Z. arugamensis* have proven easy to remove from the host body with little resistance displayed by the parasite. Manual removal by tweezer (case study, Figure 3) proves effective for smaller-sized fishes. Cruz-Lacierda and Erazo-Pagador, (2004) suggest removal of leeches from the body surface by gentle wiping across the affected area with a wet cloth; this method risks abrasion of the specimen, and damage to the mucous layer.

Handling stress needs to be considered for both these methods. If the treated animal is placed back into the same environment, re-infestation is highly probable in a short period of time. However, manual removal may be necessary in cases of extremely high parasite loads where secondary issues may present without such intervention.

Figure 3. Ventral view of a live *Periophthalmus argentilineatus* with a low parasite load; leeches can easily be removed by gentle handling of the animal and steady work with tweezers.

Varying dosages have been cited for formalin use in the control and treatment of this leech, with doses given between 50 to 250 ppm applied as a bath for 1 hour (Cruz-Lacierda et al., 2000; Cruz-Lacierda, 2001; Cruz-Lacierda and Erazo-Pagador, 2004; Hutson and Cain, 2019). Given the toxicity of this chemical and its ability to reduce dissolved oxygen, caution is urged and treatments should begin toward the lower dosage at 50 ppm when applied alongside strong aeration, with medical grade oxygen provision as a preference.

Murwantoko et al. (2017) trialled several chemical and pharmaceutical agents in their efficacy against *Z. arugamensis* infestation. Ivermectin, copper sulphate, and levamisole were identified as suitable candidates for bath-type treatments. However, this study was performed without fish subjects, and the minimal effective doses cited in this research (10 mg per litre ivermectin, 10 mg per litre copper sulphate, and 62 mg per litre levamisole for 1.5 to 3 hours) exceed safe doses recommended for fish, and as such cannot be recommended in practice. Further study of these chemicals is warranted.

Safe dosages for each of these therapeutics are orders of magnitude lower than recommended by Murwantoko et al. (2017); the only clinical recommendation in literature that is similar is that indicated by Harms (1996) for levamisole, at a dose of 50 mg per litre for 2 hours as a bath treatment for external parasites.

Other chemical therapy options for leech infestations typically employed in the aquaculture sector have apparently not yet been trialled for *Z. arugamensis*, although many of these have raised environmental concerns and have legal limitations on their usage (e.g.: many organophosphates; Noga 2010).

The extract of the plant *Dillenia suffruticosa* has been identified for having antiparasitic properties in effecting a 100% mortality of the parasite when trialled with *Z. arugamensis* (Shah et al., 2020). Although a number of other plant extracts have been successfully utilized for other species of leech and their infestations (see Bahmani et al. 2011, Gholami-Ahangaran et al., 2012; Rizky et al., 2018), these have not been tested with *Z. arugamensis*.

Environmental Manipulation - Salinity and Temperature

Salinity Variation

The environmental salinity range of *Z. arugamensis* is best described as high-end estuarine to marine and tropical to subtropical. This would indicate some degree of tolerance to environmental variation, in particular changes to salinity regimes.

Hyposalinity treatment affect hatching success of cocoons, down to about 12% success at 10 ppt, with no successful hatches at 0 ppt (Kua et al., 2014). Exposure to freshwater (0 ppt) for between 1 to 3 hours is sufficient to effect mortality in adult leeches (Cruz-Lacierda et al., 2000; Kua et al., 2014; Murwantoko et al., 2017). However, hyposalinity treatments (between 10 and 20 ppt) needed to be maintained for between 4 to 7 days to eliminate all adults and juveniles. It is evident from these results that hyposalinity may be an effective control method, while flushing the system with freshwater over one or more shorter periods would be effective for elimination.

Hypersalinity treatments may not be tolerated well by many host fish species (Gonzalez, 2012). Although cocoon hatch rate was reduced to 0% at 40 ppt, adults and juveniles were capable of surviving at this increased salinity for 4 to 7 days (Kua et al., 2014).

Temperature Treatments

The use of increased temperatures to control or eliminate *Z. arugamensis* have not proven as an effective method to date. Temperatures in excess of 35°C can be tolerated for up to 5 days by juveniles and adults, and cocoon hatch rate was noted to only decrease to 13.3% under such conditions (Kua et al., 2014). Such high temperatures may act as a stressor on the host fish species (Roessig et al., 2004), with the life cycle is completed in a shorter period of time at higher temperatures (Kua et al., 2014), thereby introducing the risk of facilitating a more rapid increase in parasite numbers.

System Disinfection and Biocontrol

Complete disinfection protocols usually involve removing the fish from the system and allowing all equipment to air-dry with exposure to sunlight (Hutson & Cain, 2019); cage culturists are advised to dry out the cages on rotation every 9 to 10 days to break the life cycle (Kua et al., 2010). Alternatively, the system may be disinfected with chlorine (Cruz-Lacierda and Erazo-Pagador, 2004) in order to eliminate remaining adults and cocoons.

Cleaner organisms have been suggested as a possible control measure (Hutson and Cain, 2019), with noted successful candidates identified in a study by Vaughan et al. (2018), particularly the cleaner shrimp *Lysmata vittata* in being effective in eliminating both adults and cocoons from controlled environments.

Figure 4. The barred mudskipper *Periophthalmus argentilineatus* on display; note the adult *Zeylanicobdella arugamensis* attached to the base of the first dorsal fin

Case Report - Hyposalinity and Hypersalinity with Manual Removal

In February 2016, a dedicated mangrove and mudskipper display enclosure was opened at uShaka Sea World, Durban, South Africa. The focal species of the display was the barred mudskipper *Periophthalmus argentilineatus*, which were acquired locally from the nearby estuarine system of the Umgeni River (29°48'36"S 31°02'07"E). About two months after introduction to the exhibit, the specimens were noted to be carrying a number of leeches on their body surface. Despite intervention wherein the leeches were manually removed, more leeches were subsequently discovered parasitizing the mudskipper specimens again approximately 10 days later. It was decided to attempt hyposalinity and hypersalinity exposure treatments in lieu of chemical treatments owing to the presence of plants and invertebrates sharing the display exhibit with the affected specimens.

Materials and Methods

Data were recorded on the daily monitoring sheets and checklists regarding animal health, leech presence and removal, treatments, and environmental fluctuations applied to the tank. Data were extracted from these monitoring sheets from the day that the mudskippers *P. argentilineatus* were added to the exhibit, 22 February 2016, until the conclusion of this case study 2 February

2018. Samples of the parasite were collected from host fishes and either preserved in buffered formalin or immediately prepared on slides for squash mounts.

Diagnosis, cControl and Treatment Attempts

The parasite was identified as *Z. arugamensis* by morphology as outlined by keys and descriptions in Sawyer et al. (1982) and Nagasawa & Uyeno (2009). Prevalence was as high as 100% outside of treatment periods, with as many as 40 leeches being removed from any single specimen during a single session of manual leech removal. Although *P. argentilineatus* was the focal species of this display during the time period of this case study, at least one other fish species, a goby *Eleotris fusca*, shared the exhibit with the mudskippers. Only a single leech was found attached to *E. fusca* during the study period. Not only is this a new host record of *Z. arugamensis* (as well as the first known instance of *Z. arugamensis* parasitizing a member of the family Eleotridae), but this also contrasts Polgar et al.'s (2009) observation that this species of leech appeared to have a natural preference for fully aquatic species rather than semi-aquatic species.

Leeches were found to non-specific with regards to attachment site; however, the ventral surface of the mudskipper presented the greatest parasite loads. The greater parasite load on the ventral surface may be due to the adult *Z. arugamensis* preference to take refuge within the substratum during egg laying (as observed by Murwantoko et al., 2017). As per the observations of Polgar et al. (2009), the leeches did not appear to detach once the mudskippers left the water. High humidity in the terrestrial portion of the exhibit likely prevented the parasites from drying out once the mudskippers left the water.

A decreased appetite was noticed at mild parasite loads. Frayed fins were only present in severely parasitized or deceased specimens. Haemorrhaging and swelling at attachment sites was not observed. In the confines of an aquarium environment, parasite numbers increased rapidly in a short space of time - even overnight - without the opportunity for the host to escape.

Some specimens succumbed to the leeches (Figure 2); the leeches did not detach from the host for some time after death (±1 hour). This may be because, despite blood coagulation that occurs after death, the anticoagulants utilized by the parasites ensure that a dead host can provide a viable blood meal for a short time period.

The first treatment that was attempted was the manual removal of the leeches from the host body. This was achieved by catching and handling the host fish and removing the parasites from its body using a pair of tweezers. This initial technique was employed in lieu of chemical or environmental treatments as it is a simple procedure with minimal risk to the host animal or the invertebrates and plants sharing the enclosure, although the excessive handling stress and potential for handling damage were cause for concern. This method is effective in regularly removing up to 100% of the leeches on a particular specimen, however, re-infestation was rapid and therefore required regular application. This method does not address leeches or eggs in the tank environment.

The enclosure has incoming water from two sources, namely one of each of freshwater and marine inlets. In order to flush a system with one of these two sources, one of the inlets is closed off entirely. This allows the opposing incoming supply to dominate the make-up of the water,

slowly changing the salinity of the exhibit water to match that of the sole incoming supply. Flushing of the enclosure with one of the water sources was done for one full day, before opening the other incoming water source and allowing the system to return to its previous salinity.

Flushing treatments were exercised once per week for a period of 3 weeks. Flushing was not performed for more than a day at a time (9 hours maximum flushing time); despite their euryhaline tolerances, this was done to prevent exposing the other exhibit inhabitants to unsuitable conditions for extended periods. Flushing protocols with either fresh or marine water were used concurrently with the manual removal technique (outlined above).

After flushing the exhibit with freshwater (< 0.5ppt) once per week for a period of 3 weeks, prevalence and individual parasite load was reduced. However, after approximately 4 weeks (25 days) without the freshwater flushing treatment, the leeches began to reappear. Conversely, when the exhibit was flushed with marine water (> 30 ppt), re-infestation took approximately 5 days to occur. These contrasting effects were mostly consistent between the freshwater and marine flush treatments, with freshwater flushing being the most effective control method between the two of them. During flushing treatments - both freshwater and marine - the maximum number of leeches manually removed from specimens was 1 leech per host per day.

Time to re-infestation after treatments varied depending on treatment method applied and environmental factors. Manual removal saw the most rapid return of parasites to the host in as little as 1 day to as long as 2 days; conversely, freshwater or marine flushing treatments only saw re-infestation after approximately 25 and 5 days, respectively.

Discussion of Case Study

It is apparent that the freshwater flushing treatment was more effective than the saltwater flushing treatment, as demonstrated by the time taken to re-infestation. This appears to be in line with the experiments conducted by Kua et al. (2014), in which *Z. arugamensis* had a low tolerance for freshwater.

The recurrence of the leeches after treatment is likely a reflection of ineffective treatment methodology and renewal of the leech life cycle. The flushing treatments, as applied in this case, were only effected for a full working day at a time (approximately 9 hours). This is sufficient time to eliminate juveniles and adults (maximum 3 hours as per Kua et al., 2014), however may not affect the cocoon egg stages. Any cocoons that remained after treatment may have still been viable to hatch, and as such re-infestation was seen within 25 days - marginally longer than the time period taken to hatch and begin searching for the first blood meal.

Another factor affecting effectiveness may have been the aquarium environment, in particular the substratum acting as an environmental refuge. The sediment used on exhibit has a high density and therefore low water permeability. This may allow the juvenile and adult *Z. arugamensis* to take refuge in a more suitable environment until such time that favourable conditions return - leeches can wait weeks between meals owing to high feeding volume and slow digestion (Hutson & Cain, 2019). Cocoons laid in the substratum would also be afforded such protection. If so, such life stages may be unaffected by treatment and control protocols applied to the water environment (Burreson, 1995).

This is not the first observation of a leech parasitizing an amphibious fish host (see Polgar et al., 2009). The utilization of the host in this case study was likely opportunistic, as mudskippers are known to expose themselves to a range of extreme environmental conditions to which parasites might struggle to adapt. However, in the limited space of an aquarium environment, and a high humidity terrestrial zone, *Z. arugamensis* could utilize an amphibious resource. Fully aquatic fish species sharing the environment were also parasitized, although to a lesser degree.

Figure 5. Several adult *Zeylanicobdella arugamensis* bundled together on the ventral surface of a mudskipper; note the presence of both light and dark individuals, displaying the variation of pigmentation in this species.

Conclusions

Z. arugamensis has proven to be a persistent parasite to deal with in a display enclosure, although as outlined in this paper simple control methods may be implemented to keep parasite loads minimal. However, complete elimination of all life stages requires prolonged and/or repeated treatments, as the various life stages of this parasite are differently affected by control and treatment methods. At least part of the population may take refuge in the substratum during egg laying or development stages, allowing them to escape treatment effects that are applied over shorter periods.

It is apparent from this case that while the exhibit is being flushed, parasite abundance on the mudskippers decreases dramatically. This may be explained by the fact that (1) both adults and juveniles are intolerant of the new environmental conditions and perish with a relatively short period, and (2) some adults and juveniles may behaviourally seek refuge in the substratum to avoid the negative effects of such environmental change.

The use of environmental manipulation, as demonstrated in this case, is of great use in controlling an infestation of *Z. arugamensis* in a display enclosure. However, complete elimination may not be entirely feasible utilizing this technique. A number of chemical treatments have proven effective in both the control and treatment of this parasite (e.g.: formalin). Chemical treatment was not opted for in this instance, however, over concerns of the effects on the cohabiting live plants and invertebrates.

As eggs may not be affected by treatments, it is recommended that any treatment protocol be repeated at least twice at 10-day intervals to ensure that the adults and subsequent hatched (non-reproductive) juveniles are exposed to treatment protocols.

Given that *Z. arugamensis* is known to be non-host-specific, can self-fertilize, and is tolerant of a wide range of environmental conditions, extreme caution is advised to ensure that strict biosecurity protocols are in place to prevent accidental introduction to other systems.

References

Bahmani, M., J. Abbasi, A. Mohsenzadegan, S. Sadeghian, and M. G. Ahangaran. 2011. *Allium sativum* L.: the anti-immature leech (*Limnatis nilotica*) activity compared to niclosomide. Comparative Clinical Pathology. 22: 1380-1387.

Burreson, E. M. 1995. Phylum Annelida: Hirudinea as vectors and disease agents. *In:* Fish Diseases and Disorders: Vol. 1. Protozoan and Metazoan Infections. Woo, P. T. .K (Ed.). CAB International, UK, pp. 599-629.

Cruz-Lacierda, E. R. 2001. Parasitic diseases and pests. *In:* Health Management in Aquaculture. Lio-Po G. D., C. R. Lavilla, & E. R. Cruz-Lacierda (Eds.), Tigbauan, Iloilo, Philippines: Aquaculture Department, Southeast Asian Fisheries Development Center.

Cruz-Lacierda, E. R., J. D. Toledo, J. D. Tan-Fermin, and E. M. Burreson. 2000. Marine leech (*Zeylanicobdella arugamensis*) infestation on cultured orange-spotted grouper, *Epinephelus coiodes*. Aquaculture. 185: 191-196.

Cruz-Lacierda, E. R., and G. E. Erazo-Pagador. 2004. Parasitic diseases. *In:* Diseases of cultured groupers. Nagasawa K., and E. R. Cruz-Lacierda (Eds.). p. 33-57). Tigbauan, Iloilo, Philippines: Aquaculture Department, Southeast Asian Fisheries Development Center.

de Silva, P. 1963. *Zeylanicobdella arugamensis* gen. nov. and sp. nov. from Arugam Kalapu, Eastern Province, Ceylon. Spolia Zeylanica. 30(1): 47-53.

Gholami-Ahangaran, M., M. Bahmani, and N. Zia-Jahromi. 2012. Comparing of anti-leech effect of *Vitis vinifera* L. plant and niclosamide on different forms of *Limnatis nilotica*. World Applied Science Journals. 20: 1,082-1,085.

Gonzalez, R. J. 2012. The physiology of hyper-salinity tolerance in teleost fish: a review. Journal of Comparative Physiology B. 182: 321-329.

Harms, C. A. 1996. Treatments for parasitic diseases of aquarium and ornamental fish. Seminars in Avian and Exotic Pet Medicine. 5(2): 54-63.

Hayes, P. M., N. J. Smit, A. M. Seddon, D. F. Wertheim, and A. J. Davies. 2006. A new fish haemogregarine from South Africa and its suspected dual transmission with trypanosomes by a marine leech. Acta Parasitologica. 53: 241-248.

Hayward, C. J. 1997. Helminth ectoparasites of sillaginid fishes (Perciformes: Percoidei) have low species richness. Folia Parasitologica. 44: 173-187.

Hutson, K. S. and K. D. Cain. 2019. Pathogens and parasites. *In:* Aquaculture: Farming Aquatic Animals and Plants (3rd ed.). Lucas, J. S., P. C. Southgate, and C. S. Tucker (Eds.). John Wiley & Sons Ltd. p 217-246.

Kua, B. C., S. Z. Abdullah, M. F. Abtholuddin, N. F. Mohd, and N. N. Mansor. 2006. Marine leech isolated from cage-cultured sea bass *(Lates calcarifer)* fingerlings: a parasite or vector? Abstract in Programme Book of the 15th Scientific Conference Proceeding. 4–6th December 2006, Terengganu.

Kua, B. C., M. A. Azmi, and N. K. A. Hamid. 2010. Life cycle of the marine leech (*Zeylanicobdella arugamensis*) isolated from sea bass (*Lates calcarifer*) under laboratory conditions. Aquaculture. 302: 153-157.

Kua, B. C., F. C. Choong, and Y. Y. Leaw. 2014. Effect of salinity and temperature on marine leech, *Zeylanicobdella arugamensis* (de Silva) under laboratory conditions. Journal of Fish Diseases. 37: 201-207.

Leong, T. S. and S. Y. Wong. 1988. A comparative study of the parasite fauna of wild and cultured grouper (*Epinephelus malabaricus* Bloch and Schneider) in Malaysia. Aquaculture. 68: 203-207.

Mahardika, K., I. Mastuti, S. Sudewi, and Z. Zafran. 2018. Identification and life cycle of marine leech isolated from cultured hybrid grouper in the northern Bali waters of Indonesia. Indonesian Aquaculture Journal. 13(1): 41-49.

Murwantoko, M., S. L. Condro, A. Isnansetyo, and Z. Zafran. 2017. Life cycle of marine leech from cultured "cantik" hybrid grouper (*Epinephelus* sp.) and their susceptibility against chemicals. Aquacultura Indonesia. 18(2): 72-76.

Nagasawa, K., and D. Uyeno. 2009. *Zeylanicobdella arugamensis* (Hirudinida, Piscicolidae), a leech infesting brackish-water fishes, new to Japan. Biogeography. 11: 125-130.

Nagasawa, K., N. Shimadzu, and Y. Ikeda. 2012. Four new host records for the fish leech *Zeylanicobdella arugamensis* (Hirudinida, Piscicolidae), with an updated host list (1963-2012). Biogeography. 14: 143-146.

Noga, E. J. 2010. Fish Disease: Diagnosis and Treatment. John Wiley & Sons, Ames, Iowa.

Polgar, G., E. M. Burreson, F. Stefani, and E. Kamrani. 2009. Leeches on mudskippers: host–parasite interaction at the water's edge. Journal of Parasitology. 95(4): 1,021-1,025.

Rizky, P. N., T. C. Cheng, and H. Nursyam. 2018. Anti-parasite activity of *Scutellaria baicalensis* and *Morinda citrifolia* extracts against *Piscicola geometra*. Earth Environmental Science. 137: 01203.

Roessig, J. M., C. M. Woodley, J. J. Cech, and L. J. Hansen. 2004. Effects of global climate change on marine and estuarine fishes and fisheries. Reviews in Fish Biology and Fisheries. 14: 251-275.

Rueckert, S, W. Hagen, A. T. Yuniar, and H. W. Palm. 2009. Metazoan fish parasites of Segara Anakan Lagoon, Indonesia, and their potential use as biological indicators. Regional Environmental Change. 9: 315-328.

Sawyer, R. T., A. Taylor, and M. J. H. Sahat. 1982. The leeches of Brunei (Annelida: Hirudinea) with a checklist and key to the known and expected freshwater, terrestrial and marine leeches of Borneo. Journal of the Brunei Museum. 5: 168-201

Seng, L. T., Z. Tan, and W. J. Enright. 2006. Important parasitic diseases in cultured marine fish in the Asia-Pacific region. AQUA Culture Asia Pacific Magazine. 2(1): 14-16.

Shah, M. D., B. A. V. Maran, M. Iqbal, F. F. Ching, M. T. M. Lal, R. B. Othman, and R. Shapawi. 2020. Antiparasitic activity of the medicinal plant *Dillenia suffruticosa* against the marine leech *Zeylanicobdella arugamensis* (Hirudinea) and its phytochemical composition. Aquaculture Research. 51(1): 215-221.

Vaughan, D. B., A. S. Grutter, and K. S. Hutson. 2018. Cleaner shrimp are a sustainable option to treat parasitic disease in farmed fish. Scientific Reports. 8: 13,959.

Rough Pomfret. Bruce Koike.

Stegostoma fasciatum BREEDING PROGRAM AT LORO PARQUE AQUARIUM

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Abstract

Loro Parque has had a successful zebra shark, *Stegostoma fasciatum* (Hermann, 1783), breeding program for five years. Four trained adults are held at the facility and simulated seasonal implantation has resulted in reproductive behaviours. Pre-copulation often resulted in copulation and 18 newborns were hatched in this five-year period time. For the newborns the mean incubation time was 145±10 days (n=18). Neonates were measured at birth. Mean total length (TL) was 26.8±0.5 cm (n=18) in the range of 29 to 24.5 cm TL. Mean weight was 74.1±4.9 gm (n=18) in the range of 90 to 62 gm. Differences, in size or weight, between females and males were not appreciable.

Introduction

The zebra shark, *Stegostoma fasciatum*, (Hermann, 1783) is a benthic shallow coastal shark that can be found in the Indo-West Pacific (Compagno, 2002). It's relatively common in aquariums and has had many reproductive successes all over the world.

Stegostoma fasciatum presents a cylindrical body with a central ridge on which we can find two dorsal fins. Laterally they have pectoral fins, and the body ends with a caudal fin that equals nearly the half of the body (Compagno, 2002). As an oviparous species they lay eggs with dark cases and large lateral tufts of fine hair that help them attach to substrate.

The International Union of Conservation of Nature (IUCN) classified *Stegostoma fasciatum* as a "vulnerable" species for the declining population trend. This makes studies and reproduction in zoological facilities so important.

Exhibition

Four adult *Stegostoma fasciatum* can be found at Loro Parque facilities. The exhibit that holds the adult zebra sharks is an 800,000 m^3 tank. It has an irregular shaped periphery and a regular depth of 4 meters. The tank also presents an acrylic tunnel running down the middle that crosses the floor. The walls are covered with many artificial corals.

It's a "closed" system but due to auxiliary cubes that gain water from the system and a routine water change (20% three times a week) it's almost an "open" system in terms of water quality (Mohan 2004). Salinity remains constant all the year around at 32 gm/L.

Temperature is a little bit more complex. Before 2014 the temperature of the water remained constant at 22ºC. In 2014 we decided to establish a breeding program. The development of simulated seasons, winter and summer, would theoretically start to activate the natural cycles

of the species. We started to gradually increase the temperature in summer up to 26ºC and decreased it in winter down to 23ºC.

Illumination follows the same logic as temperature. In 2015 we introduced the seasonal light program. As we got closer to summer the number of light hours were increased up to 16 hours and when we were getting closer to winter the number of light hours were decreased to 14 hours.

The collection within the tank contains four zebra sharks (*Stegostoma fasciatum*), three nurse sharks (*Ginglymostoma cirratum*), five sandbar grey sharks (*Carcharhinus plumbeus*), two reticulate whiprays (*Himantura uarnak*), two ocellated eagle rays (*Aetobatus ocellatus*), many southern stingrays (*Hypanus americanus*) and a plentiful variety of Indo-Pacific open seas fishes and Indo-Pacific and Caribbean reef fishes.

Parents

The four adult zebra sharks consist of two females and two males (2.2.0). Individuals were not introduced together in the tank. Females were introduced in 2009 and 2014. Males were introduced in 2000 and 2006. The average total length (TL) of the females is 199.5 cm TL while for the males it is 212.5 cm TL. The average body mass (BM) of the females is 36 kg BM while for the males it is 35 kg BM. Both averages compare to the sexual maturity range (Compagno, 2002).

Diet ration remains 7.5% body weight (BW) per week. Adults were fed 2.5% BW per day 3 times a week. Diet was based on a variety of white* fish, cephalopods and blue* fish. Vitamin supplement intake was at the rate of 0.1 pill for each kg of food (Aquavit®, International Zoo Veterinary Group, EU). **Editor's note: "white fish" include gadids, pleuronectiforms, monkfish, etc., while "Blue fish" include "oily" species such as anchovy, clupeids, scombrids, salmon, etc.*

We use target training to monitor individuals. Training allows us to maintain constant checks on individuals and assure their food and vitamin consumption. A hammock is used as a target for training and is placed at the surface of the tank. The individual enters the hammock, stops on their own in the middle of it, waits for the trainer to start the exercise and, with the help of the trainer, turns upside down (abdomen looking up) with the objective of achieving tonic immobility (TI). This TI can be negative, with different gradients from very stressed to very relaxed, or positive. After the exercise, they returned to their normal position, waited for food and were fed as a positive reinforcement. Each individual is in a different phase of training. This behavioural chain has let us obtain voluntary daily animal checks, numerous voluntary blood samples, biometrics, etc.

Copulation

One year after we started setting up the artificial seasons, in 2015, pre-copulatory behaviour began. Males follow females all over the tank and bite their caudal and pectoral fins. Two things could happen in those cases; females kept swimming away or laid down in the bottom with the male attached.

It was also observed many times that one of the males chased and bit the other male. This conduct is considered to be related to territorial behaviour and it is only noticed during the copulatory season. Nevertheless, this kind of conduct has never resulted in serious injuries and separating the individuals has not been necessary.

In best case scenarios pre-copulation was followed by copulation behaviour. Pre-copulation started in January and continued within the copulation range of time. Copulation occurred from February to June, with April being the month with the highest copulation proportion. During copulation the male, after biting the female, rotates over the body of the female and inserts one of his claspers into her cloaca. Copulation lasts from 1 to 4 minutes and afterwards they separate.

Sometimes one of the males inflated his abdomen before going after the female. And many times, the male remained with his abdomen inflated for several hours. It is believed that this type of behaviour is related to copulation (Watson, 2017).

Egg-laying

Egg-laying occurred from March to September, with May being the month with the highest egg-laying proportion. In the five breeding seasons females laid a total of 237 eggs. In 2016 they laid 36 (7 fertile), in 2017 they laid 41 (5 fertile), in 2018 they laid 38 (8 fertile), in 2019 they laid 54 (11 fertile) and in 2020 they laid 68 (13 fertile). We also must consider that some eggs were probably lost due to interaction with other fishes of the exhibition (Table 1).

When the egg-laying season starts, tufts begin to appear from the cloaca of the female. Then the female started to swim around structures with the objective of getting the tuft attached so the egg could be pulled out from the oviduct. Females laid around 2 - 6 eggs per day during a 6-day period and then stopped for approximately two weeks before starting again.

Table 1. Data obtained from the egg-laying, incubation and hatching of *Stegostoma fasciatum* (zebra sharks) at the Aquarium of Loro Parque. Collects data from the biometrics obtained at birth time.

Year	Nº of eggs	Nº of eggs with embryo	Nº of newborns	Neonate gender (m.f.u)	Mean incubation time (days)	Mean neonate TL (cm)	Mean neonate weight (gm)
2016	36	7	2	1.1.0	152±15	25.9±1.4	69.5±2.5
2017	41	5	3	1.2.0	136±3	27.3±1.2	79.3±7.5
2018	38	8	0	-	-	-	-
2019	54	11	4	4.0.0	137±2	27.4±1.6	78.5±11.5
2020	68	13	9	2.7.0	155±8	26.6±0.9	69.1±4.9
Total	**237**	**44**	**18**	**8.10.0**	**145±10**	**26.8±0.5**	**74.1±4.9**

Incubation

As soon as the eggs were noticed they were removed from the exhibition and moved to an incubation tank in quarantine. Eggs were acclimatized from the source water to the destination water, and were never exposed to air.

Once in the incubation tank, egg tufts were removed in order to have better control of the embryo development and to prevent neonates from getting tangled or swallowing them. The eggs were held mid-water in the tank, so they received adequate water flow that is essential for the correct development of the embryo. At first the yolk was checked every 15 days to monitor the development of the egg. After the first month the yolk was checked every 7 days. Infertile or damaged eggs were removed from the incubation tank as their decomposition could compromise the rest of the eggs.

Also, a pair of *Lysmata amboinensis* were added to the incubation tank to continuously clean the eggs. A negative interaction between *L. amboinensis* and the eggs was never observed.

Hatching

Hatching range was from September to January, with October having the highest hatching proportion. Mean incubation time was 145±10 days (n=18), with a range of 125-167 days. Incubation time changed throughout the years; 2 newborns were hatched in 2016 (152±15 incubation time), 3 in 2017 (136±3 incubation time), 0 in 2018, 4 in 2019 (137±2 incubation time) and 9 in 2020 (155±8 incubation time) (Table 1).

The average temperature of the incubation tank was 25ºC. Results suggested that the incubation period was temperature affected (Kormanik, 1993; Wourms, 1977). Higher temperatures result in shorter incubation time (Kunze, 2004).

Despite all the efforts to hatch the eggs as successfully as possible, in 2018 we had decomposition of all the eggs. It is probably that a fungus, that could never be identified, entered the hatching system and resulted in a decomposition of the eggs. This probably spoiled the water quality making the whole process speed up.

After the incubation period, newborns hatched on their own and would be found in the incubation tank. Hatching is more common at night, so the newborns were usually found first thing in the mornings. When the newborns were noticed they were moved to their own aquarium. Manual assistance for opening an egg was used when the yolk was no longer noticed on the embryo and the mean incubation time had been exceeded for two weeks. Nevertheless, experience gave us more patience and the first newborns of 2019 all hatched on their own.

Some newborns were born with little yolks, but associated problems were not noticed. Yolk was absorbed in a few days and it was after this that they started eating. Nevertheless, quality of the water and good maintenance of the aquarium were always a priority.

Juveniles

Neonates were measured at birth. Mean total length (TL) was 26.8±0.5 cm (n=18) with a range of 29 to 24.5 cm TL. Mean weight was 74.1±4.9 gm (n=18) with a range of 90 to 62 gm. Differences, in size or weight, between females and males were not appreciable.

Newborns are maintained by themselves, or if necessary, with other newborns, attempting to prevent intraspecific aggression (Kunze 2004). Coral sand was used on the floor of the entire aquarium for prevention of the appearance of dermatitis or pressure sores on the abdomens of the newborns (Christopher 2009).

Feeding was tried on the birth date and, in most of the cases, was successful. Newborns are fed up to 4% body weight (BW) per day divided in four food rations over the first two weeks. After the first two weeks they were fed two times per day. If the abdomen of a newborn looked full, it was possible to skip one meal.

Diet is based on prawn, shrimp, squid and mussel (Watson, 2017). Following the advice of Silvia Lavorano (Genova Aquarium), blue and white fish was not included on diet until the newborns were two months old, in order to avoid intestinal problems.

Inappetence periods are not unusual for newborns, lasting from one to several days. When inappetence occurred, we made daily checks and tried to feed them several times a day. Only on some occasions did we make the decision to do force-feeding using small cannulas and fish porridge.

Target training was introduced very early. Two months after the birth date we started with target assimilation. The target consists of a small hammock that is placed on the floor of the aquarium. Pups were led to the hammock with the final goal that they entered and were fed inside. Over time training evolved to the same program used with the adults. This training allows us to make closer checks on the animals on daily basis, and allows us to take non-stressful volunteer biometrics and blood samples.

Medical Treatments

Pre-copulatory behaviour frequently leads to injuries to the caudal and pectoral fins of the adults. To prevent the injuries from getting worse, medication was added to the diet. Pure hydrolysed collagen (Movial Plus Fluidart®, Actafarma Lab. EU) at a dosage rate of 540 mg/kg BW per day, three days per week were supplied during the pre-copulation season.

Despite all our efforts mortality occurred in two newborns. Necropsy proved that wounds related to interspecific encounters became septic. No external signs were noticed on the individuals prior to death. After this case we had another wound accident in one of the newborns and decided to intercede and provide preventive treatment. Ceftazidime (Ceftazidima Normon®, Normon EU) at 20 mg/kg BW was supplied via an intramuscular route every 72 hours for 5 doses. The newborn did not show any negative symptoms and remained healthy.

One of the pups had an inappetence period of 5 days. In this case, inappetence was accompanied by apathy and impaired swimming and breathing, so we decided to apply treatments. Ceftazidime (Ceftazidima Normon®, Normon EU) at 20 mg/kg BW was supplied via an intramuscular route every 72 hours for 5 doses. We also added Complex B (Hydroxyl®, Almirall EU) at a dosage rate of 0.07 mg/kg BW per day to the fish porridge. Blood samples

were taken from the pup which was not eating, and from a healthy one. Samples were taken at the beginning and at the end of the treatment to compare the results. After 15 days of fasting the pup started eating again. Blood values, behaviour, swimming and breathing returned to normal.

Future Directions

Future reviews will study the development of the measuring, feeding and training of the newborns and will also examine the results of blood samples and ultrasounds on adults and pups. Emphasis will be placed on knowing the genetic pools distributed among all aquariums and using essential tools such as ZIMS.

Learning about the biological cycle of endangered species gives us the opportunity to apply better programs that will also act over ecosystems and all the species within them. *Stegostoma fasciatum* is one of the most precious species in the ocean and in order to protect them correctly it is our duty to learn as much as possible from them.

Acknowledgments

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Literature Cited

Christopher, L., and H. Thomas. 2009. Notes on captive propagation and rearing of zebra sharks, *Stegostoma fasciatum* at John G. Shedd Aquarium. Drum and Croaker: 40: 3-8.

Compagno, L.J.V. 2002. Sharks of the World: An Annotated and Illustrated Catalogue of Shark Species Known to Date. Volume 2: Bullhead, Mackerel, and Carpet Sharks (Heterodontiformes, Lamniformes and Orectolobiformes). FAO Species Catalogue for Fishery Purposes. 1(2): 269p.

Kormanik, G.A. 1993. Ionic and osmotic environment of developing elasmobranch embryos. Environmental Biology of Fishes. 38: 233-240.

Kunze, K., and L. Simmons. 2004. Notes on reproduction of the zebra shark, *Stegostoma fasciatum*, in a captive environment. *In:* The Elasmobranch Husbandry Manual: Captive Care of

Sharks, Rays and their Relatives. Ohio Biological Survey. p 493-498.

Mohan, P. J., and A. Aiken. 2004. Water quality and life support systems for large elasmobranch exhibits. *In:* The Elasmobranch Husbandry Manual: Captive Care of Sharks, Rays and their Relatives. Ohio Biological Survey. p 69-88.

Watson, L., and M. Janse. 2017. Reproduction and husbandry of zebra sharks, *Stegostoma fasciatum*, in aquaria. *In:* The Elasmobranch Husbandry Manual II: Recent Advances in the Care of Sharks, Rays and their Relatives. Ohio Biological Survey. p 421-432.

Wourms, J. P., 1977. Reproduction and development in chondrichthyan fishes. American Zoologist 17: 379-410.

Personal communications

Lovarno, S. 2016. Acquario di Genova, Area Porto Antico -Ponte Spinola, 16128, Genova, Italy, Europe.

Pacific Ratfish. Bruce Koike.

HUSBANDRY TECHNIQUES AND MEDICAL INTERVENTION IN THE CARE OF GREEN MORAYS (*Gymnothorax funebris*) AT NEW ENGLAND AQUARIUM

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Introduction

The Giant Ocean Tank (GOT) is a 200,000-gallon, 23 ft deep and 40 ft wide, cylindrical tank in the center of the New England Aquarium. Constructed in 1969, it has housed marine species since 1970. Today, it represents a Caribbean reef ecosystem that includes an artificial reef built and installed in 1984, though more recently renovated in 2013. At present, there are about 100 different species of bony fish, elasmobranchs, and sea turtles residing in the Giant Ocean tank including two green moray eels (*Gymnothorax funebris*) and one spotted moray eel (*Gymnothorax moringa*).

Green moray eels have been exhibited continually in the GOT since 1973 with as many as seven cohabitating at one time. Green morays are the largest species of moray eel, reaching 8 feet (2.5 m) in length and 60 lbs (29 kg) (Robins and Ray, 1986). The species is well known for its bright green coloration and like other morays, pharyngeal jaws; a second set of jaws in the throat, which assists in pulling prey into the esophagus. These adaptations are advantageous as predators in temperate and tropical regions where their elongated body makes it simple to penetrate the crevices of reef habitat in search of hiding fishes (Moyle and Cech, 1988). Though typically seen as menacing to aquarium visitors, in our experience they are usually quite docile and considered a straightforward species to care for.

Husbandry

In the Giant Ocean Tank, moray eels are offered food daily through target feeding by staff during morning feeding dives. The prepared eel diet is placed in a blue catch bag distinct from other feeding equipment used on exhibit and brought to each eel. Individual food items are stick-fed using a neon yellow fiberglass rod and offered to the eel. By using unique feeding equipment, the eels associate those items as their feeding targets, typically eliciting an efficient feeding where the eels are aware which diver has their prepared diet. Typical menu items are de-penned and de-beaked squid (*Illex* sp.) and capelin (*Mallotus villosus*) for adult eels, white shrimp (*Penaeus* sp.) and other smaller items are incorporated for younger eels or smaller species. The occasional

herring (*Clupea* sp.) or mackerel (*Scomber scombrus*) steak is offered if attempts to stimulate an appetite or administer vitamins or medications are being made. Due to documented cases of ocular lipid deposition in moray eels in human care (Clode et al., 2012), a primary diet of lean food items is offered, reserving options with higher fat content only for unique circumstances. Appetite can vary through the year possibly due to several factors including the age of the eel, season, or other environmental conditions. GOT eels sometimes eat every day and at other times will not eat for an extended period of weeks or more. Unaccounted for during these times of fasting is the potential for occasional predation on the living collection which may go undocumented by staff during census counts. Historical care records from the Giant Ocean Tank show stints of two or more months of fasting, and often an eel will go two weeks or more several times per year. Reports from other institutions even mention periods of over three months of an eel going off food (Cameron Park Zoo, pers. comm.). This behavior is also documented to an extent in wild individuals, where moray eels may forage infrequently, returning to their shelter sites where they can remain for days with little movement or energy expenditure (Hobson, 1974; Abrams et al., 1983).

Along with daily feedings, aquarists also monitor and observe them daily. Observations are made of body condition, posture, respiration, and gill coloration if able to obtain a good position without disturbing the eel. Scuba-certified aquarium veterinary staff will also conduct 'wet rounds' on a monthly basis. Hands-on preventative care work-ups, including morphometrics, physical examination, and bloodwork are also routinely performed. Of course, unscheduled exams may also occur for medical reasons. We will look at a case study of a particular green moray eel later.

Medical Intervention

The Giant Ocean Tank has a unique design, where the entire 360° surface of the exhibit is open and accessible to the public. This makes animal management somewhat difficult, and strategies to minimize visitor disturbances must be employed. Some examples are avoiding operating hours, temporarily closing off the top of the exhibit, and/or using stanchions to temporarily block off the pathway between the dive platform and behind-the-scenes area. Exams generally take place in the "splash room" (a support area behind the scenes for the Giant Ocean Tank) or Aquarium Medical Center (AMC), except on occasions when public viewing is welcomed and even made as part of the daily programming. This typically is reserved for sea turtle exams, not for animals like moray eels which pose more logistical complications and are higher risk procedures.

How does one collect and remove a moray eel? Equipment needed (at New England Aquarium) is included in Table 1. Eel collection is done by two aquarists/divers with prior eel collecting experience, and sometimes a third observing for training purposes. Both aquarists wear HexArmor® gloves for the entirety of the collection process. One is designated as the bag manager and the other is designated as the eel handler. Depending on the location of the eel, collection can be either quite easy (out in the open) or very difficult (eel mostly tucked in the reef). Demeanor of the eel also plays a role in deciding how assertive the divers need to be when approaching the animal for collection. The bag manager opens the catch-bag wide and approaches the eel towards the head. The eel handler approaches from behind (if possible), grabs with both hands around the body near mid-section, and guides the eel into the bag (Figure 1). Once the eel is completely in the

Table 1. Required equipment.

Equipment	Description
Eel collecting bag	15” diameter opening with length extended to 40”
HexArmor Gloves	Anti-puncture gloves to ensure staff safety during collection and handling
Isolation Barrel	75-gallon repurposed pickling/rain barrel modified for fish holding with perforations and viewing windows
Exam container	90-gallon rectangular bin with fiber-angle reinforced frame and custom PVC sheet lid
Exam table & Stretcher	Perforated PVC sheet with 1” Schedule 80 PVC frame. 4’ black vinyl eel stretcher with 6’ stretcher poles
Dolly/Trucks	Suncast platform truck for transport of exam container to aquarium medical center
Hoist	Lodestar Model L electronic chain hoist
Drum sling	Fabric sling with steel hooks, attached to carabiner and oblong ring
O2 cylinder & airstones	Industrial grade oxygen with ¼” airline and airstones
DO meter and probe	Hach meter HQ30d & probe LDO101
Submersible pump	Danner Model 2 utility pump with control valve and tubing

bag (head to tail), the bag manager closes the open end and manually secures it with both hands to avoid the eel from escaping. Both divers then transfer the eel to an empty barrel (Figure 2) and put the eel and bag together into the barrel, ensuring the bag is not clipped shut so the eel is able to exit the bag while in the barrel. The divers then secure the lid and the barrel is then brought to the surface gradually to allow the eel to acclimate to the changing depth. For removal from the exhibit, the barrel handles are attached to the drum sling connected to the hoist. As the barrel is lifted up and out of the water, most of the water in the barrel will drain over the exhibit, except for the bottom ~30 gallons, enough to fully submerge the eel (Figure 3). It is then moved along the hoist track (Figure 4) to the top of the stairs adjacent to the behind-the-scenes area known as the splash room. Once carried into the splash room, doors are closed and signs are mounted notifying others that they should not enter unless granted permission. The barrel is laid on its side to let the remaining water drain out, and then lifted into the examination box containing anesthetized water (Figure 5). The eel is observed through a viewing window in the barrel lid until medical staff feel confident that the eel is sufficiently unconscious. The barrel is opened and slowly removed leaving the eel in the box by itself. The lid to the exam container is secured as the barrel is being removed as a safety precaution to avoid any eel escapes. From this point, the lid can be removed and any necessary medical intervention can be conducted while staff monitor dissolved oxygen levels and eel respiration (Figure 6). After the exam is finished, the eel is gently slid back into the barrel, the barrel is lifted out of the eel box, and exhibit water is added to the barrel to flush out the anesthesia and gradually recover the eel. Once the eel is awake and respiring on its own with minimal labor, barrel and fish are returned to the GOT via the hoist, where it is monitored throughout the day by staff. Eels are generally monitored for a few hours following exams prior to release back to the exhibit, ensuring they are fully awake and recovered from the procedure.

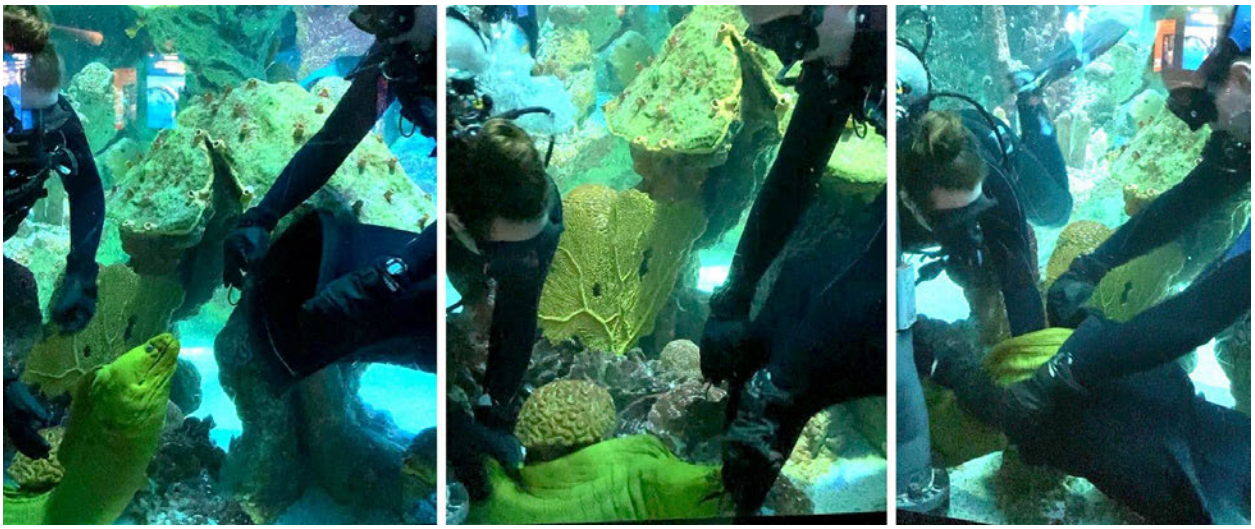


Figure 5. Moray eel collection in Giant Ocean Tank, NEAq. Photo credit Fritz McGirr.

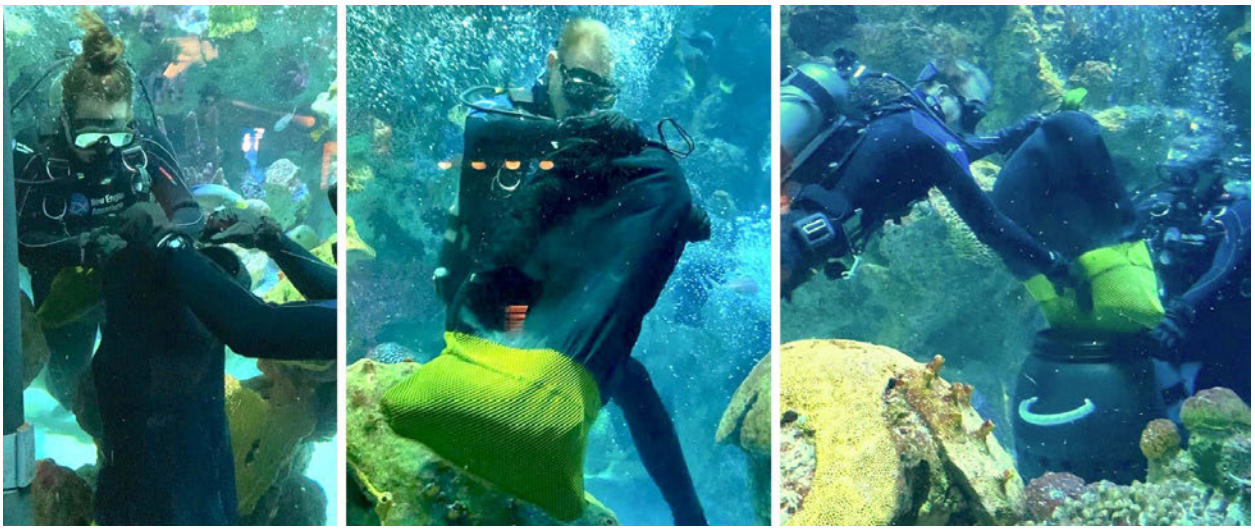
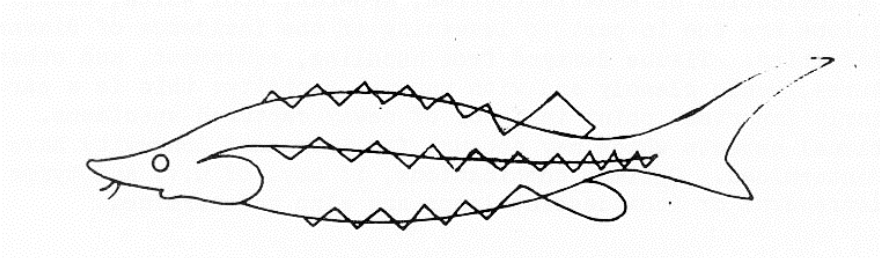


Figure 2. Moray eel bag to barrel transfer in Giant Ocean Tank, NEAq. Photo Credit Fritz McGirr.



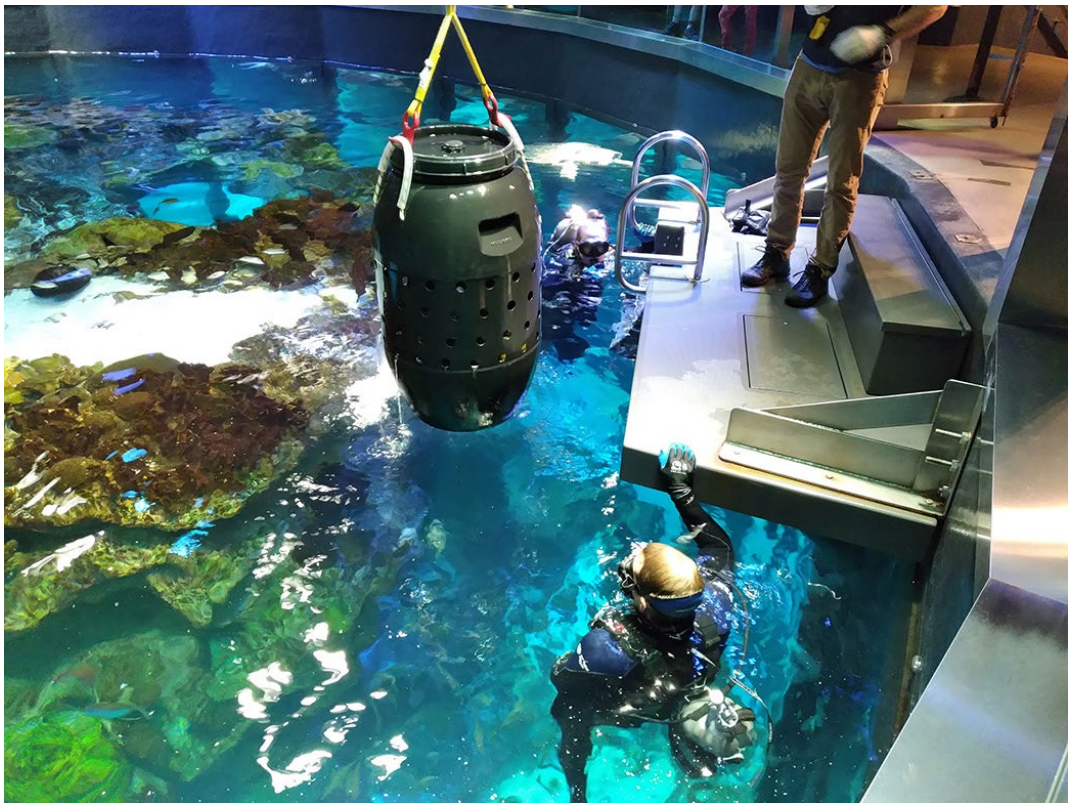


Figure 3. Barrel is removed from the GOT via electronic hoist. Photo credit Samantha Bluhm.

Figure 4. Barrel moves along the hoist track to behind the scenes area. Photo credit Samantha Bluhm.

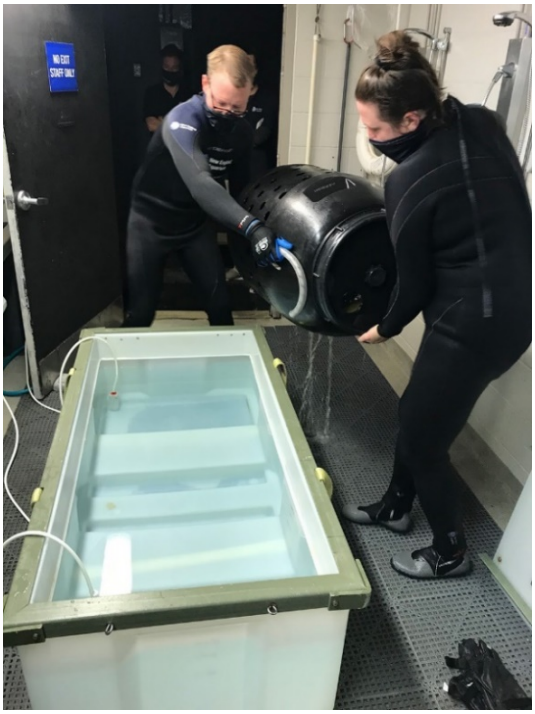


Figure 5. Barrel and eel being lifted into eel box with anesthetized water. Photo credit Samantha Bluhm.

Figure 6. Moray eel examination and blood draw. Photo credit Samantha Bluhm.

Case Study: Green Moray Eel #87557

Green moray eel #87557, also known as "Thomas" was acquired in 2008 from an aquarium hobbyist in Billerica, MA when its owner could no longer provide appropriate care and the New England Aquarium was contacted to step in. It was estimated to be 1-2 years old based on size (Figure 7). After a 2-month quarantine, in January 2009 it was introduced into the GOT population. Over 3 years later, in 2012 the eel was moved to the temporary Tropical Ocean Exhibit (TOE) for the GOT renovation that took place between the fall of 2012 and the summer of 2013. During this time, two large adult green moray eels were acquired from the New York Aquarium and added to the TOE. Not long after, Thomas sustained numerous lacerations (Figure 8) that required immediate medical attention. A majority of the wounds were fully healed 4 weeks later. By the summer of 2013, the renovation was complete and the eel was returned to the GOT. The following year, in 2014, it required sutures again when a 2" wide open wound of unknown origin was observed. In 2018, medical intervention was again required when GOT staff became concerned about a discolored left nostril. Biopsy confirmed this was chronic ulcerative nasal dermatitis, a benign inflammatory/reactive process most consistent with trauma, as might occur with rubbing, and revealed no infectious agents. The nostril remains discolored to this day.

Figure 7. #87557 in 28-gal bin prior to transport to NEAq.

Figure 8. Lacerations were sustained from an assumed melee with a conspecific.

In November of 2019, Thomas was observed with abnormally pale gill coloration and had reportedly been anorexic for several weeks. The eel was captured for examination by staff veterinarians and was determined to have severe anemia (packed cell volume (PCV) 3%). The remainder of its bloodwork was unremarkable and blood culture was negative. Differentials for anemia in this case included chronic disease (infection, inflammation, hepatic, renal, other), nutritional deficiency secondary to fasting vs. hemolysis (transient hypophosphatemia, immune mediated, other). Treatment was initiated with intramuscular injections of iron dextran (to cover for potential iron deficiency anemia), Ceftiofur (antibiotic to cover for potential infection), Vitamin B complex and Vitamin C (to cover for vitamin deficiency anemia, help stimulate appetite, and for immune support), and tube feeding (1-1.5% body weight) via orogastric tube for nutritional support. The eel was caught and handled once weekly for a total of three treatments, during which anemia resolved (PCV normalized to 19%), gill color improved, and the animal began eating.

The eel remained stable for about 6 weeks, but anorexia, pale gills, and anemia were again noted in January of 2020. Further diagnostics including full body radiographs, gastrointestinal endoscopy, and colonoscopy were unremarkable. Repeat blood culture grew *Staphylococcus warneri*, which is a commensal organism in teleost epidermis, but has been known to be pathogenic in some cases. Treatment was restarted with injections of iron dextran, Vitamins B and C, and florfenicol (antibiotic based on culture and sensitivity), along with tube feeding and administration of an appetite stimulant (Carpromorelin). The eel also received two doses of a steroid, triamcinolone, in an attempt to stimulate appetite and treat any underlying inflammation. After 3 weeks of treatment, anemia again resolved and gill color normalized. This eel had been doing well for several months, however anemia and pale gills recurred in September 2020, despite having normal appetite. In light of good appetite, the eel was started on oral iron supplementation (ferrous sulfate) administered in food twice weekly for 5 weeks. PCV was noted to be normal 3 weeks after treatment and 2 weeks after discontinuing treatment. At this time, the underlying cause of recurrent anemia in this eel remains unknown, but the main differentials include transient nutritional deficiency during period of anorexia (iron, vitamin, hypophosphatemia, other) versus secondary to underlying chronic disease (underlying mild hepatic, renal, neoplasia, inflammatory, or other). We plan to continue to monitor closely and will perform every 6-month routine rechecks and bloodwork if eel remains stable.

Acknowledgements

The authors would like to thank the Fishes and Animal Health Departments of the New England Aquarium as well as our dedicated Giant Ocean Tank team of staff and volunteers that made this paper possible.

References

Clode, A. B., C. Harms, M. H. Fatzhinger, F. Young, C. Colitz, and D. Wert. 2012. Identification and management of ocular lipid deposition in association with hyperlipidaemia in captive moray eels, *Gymnothorax funebris Ranzani, Gymothorax moring* (Cuvier) and *Muraena retifera* Goode and Bean. Journal of Fish Diseases. 35: 683-693.

Lieske, E. and R. Myers. 1994. Collins Pocket Guide. Coral reef fishes. Indo-Pacific and Caribbean including the Red Sea. Harper Collins Publishers. 400 p.

Moyle, P. B., and J. J. Cech. 1988. Fishes: An Introduction to Ichthyology. Prentice Hall, Englewood Cliffs, N.J.

Robins, C.R. and G.C. Ray. 1986. A Field Guide to Atlantic Coast Fishes of North America. Houghton Mifflin Company, Boston, U.S.A. 354 p.

THE END OF THE DALLAS AQUARIUM/CHILDREN'S AQUARIUM AT FAIR PARK?

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In 1936, in the grip of the great depression, a community of people moved from their homes in Texas before their community was forever entombed in water. Their town would soon become a lost city, the diaspora of its inhabitants took with them remnants of their shared culture and history dating back to the 1850's when Texas was still very much a wild-west frontier.

Every civilization has cities and cultures lost to time, one thinks of Pompeii, Xanadu, Macchu Picchu, Calakmul, and Tulum; in Texas there is Bluffton, located at the bottom of Lake Buchanan in the Hill Country outside Austin. In 2009, just as the City of Dallas was considering closing the Dallas Aquarium, historic droughts exposed the tombstones of the people of Bluffton, the only tangible remnants of their shared history, before swallowing them back up again when rains came to the arid lands. That year the venerable aquarium got a reprieve, and was renovated and reopened, ostensibly to last at least another 75 years in educating and inspiring the people of the city, many who would otherwise never come face to face with the wonders of the sea. Sadly, history did not repeat itself in 2020 amid the SARS-CoV-2/COVID-19 global pandemic.

When a city is lost, through natural disaster or through a confluence of human failings, the sum of the institutional knowledge that disappears forever is far greater than it would appear at first glance. The year the Dallas Aquarium opened, archeologists in what is now Iraq unearthed a curious piece of pottery dating to at least 224 BCE from the ruins of Ctesiphon, one-time capital of the Parthian empire. This artifact contained rods of two dissimilar metals and residue of an acidic fluid: in other words, an anode, a cathode, and an electrolyte. Is it possible the ancient Persians developed a battery, something western science would not re-create for another two millennia? What priceless knowledge and facts which could have changed the world were lost when the Library of Alexandria burned? What secrets did the Mayans of Calakmul and Tulum possess that were lost to time? While such comparisons are rife with hyperbole, the sum loss of institutional knowledge, great and small, is always tragic. Once a culture is lost, whether that culture be an entire city, a culture, or a venerable institution, it can never be fully regained by a society, despite their best intentions.

Eighty-four years of experience are entrusted to the current staff of the Children's Aquarium at Fair Park, which opened as the Dallas Aquarium in 1936 and announced that it was closing its doors in 2020. These dedicated aquarists bear the torch carried by generations past, and will keep some part of this legacy alive through teaching and mentoring other aquarists in other facilities. The collective traditions and wisdom of the best way to keep fishes in this art-deco building have been refined over generations, and passed down to successive cohorts of aquarists to the present day. Many of the larger successes and discoveries have been communicated to the aquarium community over the better part of the last century, but as we all know, the concise data distilled down into a technical paper or conference presentation represents the tip of the metaphorical iceberg, the body of that knowledge lies with the people who did the work, and the people they trained to replace them, and so on, and so on. As such, every time we lose an institution in our aquarium community, we lose a huge body of culture, and we lose thousands of collective tips and tricks that refine this hybrid of art and science we call animal husbandry.

In recent years we have lost a number of venerable aquaria of the 'old guard', the pioneering facilities where the very fundamentals of husbandry that we practice today were developed and refined. Over time, bigger and (arguably) more impressive facilities have replaced aging ones, and we can only assume that in turn they too will one day be replaced by a new generation of aquaria, so it goes. The London Zoo Fish House (1853), the Belle Isle Aquarium (1904), The National Aquarium in Washington DC (1873), SeaArama in Galveston (1965), Sea World of Ohio (1970), and the original aquariums of New York, Seattle, and Scripps all come to mind as facilities lost but to history and our collective memories.

Some of these old guard institutions have received a new lease on life through extensive renovations, such as the Steinhart Aquarium (1923) and the Toledo Zoo Aquarium (1939); these will hopefully persist for many years to come. It is also noteworthy that the Belle Isle Aquarium re-opened in 2012 after seven years in mothballs; it is the fervent hope of the authors the Dallas Aquarium at Fair Park might see a similar rebirth in the future. In fact, since the announcement that the facility would close in mid-2020, several efforts have sprung up to save the facility, so despite the fact that the disposition of the animal collection is currently underway there may yet be a glimmer of hope. For an excellent review of aquarium history as it relates to openings and closings, the reader is directed to Mohan (2020). In the event the institution is not granted an eleventh-hour reprieve, the authors will here encapsulate a summary of the history of the institution, so that its memory is not so lost to time as the city of Bluffton.

History of the Dallas Aquarium

The Children's Aquarium at Fair Park (originally named the Dallas Aquarium) was formally established in 1936 at the corner of 1st Avenue (formerly Centennial Dr.) and Martin Luther King Jr. Boulevard (formerly Forest Ln.). While the current aquarium celebrated its 84th anniversary in 2020 the history of public fish display at the site stretches back more than a century and is inexorably intertwined with the history of the State Fair of Texas.

The original exposition of fishes at the State Fair of Texas came with the establishment of the State Fish Hatchery on the fairgrounds through the efforts of Colonel William G. Sterett, head of the Texas Fish, Game, and Oyster Commission (now Texas Parks and Wildlife). Very little information on the original State Fish Hatchery exists, save for a few blurry photographs taken

during construction and newspaper descriptions of an outdoor water garden with shell-lined pathways around concrete ponds with stone waterfalls where fishes were set upon display for the masses visiting the fair. The hatchery/exposition began construction in 1913 and closed to the public in 1934 when operations shifted to a new hatchery under construction at White Rock Lake. During its history the hatchery featured a variety of sport fishes outdoors in the ponds and through the urging of Col. Sterett even displayed more diminutive native and exotic fishes in a small aquarium building on site. The display of smaller aquarium specimens in tanks rather than ponds proved so popular, it was later expanded to a series of tanks in wood cabinetry patterned after displays in the New York Aquarium located in the of the Dallas Museum of Natural History (now the Perot Museum of Nature and Science) prior to the official opening of the current aquarium. There was a movement among some citizens to name the city's new aquarium the William G. Sterett Aquarium, but ultimately the facility came to be called the Dallas Aquarium.

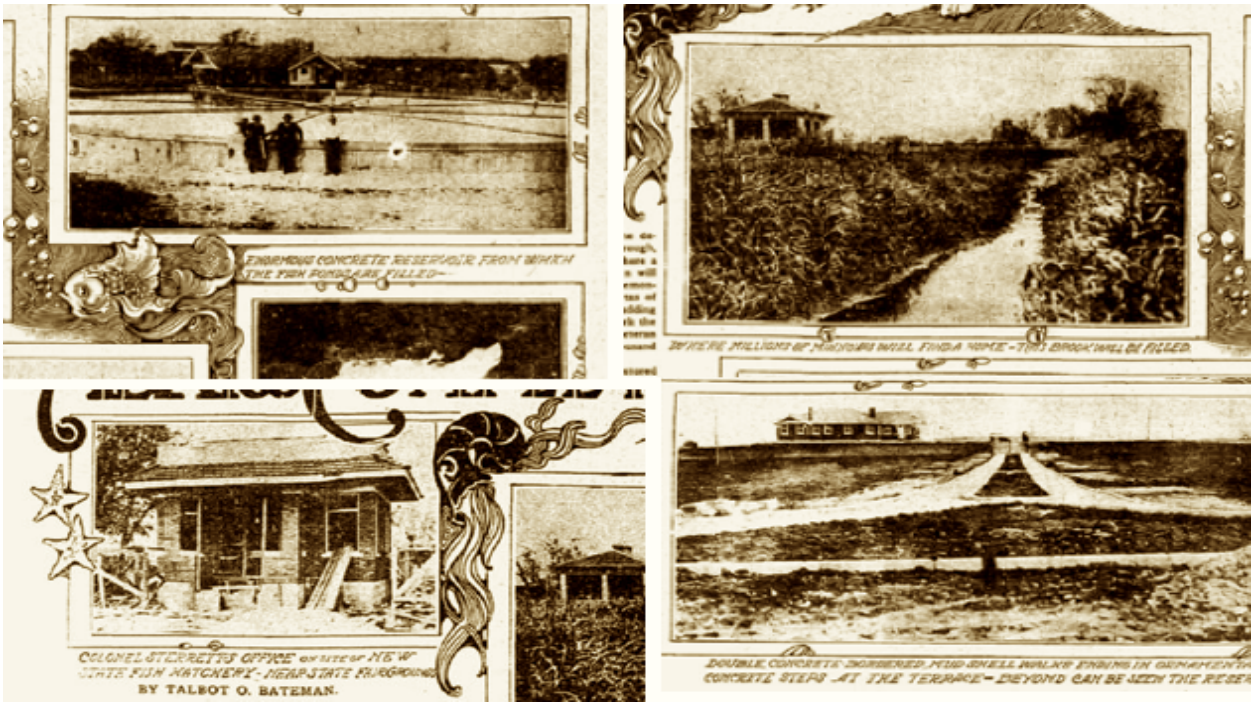


Figure 1. The Fish Hatchery at the State Fair of Texas (1913-1934) pictured in 1913 while under construction. Water was sourced from a well (top right) and diverted to numerous ponds (top left and bottom right), controlled by machinery in a modest pump house and attendant's office (bottom left). Black basses and catfish were reared in these ponds, and stocked into local lakes. Later a small enclosed display aquarium was added, and in 1935 cabinetry and aquaria were set up for display in the rotunda of the Dallas Museum of Natural History to drum up excitement for the new aquarium that would open in 1936. The fish stocking duties were taken on by the White Rock Lake Fish Hatchery starting in 1937, which was also overseen by the aquarium.

STATE FISH HATCHERY MAY MAKE DALLAS PEOPLE FISH FANCIERS

extent the specialized effort has made to procure queer and curious fish by people willing and able to pay for them.

The hobby for keeping aquaria in which wonderful examples of tiny fish are bred is becoming more and more popular throughout the United States every year. Men who have the hobby are no longer content simply to keep their aquaria as decorative objects. They are engaged instead in breeding fish in hundreds of varieties by cross-breeding, among the goldfishes, in obtaining remarkable and interesting new varieties. The amateur toy fish fancier of today breeds his tiny treasures for "points" as carefully as the owner of kennel books after the development of his prize winning dogs or as the stock farm manager watches and guards his prospective entries for the turf or the horse show.

Some of the men connected with the Aquarium Society have more than a hundred aquaria, in which they keep as many as 30 varieties of the small fish. The New York amateurs devote themselves largely to the small tropical fishes, which they purchase from all parts of the world. Goldfish are not so popular with the aquarium owners of New York. Philadelphia is the great center for goldfish competition. More remarkable goldfish are owned there than in any other city of the country.

While the prize aquarium fish has never yet attained to the price of a prize dog, or even of a prize cat, as much as $1,000 is asked for a pair of prize winners of the aquarium world which measure about four inches in length. This price is asked for a pair of large lion heads which are diploma winners. Such a price is very unusual; the range of prices for the desirable pairs is ordinarily from $10 to $100.

To obtain the tiny beauties which are in demand for the aquaria, traders ex-

COL WILLIAM G STERRETT, GAME, FISH AND OYSTER COMMISSIONER, OF TEXAS THROUGH WHOSE EFFORTS THE STATE HATCHERY AT FAIR PARK WAS ESTABLISHED AND WHICH IS TO BECOME A MOST BEAUTIFUL PIECE OF LANDSCAPE GARDENING

Figure 2. "Colonel" William G. Sterrett, newspaperman, politician, vocal opponent of the temperance movement, and friend of Theodore Roosevelt. Sterrett was an avid outdoorsman and head of the Texas Game, Fish and Oyster Commission from 1910-1920, where he was the driving force behind the creation of a hatchery for fish display at the Texas State Fair and a strong proponent for the creation of the first aquarium in Texas. Sterrett died in 1924, and there was a movement to name the aquarium after him when it opened in 1936, though it eventually came to be called the Dallas Aquarium. Excerpt from an article "State Fish Hatchery May Make Dallas People Fish Fanciers" in the Dallas Morning News, 1913. Similar news stories of the day heralded his attempts to bring "Finny Folk", "Beautiful Beasts", and "Wonderous Wildlife" to the people of Dallas, among additional alliterative appellations.

In 1935 the state began preparing for the celebration of the Texas centennial and Fair Park was selected as the site for the event. The centennial was to be an exposition on the scale of a world's fair and a slew of new buildings were to be constructed before the ceremonies were opened amid a presidential visit by Franklin D. Roosevelt in 1936. These new structures included the aquarium, Dallas Museum of Fine Arts, Horticulture Museum, Hall of State, among others. These projects as a whole were overseen by renowned architect George Dahl and collectively represent one of the best collections in the country of extant art deco architecture. The architectural firm

responsible for the original design of the aquarium itself was Fooshee, Cheek, Thompson, Flint and Broad and the general contractor was Thomas Bates and Sons. The original budget for construction was $165,000 but final cost of the project was in excess of $232,000. The building's exterior was patterned after the 1933 Seattle Art Museum (now the Seattle Museum of Asian Art) and the interior layout and service corridors were inspired by the 1930 John G. Shedd Aquarium in Chicago. At the time of opening the building featured a number of technological advances such as the first large scale aquarium chiller to provide cool water for salmonid fishes and never before displayed arctic rarities such as the Alaskan blackfish (*Dallia pectoralis*). Water was supplied by an on-site well and a water tower and complex distribution system were included in the original construction.

In 1935 the state began preparing for the celebration of the Texas centennial and Fair Park was selected as the site for the event. The centennial was to be an exposition on the scale of a world's fair and a slew of new buildings were to be constructed before the ceremonies were opened amid a presidential visit by Franklin D. Roosevelt in 1936. These new structures included the aquarium, Dallas Museum of Fine Arts, Horticulture Museum, Hall of State, among others. These projects as a whole were overseen by renowned architect George Dahl and collectively represent one of the best collections in the country of extant art deco architecture. The architectural firm responsible for the original design of the aquarium itself was Fooshee, Cheek, Thompson, Flint and Broad and the general contractor was Thomas Bates and Sons. The original budget for construction was $165,000 but final cost of the project was in excess of $232,000. The building's exterior was patterned after the 1933 Seattle Art Museum (now the Seattle Museum of Asian Art) and the interior layout and service corridors were inspired by the 1930 John G. Shedd Aquarium in Chicago. At the time of opening the building featured a number of technological advances such as the first large scale aquarium chiller to provide cool water for salmonid fishes and never before displayed arctic rarities such as the Alaskan blackfish (*Dallia pectoralis*). Water was supplied by an on-site well and a water tower and complex distribution system were included in the original construction.

Upon opening, the aquarium focused its displays on freshwater fishes native to Texas, though smaller tanks showcased such rarities as Amazon fishes, Asian species, fancy goldfish, and coastal oddities such as seahorses and pipefish. The aquarium was among the first in the world to successfully breed and rear seahorses in 1937, and contributed much towards the knowledge of the culture of fishes through breeding at the aquarium and the associated fish hatchery at White Rock Lake. Through the 1950s the aquarium continued to acquire and display specimens rare for their time such as some of the first arowana (*Osteoglossum* spp.), Siamese fighting fishes (*Betta splendens*), Mexican blind cavefish (*Astyanax fasciatus mexicanus*), Mexican Swordtails (*Xiphophorus spp.*), Hellbenders (*Cryptobranchus alleganiensis*), and many other rarities that are now commonplace in aquaria. The facility had the distinction of being the first to display all three species of lungfish at the same time, owing to overseas contacts the curators made serving in the Pacific theater during WWII. In 1950 The institution was also the first facility outside Asia to display Japanese Giant Salamanders, *Andrias japonicus,* as one of the aquarists (Donald Blair) was the son of a US Army officer (Major Daniel Blair) who oversaw the city of Tottori during the postwar occupation. In the 1960's aquarium staff was credited with discovering species new to science such as the basslet *Lipogrammus klayi* from the deep reefs off Curacao, named after aquarist Gerrit Klay who collected the type specimens from depths over 300 fsw (91m) in Curaçao

(diving on air). Gerrit Klay would later work at the Cleveland Aquarium, and then open his own facility, Shark-Quarium in Marathon Key, FL. Gerrit would go on to distinguish himself in refining the techniques used for capture, transport, and display of captive elasmobranchs, many of which form the foundation of shark husbandry as we know it today.

The aquarium expanded into salamander breeding and conservation activities in the 1970s through the 1990s working with a number of Texas cave-dwelling species of the genus *Eurycea*, including the Texas Blind Salamander, *Eurycea rathbuni*, and Comal Springs salamander, *Eurycea nana,* and the aquarium was the first to breed both species. The salamander work was initiated by Dr. Glen Longley of Southwest Texas State University (now Texas State University) in response to droughts causing drastic reductions in flow to Barton springs in Austin, TX. David Roberts of Dallas Zoo herpetology department and Dave Schleser of the aquarium partnered together to investigate the husbandry and reproduction of some of these salamanders. After noting that salamanders in Barton springs congregate near bubbling upwellings from the Edwards aquifer, they devised an artificial upwelling consisting of limestone rocks stacked in an acrylic cylinder with flow of cool, calcium-rich hard water from the aquarium's well coming up from the bottom (detailed in Roberts et al., 1995). It wasn't long before they noticed eggs on the rocks, and this work so impressed Dr. Longley and wildlife authorities that permits were amended and the aquarium was given Texas blind salamanders with which to keep and breed, and the rest is history.

llas Morning News

LOCAL NEW
SPORTS
MARKETS
WANT ADS

AS, TEXAS, SATURDAY, SEPTEMBER 28, 1935 Oldest

Centennial to Build Only Aquarium in Southwest

e second aquarium west of the ssippi, to be erected by the Park | Cheek, Flint & Broad and H. B. Thomson. The structure will have | There will be no salt water tanks. Refrigerating equipment will provide | stantly. The Dallas aquarium will a model for its size and will be

Figure 3. Announcement of the construction of the aquarium, and conceptual sketch in the Dallas Morning News, September 28, 1935. The full article (available through DMN archives) captures the excitement of this announcement. At the time, only a handful of major public aquaria existed in the country, including the Belle Isle Aquarium, John G. Shedd Aquarium, New York Aquarium, National Aquarium, and Steinhart Aquarium. Truly bringing the sea to the inland residents of Dallas was a monumental undertaking for the time.

Figure 4. Opening day of the Dallas Aquarium, June 6th, 1936. Photo Dallas Aquarium Archives.

Figure 5. The White Rock Lake Fish Hatchery, once managed by the aquarium. The earthen ponds have long since been drained and fallen into disuse, but are still visible and part of a network of hiking trails at the base of the dam in White Rock Lake Park. The hatchery turned out thousands of bass and sunfish yearly to stock White Rock Lake, Bachman Lake, and other nearby reservoirs. Aquarium director Pierre Fontaine and others wrote a monograph on Texas fish hatchery operations (Cheatum, 1943). Photo Dallas Historical Society.

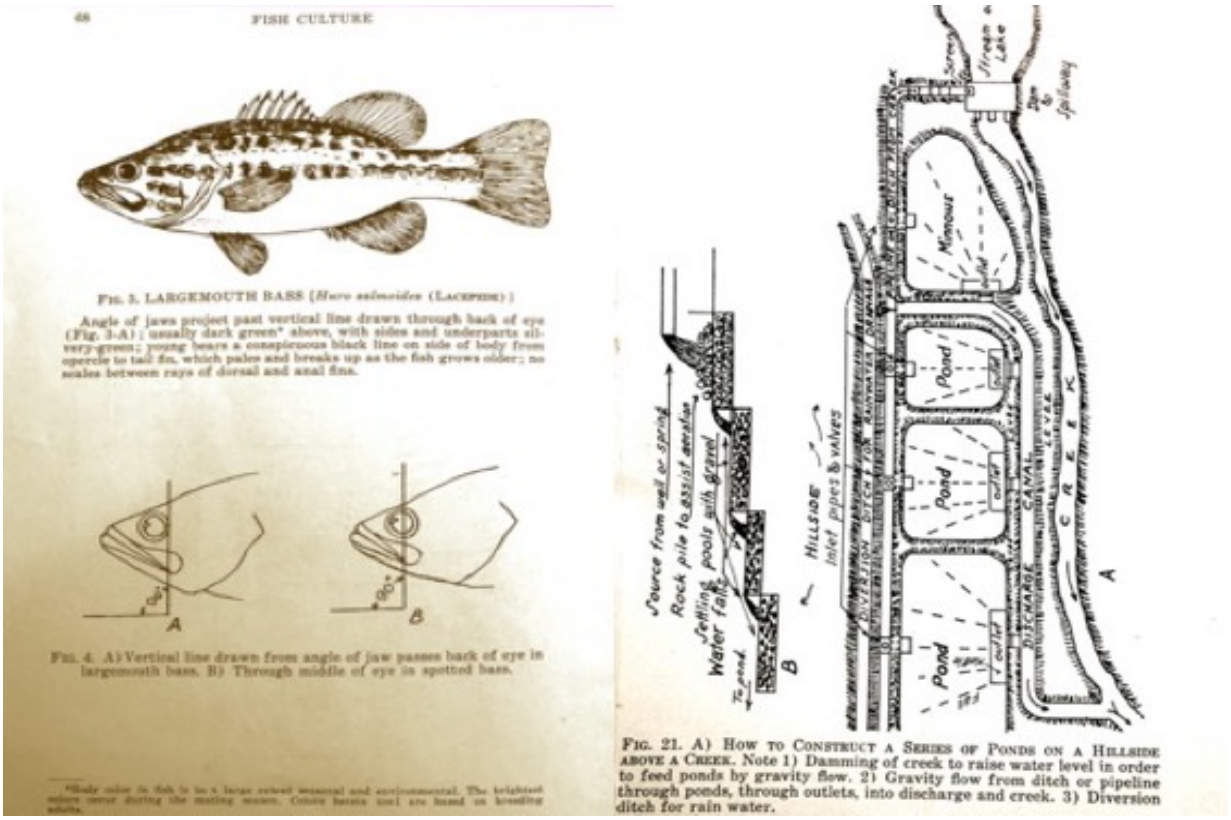


Figure 6. Illustrations by Pierre Fontaine on game fish species and hatchery construction, from the monograph on Texas fish hatchery operations he co-authored based on his experience managing the Dallas Aquarium and the White Rock Lake Fish Hatchery (Cheatum et al., 1943). Fish culture was well known in other states but Texas had unique challenges to establishment of fish hatcheries; namely droughts which make natural water supplies irregular, high summer temperatures that affect dissolved oxygen, turbid river waters, and a lack of natural lakes (only one natural lake exists in Texas). This manual helped refine fish culture strategies tailored to the environment of the state, in the days before recirculating aquaculture systems, pelletized feeds, and other advances that would come in later decades.

Figure 7. Alligator snapping turtles, *Macrochelys temmnicki*. The iconic king of N. American turtle species ranges into East Texas near Dallas and the aquarium has held five of these massive animals in its 84-year history. The largest, a specimen held from 1996-2016 weighed in at a maximum of 224lb (102kg), just shy of the world-record 235lb (107kg). It was once thought that this species lived in excess of 100 years, but modern aging techniques show that 30-35 years is an average longevity. Clips from the Dallas Morning News, from the Dallas Aquarium Archives.

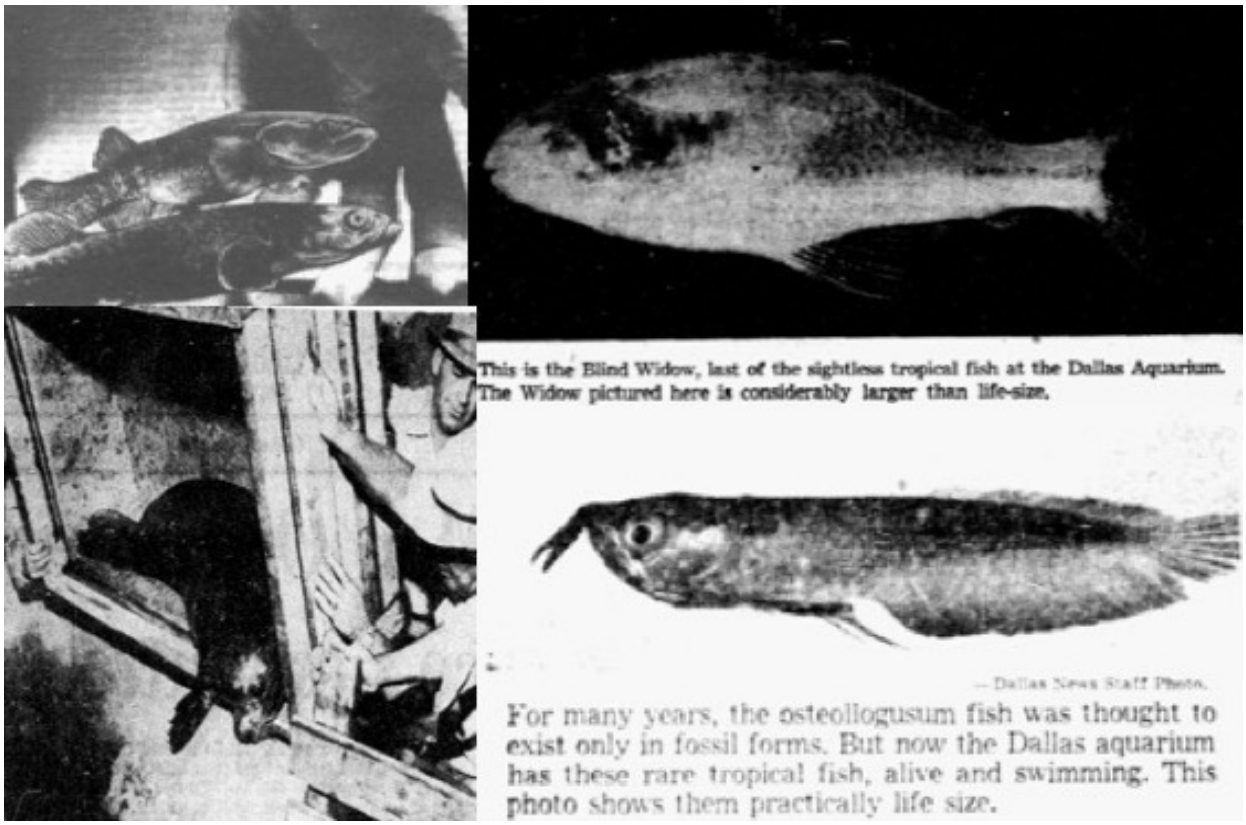


Figure 8. Some of the rare specimens displayed in the early days of the aquarium (1936-1950). Many of these are fairly common nowadays, but were so uncommon when they debuted that these were truly rare marvels that most people had never heard of, let alone seen. Top left, the Alaskan blackfish, *Dallia pectoralis*, which had never been displayed before in an aquarium and were actually shipped to Dallas frozen solid in a block of ice, where they were then thawed and revived. Top right, a Mexican blind cavefish, *Astyanax fasciatus mexicanus*, and bottom right, an arowana, *Osteoglossum* sp., both rare at the time. Bottom left, a harbor seal, *Phoca vitulina*, kept for many years in freshwater, in a very small tank, certainly practices normal at the time, but no longer considered good husbandry.

In the early 1990s aquarium staff partnered with the New York Aquarium and began working with desert fishes of the American west and Northern Mexico. Their efforts resulted in establishment of captive populations of over a dozen species of pupfishes and goodeids that had since become critically endangered or extinct in the wild. These efforts continue today through the AZA Freshwater Fishes TAG and until recent years the aquarium was active in maintaining several Mexican pupfishes of the genus *Cyprinodon* and *Megupsilon aporus*.

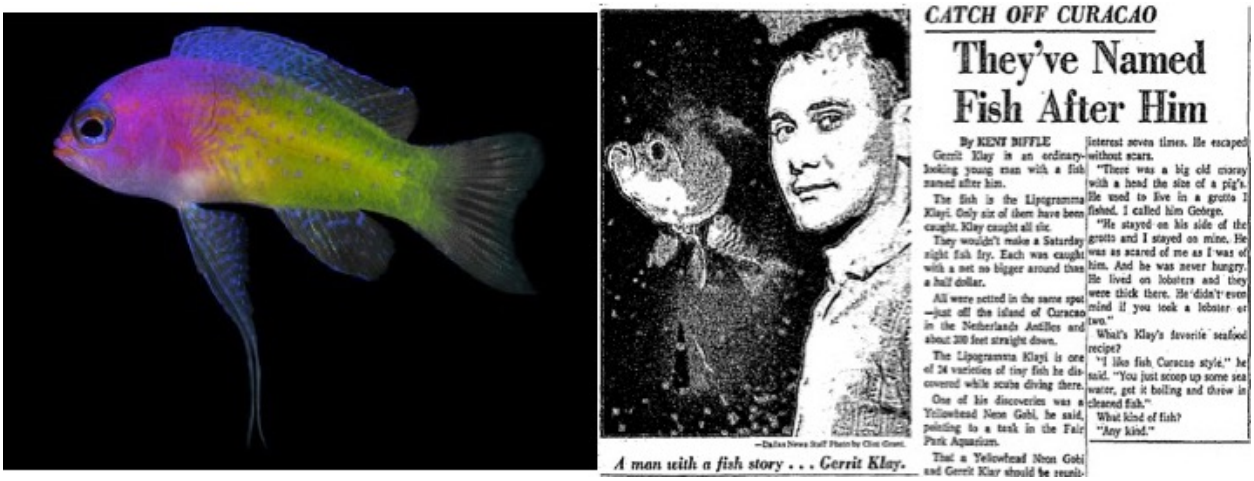
The work with pupfishes began at the request of Dr. Salvador Contreras-Balderas at the Universidad Autonoma de Nuevo Leon as the plight of the endemic desert fishes of the Sandia valley in México became apparent in the late 1980's and early 1990's. Dr. Paul Loiselle of the NY Aquarium and Dr. Dave Schleser and Charles Yancey of the Dallas Aquarium traveled to México and collected some of the last surviving specimens of *Cyprinodon veronicae, C. longidorsalis, C. alvarezi,* and *Megupsilon aporus* with Dr. Contreras-Balderas and Dr. Arcadio Valdez Gonzalez. Just a year later these springs were dry, the aquifers having been pumped dry for industrialized alfalfa farming. Before returning to the US, the visiting Americans also presented the university with equipment and supplies, and a check for $1,000 USD raised by the

Dallas Zoological Society to further their conservation efforts. The aquarists returned with the pupfishes, as well as several imperiled goodeids from the breeding programs at the university, and began to breed and recruit other AAZPA facilities to form the original Desert Fishes Program of the Freshwater Fishes TAG, Drs. Schleser and Loiselle also presented their work at the Desert Fishes Council meetings, gaining the support and partnership of researchers and academics.

In the mid 2000's the aquarium became involved in freshwater mussel conservation as the invasive zebra mussel threatened to expand its range into North Texas. Over a period of ten years the aquarium conducted hundreds of surveys at 87 locations in partnership with the Texas parks and Wildlife Department, collecting data on 10,995 individual animals of 31 species. These surveys also documented previously-unknown populations of several imperiled species such as the Texas heelsplitter, *Potamilus amphichaenus.* During severe droughts the aquarium conducted "rescue operations" in which over 2,500 mussels from survey sites were relocated into water as flowing streams became hot mud in the hot summer months. In partnership with Texas Tech University and Texas A&M University, mussel propagation research was carried out from 2010-2016, resulting in the breeding of eight species, including the first-ever propagation of the imperiled *Potamilus metnecktayi* and *P. amphichaenus* (Bosman et al., 2015).

Figure 9. Pupfishes and salamanders. Top left, *Cyprinodon veronicae*, a critically endangered pupfish from Northern México. Bottom left, *Eurycea rathbuni*, the Texas Salamander (photo US Fish & Wildlife Service). Right four imperiled pupfish species, males (top) and females (bottom), clockwise from top left, *Megupsilon aporus, Cyprinodon veronicae, Cyprinodon fontinalis,* and *Cyprinodon longidorsalis*.

Figure 10. Opening of Desert Fishes Exhibit, 1991. Left to right: unknown suit, Senior Aquarist Charles Yancey (who served the aquarium for over 30 years), unknown, Nell Ann Rose, Curator Dave Schleser, unknown, Dallas Zoo Director Rich Buickerood, unknown suit. Rich has the distinction of being the only zoo director to have been bitten by an alligator gar while leading a tour of the aquarium, after putting his hand too close to the tank. Photo Dallas Zoo Archives.



CATCH OFF CURACAO

They've Named Fish After Him

By KENT BIFFLE

Gerrit Klay is an ordinary-looking young man with a fish named after him.

The fish is the Lipogramma Klayi. Only six of them have been caught. Klay caught all six.

They wouldn't make a Saturday night fish fry. Each was caught with a net no bigger around than a half dollar.

All were netted in the same spot—just off the island of Curacao in the Netherlands Antilles and about 300 feet straight down.

The Lipogramma Klayi is one of 24 varieties of tiny fish he discovered while scuba diving there.

One of his discoveries was a Yellowhead Neon Gobi, he said, pointing to a tank in the Fair Park Aquarium.

That a Yellowhead Neon Gobi and Gerrit Klay should be reunited seven times. He escaped without scars.

"There was a big old moray with a head the size of a pig's. He used to live in a grotto I fished. I called him George.

"He stayed on his side of the grotto and I stayed on mine. He was as scared of me as I was of him. And he was never hungry. He lived on lobsters and they were thick there. He didn't even mind if you took a lobster or two."

What's Klay's favorite seafood recipe?

"I like fish Curacao style," he said. "You just scoop up some sea water, get it boiling and throw in cleaned fish."

What kind of fish?

"Any kind."

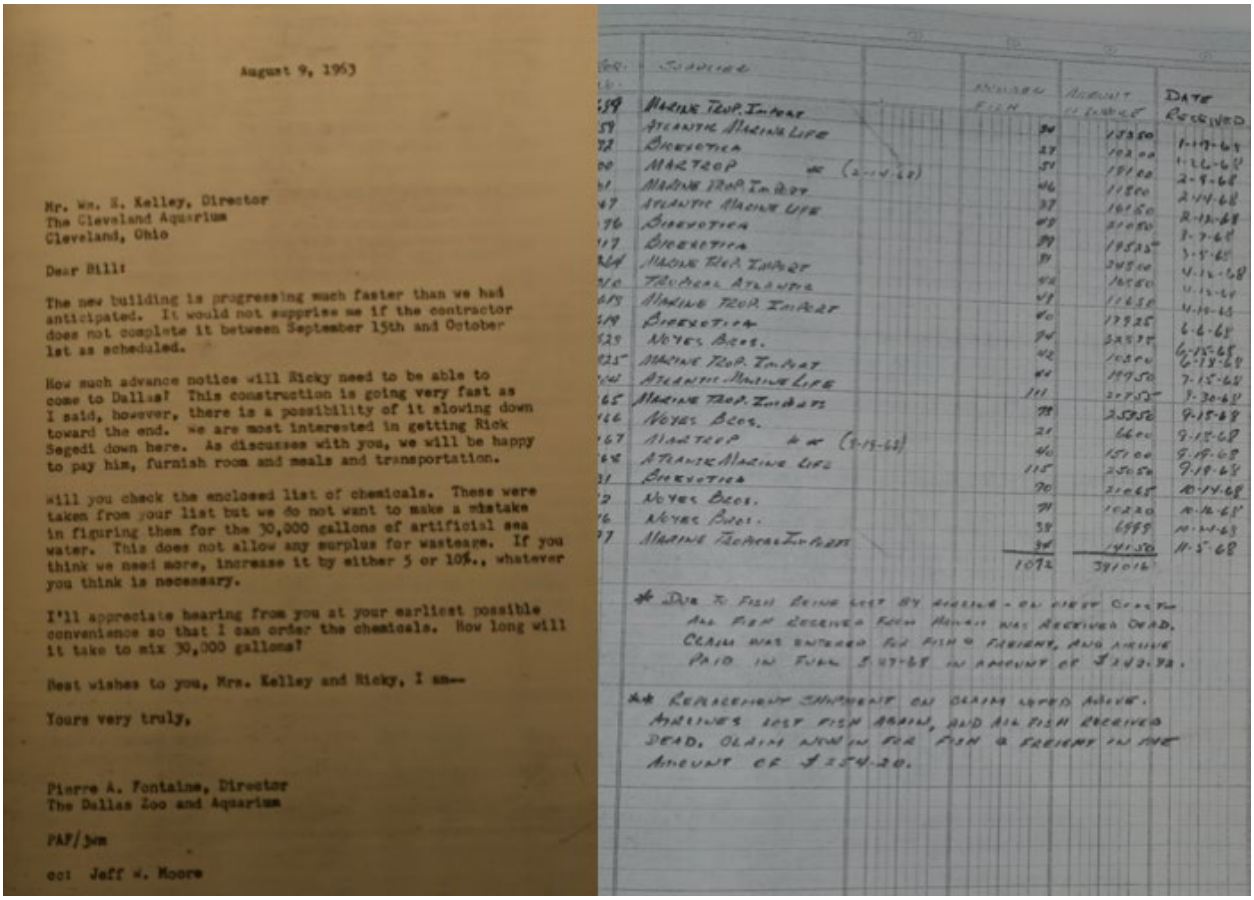
A man with a fish story . . . Gerrit Klay.

Figure 11. The deep-water basslet *Lippogramma klayi,* image (left) Ross Robertson from Smithsonian Tropical Research Institution https://biogeodb.stri.si.edu/caribbean/en/pages/random/4043. At right: excerpt of announcement of description of the species (right) from the Dallas Morning News March 29, 1964 featuring aquarist Gerrit Klay. The species was formally described by Randall (1963) with two other deep water basslets from Curaçao.

A number of renovations and improvements have taken place in the 84-year history of the facility, beginning with the replacement of the original ceiling in 1951 at a cost of $2,500 to dampen the noise created by the crowds visiting the aquarium. Minor improvements were

probably made over the course of the next dozen years but none were major enough to warrant press attention or creation of records for posterity.

A major expansion and renovation came in 1963-64, when a marine wing was added on to the north end of the aquarium. James Cheek, who participated in the original construction, was the architect chosen to design the expansion with a budget of $150,000. The general contractor responsible for completing the expansion was D&M Construction Co. During this time period the exhibit space of the facility was nearly doubled to allow for the display of numerous marine creatures from the Texas coasts and far-flung Pacific shores. The roof and skylights were rehabilitated, and the innovative water chilling system installed in 1936 was upgraded with new compressors and equipment.



August 9, 1963

Mr. Wm. E. Kelley, Director
The Cleveland Aquarium
Cleveland, Ohio

Dear Bill:

The new building is progressing much faster than we had anticipated. It would not surprise me if the contractor does not complete it between September 15th and October 1st as scheduled.

How much advance notice will Ricky need to be able to come to Dallas? This construction is going very fast as I said, however, there is a possibility of it slowing down toward the end. We are most interested in getting Rick Segedi down here. As discussed with you, we will be happy to pay him, furnish room and meals and transportation.

Will you check the enclosed list of chemicals. These were taken from your list but we do not want to make a mistake in figuring them for the 30,000 gallons of artificial sea water. This does not allow any surplus for wastage. If you think we need more, increase it by either 5 or 10%., whatever you think is necessary.

I'll appreciate hearing from you at your earliest possible convenience so that I can order the chemicals. How long will it take to mix 30,000 gallons?

Best wishes to you, Mrs. Kelley and Ricky, I am--

Yours very truly,

Pierre A. Fontaine, Director
The Dallas Zoo and Aquarium

PAF/jm

cc: Jeff W. Moore

Figure 12. The 1963-64 addition of the Marine Wing, an addition onto the north side of the building allowed for display of Caribbean and Gulf of Mexico species, a rarity for inland aquaria at the time. The Marine Wing also has historical notability as being the first large-scale use of synthetic seawater at a public aquarium. The letter at left to William E. Kelley, then director of the Cleveland Aquarium, discussed coordinating a visit by Rich Segedi to mix the initial 30,000-gallon batch of artificial sea water, the largest ever made at the time, using their improved recipe based on the Frankfurt formulation, an early attempt used in small-scale at the Frankfurt Zoo in Germany. Kelley would go on to found Aquarium Systems Inc. the following year with artificial seawater (Instant Ocean™) as the flagship product. At right is a copy of curator Jeff Moore's ledger for animal purchases, totaling $3,901 ($33,120 in today's dollars), for this major expansion of the aquarium's collection; for most of these species this represented the first time they had ever been kept in artificial seawater. This effort was truly experimental, and these aquarists were pioneers in our field, staking their reputations and the fate of their institution on novel techniques.

The expansion made the aquarium the third largest in the nation (at the time) and the first facility to rely exclusively on artificial seawater. The cost of specimens acquired for the new marine wing totaled just over $3,900 for 1,500 marine specimens representing 78 species including sharks, rays, moray eels, sea turtles, and other animals never before displayed in the southwest United States.

Figure 13. Photos of the construction of the Marine Wing taken by Jeff Moore in 1963. Top left, the North end of the 1936 building removed for expansion (top right, bottom left). The Marine Wing would make the Dallas Aquarium the first facility in the world to rely on artificial seawater, a radical concept at the time. Many firsts were achieved, including the first elasmobranchs to be housed in artificial seawater, and the first inland facility to house sawfishes. At bottom right between the aquarium and the classic Ford pickup one can see the Fair Park Bandshell, a 4,000-seat amphitheater where Texas guitar legend Stevie Ray Vaughn would play one of his first concerts (while still in high school) just a few years after this photo was taken.

Since the re-opening of the expanded facility in 1964 the aquarium continued to display a diverse collection of aquatic life with much success in husbandry as evidenced by breeding successes and longevity of the collection; but the by the 1980s and 1990s it was becoming increasingly apparent that the building was steadily falling into a greater state of disrepair. In 1991 the institution's AAZPA accreditation was tabled citing concerns of the structural integrity of the roof and ceiling in the marine wing, lack of ADA compliant restrooms, and other concerns.

In response to these concerns a multifaceted program of repairs and improvements was begun, starting with roof repairs and the removal of the disintegrating barrel-vaulted ceiling over the marine wing in 1991 and construction of a new expansion including a new 12,000-gallon Amazon exhibit, public restrooms, and facilities for research and breeding of species of conservation concern which was completed in 1994. Rehabilitation and replacement of many of the concrete tanks from the 1964 renovations were also begun, and these were completed in 1996. Around the same time the aquarium added the adjacent 1930 Christian Science Monitor Building as an education annex to hold classes, camps, and other activities. Work began on the annex in 1996 with foundation repairs and culminated with a complete renovation and restoration of the building in 1999. The crumbling concrete vats in the aquarium proper used to mix and store seawater were repaired and retrofitted with fiberglass inner tanks and backfilled with gravel for structural support in 2000.

Figure 14. Jeff Moore, longtime curator of the Dallas Aquarium at Fair Park. At left, Jeff receives a sawfish from SeaArama in Galveston Texas in 1977, this animal replaced one that was shipped to the Vancouver Aquarium, transported by Dallas Aquarium alumni Gerrit Klay. See D&C (Hewlett, 1977), pp.41-43 for an account of this transport. At middle Jeff showing a child a *Macrobrachium* sp. and its molt in 1971. At right Jeff painting a mural on the glass of the front doors of the aquarium in 19XX. Jeff authored numerous D&C articles, including 1959 p.8, 1963 p. 14, and 1965 p.8 (Moore, 1959; Moore 1963; and Moore, 1965).

As a result of these problems the aquarium was nearly closed, but between 2001 and 2009 the City of Dallas Parks and Recreation Dept. and Dallas Zoo leadership developed a plan to completely renovate, expand, and re-open the aquarium. The facility was to be re-branded as a Children's Aquarium to fill a niche market identified as a best fit amid the other local facilities (Dallas Zoo, Fort Worth Zoo, and the Dallas World Aquarium). A master plan was commissioned of Brown, Reynolds, and Watford Architects, Inc. and two major bond packages were presented to the Dallas voters in 2002 and 2004 totaling 8.2 million dollars to save the facility. In 2009 after the State Fair of Texas the facility was closed to the public for the first time since the 1963-64 renovation so that the bulk of the animal collection could be dispositioned and work begun.

Figure 15. Pierre Fontaine and Marion Toole. Pierre Fontaine (left in 1936, center in 1968) was the Dallas Aquarium's first (and third) director (1936; 1939-1968), and later also oversaw the Dallas Zoo (1953-1968) and was president of AAZPA (later AZA) until his death in 1968. Marion Toole (right) succeeded Fontaine as Director of the Dallas Aquarium and the White Rock Lake Fish Hatchery, and left in 1939 to take charge of the state's hatchery operations, and he played a role in the incorporation of the Texas Game, Fish, and Oyster Commission, the State Parks Board, and elements of the Civilian Conservation Corps into the organization now known as the Texas Parks and Wildlife Department.

Figure 16. The aquarium as it appeared in the late 1970's (photo Jeff Moore) and after re-opening in 2010 rebranded as the Children's Aquarium at Fair Park (photo Cathy Burkey, Dallas Zoo).

Figure 17. The aquarium as it appeared in the 1970's. At right the gallery of smaller tanks and cases filled with sea shells and artifacts would come to be replaced by a larger Amazon exhibit. At right the largest (shark) exhibit of the 1964 Marine Wing. Note the handrails that kept visitors three feet from the exhibits that were part of the original 1936 design, and the taxidermized specimens on the wall. Most of these stuffed fishes were donated in the 1940's and 1950's by local sportsmen and many are still housed at the aquarium. The sailfish on the right was caught in 1939 in Ft. Lauderdale, FL and the mount was done by J.J. Reese, a prolific taxidermist of the period.

Figure 18. The aquarium as it appears in the mid-1990's. The wall of small (10-30 gallon) tanks and glass display cabinets featuring artifacts as seen in Figure XX was replaced with a large amazon-themed display (left) as part of the addition of the Breeding Lab and ADA compliant restrooms in 1994. Smaller displays at right in the "lobby house" which was added in the 1980's and allowed the collection to expand dramatically to include many more small species.

Also in 2000 the antiquated wiring in the oldest section of the freshwater wing was replaced. The final major improvement came in 2001 with a $499,000 investment in shoring up structural problems in the exterior wall of the aquarium and the restoration and cleaning of the bas-relief carvings and architectural details of the aquarium façade. While these improvements from 1991-2001 went a long way towards upgrading the building ultimately, they fell short of what was needed and led to the AZA declining accreditation to the facility in 2001 amid numerous structural concerns.

Figure 19. Expansion of the aquarium in 2010, a 58,000 gallon shark tank and 8,000 gallon stingray touch tank were added. At left, the new tanks under construction; middle shows saltwater being mixed for the first time in the exhibit. At right, unloading the last stingrays late at night under the lights of the Ferris wheel the night before the re-opening. More detailed information on the renovation and metamorphosis from the Dallas Aquarium to the Children's Aquarium at Fair Park is included in a D&C article in Vol. 44 (Christie, 2013).

The 2009-2010 renovation and expansion project was designed by Halff and Associates of Dallas and Lyons-Zaremba, Inc. of Boston. The contract for construction was awarded to Phoenix One Restoration and Construction of Dallas, a company that specialized in restoration of aged buildings and had previously renovated several museums of the same vintage within Fair Park. The total project cost 10.4 million dollars and included a complete overhaul of building plumbing and mechanical systems, installation of modern life support systems (LSS) for all exhibits, roof repair, skylight replacement, asbestos abatement, removal of lead paint, and a 5,000 square foot expansion including a 58,000-gallon shark exhibit and a stingray touch tank. Modernization of the plumbing and filtration also included state-of the industry computerized controls with numerous alarms and a web interface allowing remote operation, monitoring, and automated data logging of the building's LSS. In addition to modern LSS a state-of-the industry water treatment system was also installed; these advancements together allow the facility to conserve 4.2 million gallons of water per year over its previous usage.

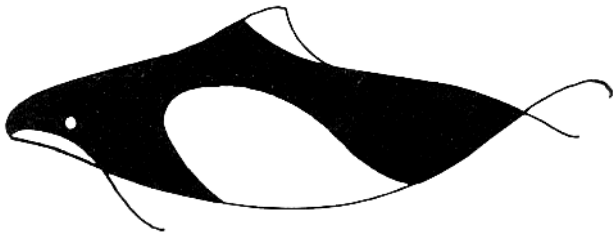


Figure 20. The psychedelic frogfish, *Histiophryne psychedelica*, the first known specimen was displayed at the Dallas Aquarium at Fair Park in the 1990s, before being preserved and sent to ichthyologist Dr. Theodore Pietsch, who described the stunning new species from the Dallas fish and additional specimens collected in Indonesia (Pietsch et al., 2009). Photos: David Hall, Seaphotos.com, used under a Creative Commons license CC 3.0.

Improvements to the aquarium's interior had to be in balance with the wishes of the Texas Historical Commission (as the building is a registered historic landmark), but significant improvements were made to modernize the look of the facility by removing the handrails that separated visitors from tanks and by raising the floor to allow children a better view of the display animals. Geometrically irregular islands housing tanks in the middle of the lobby with brightly colored kid-friendly graphics serve to add additional exhibit space and make the space feel less like a long hallway which encourages wandering and exploration by children. Teacher's stations were installed throughout the building allowing kids to touch artifacts and participate in educational activities. Technological improvements included installation of large LCD screens through the aquarium and addition of a public address system and wireless microphone capability for general announcement and for use during feeding demonstrations. A gift shop was installed and an existing room was remodeled to serve as an educational space hosting classes, camps, and birthday parties. Landscaping surrounding the building was also improved, and a fountain and banner were added to the front walkway from 1st avenue.

Prior to 2009 the facility totaled 24,367 square feet with 6,144 square feet of public space and held 152,000 gallons of fresh and sea-water. After re-opening in 2010 the facility totals 29,354 square feet with 11,125 of public space and holds 235,000 gallons of water. Until its closing the aquarium served as a place for children and adults alike to explore and learn about the diversity of aquatic life not just of Texas, but all the world's oceans.

Figure 21. Charles J. Yancey (left), Senior Aquarist for over 30 years who mentored countless staff, and staunch advocate for desert pupfishes (pictured here with one of the last two *Megupsilon aporus*, which he saved from extinction in 1992 and bred through 27 consecutive generations at the Dallas Aquarium). Charles is a supremely talented aquarist and one of the most interesting people ever to work in an aquarium anywhere (in the words of Hunter S. Thompson: "one of god's own prototypes"). At right, walking batfish, *Ogcocephalus cubifrons*. The aquarium conducted research into the husbandry of these fishes for 25 years, enabling their captive life expectancy to be measured in decades rather than months (see Schleser and Alvarado, 1992; Christie et al., 2016); the aquarium was also the first to document reproduction in any member of the family Ogcocephalidae (Christie et al., 2020). A clip of this reproduction taken by Children's Aquarium alumni John Foster was featured by comedian Ze Frank in an educational (and humorous) video available at this link: https://www.youtube.com/watch?v=B6Lh-TB2_mA

In another 84 years we wonder how many people will remember the impact that the Dallas Aquarium at Fair Park had on our industry, will as many people remember the aquarium as remember Bluffton? The practice of aquarium-keeping as we know it was refined and advanced here, and part of that will be forever ingrained in the very DNA of the science of modern aquatic animal husbandry. We would like to think that some of the legacy of discovery, conservation, and stewardship will live on through the memories of the more than 15 million visitors who came to the aquarium over 84 years.

A public aquarium serves as a window to the sea for many visitors who will never have the chance to dive or snorkel the world's oceans, and most importantly as the first face-to-face encounter many young visitors have with fishes and other marvels of aquatic and marine wildlife. If only 1% of aquarium visitors since 1936 were inspired to care about wild animals and wild places, then perhaps more than a hundred thousand people were changed by their experience visiting the aquarium in Fair Park. Long after the tanks are drained and the doors closed, the legacy of the aquarium will live on in the memories of visitors, the skills acquired by aquarists, and the other aquaria who carry on this noble work of striving to inspire the public to share an appreciation for the denizens of the sea, found only beneath the waves.

Acknowledgements

This work is dedicated to all the staff, living and deceased, who served the Dallas Aquarium throughout its history, including Pierre Fontaine, Jeff Moore, Marion Toole, Les French, Gerrit Klay, Mark Yarbrough, Allen Dixon, Jimmie Davenport, Dr. John Anderson, Lawrence Curtis, Glynn Drake, Lee House, Donald Blair, James B. Murphy, Nell Ann Rose, Tom Jordan, Charles J. Yancey, Martin P. Conricote, Eric Julius, Zelda Montoya, John W. Foster IV, Lyssa Torres,

Chris Fenwick, Helen Arceneaux, Phi Nguyen, Bob Huntington, Brian Potvin, Joseph Mazzola, Jeremiah Seymour, Kari Kolton-Zajackowski, Ken Billin, Ellen Zhao, Jake Morrison, Rafael Calderon, Alicia Byers, Chase Stryhal, James Stryhal, Jessica Nishimoto, Natali Berry, Laura Gratke, Laura Wandel, Savannah Dosher, Jorge Chavez, Deadre Henderson, Courtney Dunn, Allissa Rodriguez, Dr. Richard Cohen, Flora Cohen, Janelle Barron, and many, many others.

Figure 22. The Children's Aquarium at Fair Park staff in 2015. Back row, from left to right: John Foster, Martin Conricote, Chris Fenwick, Barrett Christie, Stephen Walker. Front row, from left to right: Bob Huntington, Eric Julius, Zelda Montoya, Charles Yancey. Photo Stephen Walker.

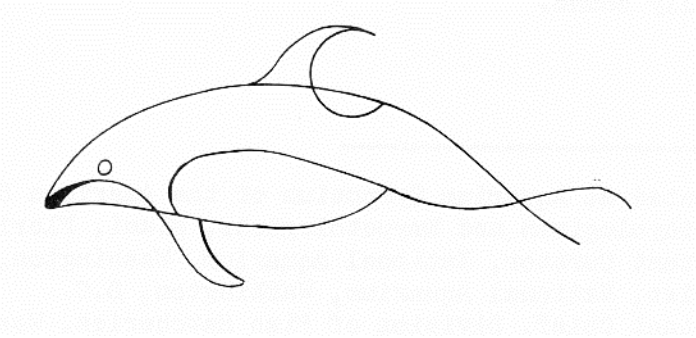


Figure 23. The current (and possibly final) staff of the Children's Aquarium at Fair Park, who are presently overseeing the sad duties of dispositioning the entire animal collection and moth-balling the facility. Pictured on the front portico to allow for social distancing (note the intricate carvings of Gulf of Mexico marine life over the front doors). Back row, left to right: Savannah Dosher, Jorge Chavez, Deadre Henderson, Alicia Byers, Alissa Rodriguez. Front row, left to right: Chase Stryhal, Jessica Nishimoto, Natali Berry, Laura Gratke, Laura Wandel.

References

Bosman, B., B. Christie, M. Hart, J. Morton, and C. Randklev. 2015. Host confirmed for *Potamilus amphichaenus* and *Potamilus metnecktayi*. Ellipsaria. 17(4): 15-16.

Cheatum, E. P., Fontaine, P. and Longnecker, W.M., 1943. Fish Culture in North-Central and Northeast Texas. Southern Methodist University Press. 80p.

Christie, B. L. 2013. The metamorphosis of the Dallas Aquarium at Fair Park into the Children's Aquarium at Fair Park, with historical annotations. Drum and Croaker 44: 4-15.

Christie, B. L., P. Z. Montoya, L. A. Torres, and J. W. Foster. 2016. The natural history and husbandry of the walking batfishes (Lophiiformes: Ogcocephalidae). Drum and Croaker 47: 7-40.

Christie, B. L., L. A. Torres, and J. W. Foster. 2020. First estimate of the fecundity of *Ogcocephalus cubifrons* (Lophiiformes) with notes on spawning behaviour. Cybium. 44(1): 69-71.

Hewlett, K. G. 1977. Saga of a sawfish. Drum and Croaker. 17(1): 41-43.

Mohan, P. 2020. Trends in aquarium openings and closings in North America: 1856-2020. Drum and Croaker. 51: 86-98.

Moore, J. 1953. News from the aquarium world: Dallas, TX. Drum and Croaker. 2: 8.

Moore, J. 1963. Longevity at the Dallas Aquarium. Drum and Croaker. 5(1): 14.

Moore, J. 1965. Dallas Aquarium. Drum and Croaker. 7(1): 8.

Pietsch, T., Arnold, R. and Hall, D., 2009. A bizarre new species of frogfish of the genus *Histiophryne* (Lophiiformes: Antennariidae) from Ambon and Bali, Indonesia. Copeia. 2009(1): 37-45.

Randall, J. E. 1963. Three new species and six new records of small serranoid fishes from Curaçao and Puerto Rico. Studies on Fauna of Curacao and other Caribbean Islands. 19(1): 77-110.

Roberts, D. T., D. M. Schleser, and T. L. Jordan. 1995. Notes on the captive husbandry and reproduction of the Texas salamander *Eurycea neotenes* at the Dallas Aquarium. Herpetological Review. 26(1): 23-24.

Schleser, D. M., and T .P. Alvarado. 1992. Suggested aquarium husbandry for ogcocephalidae – the walking batfish. American Association of Zoological Parks and Aquariums Regional Conference Proceedings. (92): 774-777.

Appendices I-III: Timelines of Governance and Renovations (1936-2020) and Leadership (1913-2020) at the Dallas Aquarium and Related Facilities (Fair Park Fish Hatchery/White Rock Lake Fish Hatchery)

Timeline of Governance

1936-1954 .....Independent Organization within the City of Dallas Parks Dept.
1954-1981 ..... Associated with the Dallas Zoo, City of Dallas Parks Dept.
1981-1989 ..... Associated with the Dallas Museum of Natural History, City of Dallas Parks Dept.
1989-2009 .....A department of the Dallas Zoo, City of Dallas Parks Dept.
2009-2020 ...A department of the Dallas Zoo, Dallas Zoo Management, Inc. 501(3)(c) non-profit.

Timeline of Renovations/Improvements

1951..... Replacement of ceiling
1963-64 .....Addition of marine wing
1991..... Roof repair and marine wing ceiling removed
1993-94 .....Expansion of aquarium including Amazon exhibit, breeding lab, restrooms
1994-96 .....Repair and replacement of concrete tanks in marine wing
1996.....Aquarium adds annex, foundation work done to building
1998-99 ..... Aquarium annex renovated and restored
2000..... Seawater mixing/storage vats replaced, electrical wiring in freshwater wing replaced

2001..... Structural repairs to exterior walls and restoration of bas-relief architectural details
2001-02 ..... Master plan developed by Brown, Reynolds, and Watford Architects, Inc.
2002-04 ..... Bond packages voted on by Dallas electorate to renovate and expand aquarium
2009-10 ..... Aquarium closed for major renovations and expansion
2020..... Aquarium Permanently Closed?

Timeline of Aquarium Leadership
1913-2020

Robert Goodfellow Superintendent of the State Fish Hatchery at Fair Park	1913-1915
J. L. French Superintendent of the State Fish Hatchery at Fair Park	1915-1923
M.L. Cartwell Superintendent of the State Fish Hatchery at Fair Park	1923-1934
Pierre A. Fontaine Director of the Dallas Aquarium	1936
Marcus Evans Superintendent of the White Rock Lake Fish Hatchery	1937-1938
Marion Toole Director of the Dallas Aquarium	1936-1938
Marion Toole Director of the Dallas Aquarium Superintendent of the White Rock Lake Fish Hatchery	1938-1939
Pierre A. Fontaine Director of the Dallas Aquarium Superintendent of the White Rock Lake Fish Hatchery	1939-1953
Pierre A. Fontaine Director of the Dallas Aquarium Director of the Dallas Zoo	1953-1968
Les French Superintendent of the Dallas Aquarium	1953-1955
Jeff W. Moore Superintendent/Curator of the Dallas Aquarium	1955-1981
Allen Dixon	1981-1984

Superintendent/Curator of the Dallas Aquarium

Larry Calvin 1984-1986
Interim Manager of the Dallas Aquarium (…demoted from position as zoo director)

Louis Gorr 1980-1986
Director of the Dallas Museum of Natural History & Dallas Aquarium at Fair Park

Henry Schulson 1986-1987
Director of the Dallas Museum of Natural History & Dallas Aquarium at Fair Park

Steve Robertson 1986-1989
Curator of the Dallas Aquarium

Warren Iliff 1989-1991
Director of the Dallas Zoo and the Dallas Aquarium at Fair Park

Dr. James B. Murphy 1989-1990
Curator of Herpetology and the Dallas Aquarium

Dr. David M. Schleser 1991-1997
Curator of the Dallas Aquarium at Fair Park

Richard W. Buickerood 1992-2006
Director of the Dallas Zoo and Dallas Aquarium at Fair Park

Brian J. Potvin 1997-2011
Curator of the Dallas Aquarium at Fair Park

Gregg Hudson 2006-Pres.
Director of the Dallas Zoo and the Dallas Aquarium/Children's Aquarium at Fair Park

Stephen D. Walker 2010-2017
General Manager of the Children's Aquarium at Fair Park

Ruston W. Hartdegen 2017-Pres.
Curator of Herpetology and Aquatics, the Dallas Zoo and Children's Aquarium at Fair Park

THE 1955 JOINT SYMPOSIUM ON AQUARIA: THE FIRST PUBLIC AQUARIUM MEETING IN NORTH AMERICA?

As documented in Copeia, 1955 (4): 318-319.

Annotated by Pete Mohan

In the absence of abstracts from the cancelled 2020 Regional Aquatics Workshop (RAW), or any announcement of a 2021 RAW (virtual presentations may yet occur), I've pulled a bit of conference history from my files.

For sixteen years (1955-1970), the public aquarium community held its "Annual Aquarium Symposium" in conjunction with the annual meeting of the American Association of Ichthyologists and Herpetologists (ASIH). The list of papers presented here will be followed by records from other years in future issues of Drum and Croaker. These symposia are the earliest regular meetings of North American public aquarium professionals of which I am currently aware. As noted in this issue's "Drum and Croaker 50 Years Ago," these annual presentations moved to AAZPA (now AZA) in 1971. Most aquarium husbandry presentations have migrated to RAW over subsequent decades, as AZA's focus on other aspects of zoo and aquarium operations has broadened.

Many of the early founders and contributors to Drum and Croaker (D&C) were also presenters at these symposia. It is likely that some of more humorous contributions to the early issues of D&C were inspired by Dopeia, a spoof version of ASIH's journal Copeia, that ran from 1940 to 1990.

The last sentence in the account of the "Evening Smoker" (below) sums up the value and importance of the social aspects of in-person meetings. Whether we are directors, curators, or aquarists, we look forward to our evenings at AZA, RAW, NAC, NAW, EUAC, IAC, (add your regional conference acronym here), etc. While the COVID-19 crisis of 2020-2021 has kept us all physically apart, we've been able to continue to share information via virtual conferences. Technology has been a true blessing but can't replace handshakes, hugs, mingling, or the din of a crowded room. I hope to clink glasses with you all in person in 2022.

The Joint Symposium on Aquaria
June 28, 1955
Morrison Planetarium Auditorium
California Academy of Sciences

<u>The afternoon session was moderated by Dr. Earl S. Herald.</u>

"AQUARIUM DESIGN, CONSTRUCTION, AND MATERIALS

The Multisystem Aquarium: The New York Aquarium.
Christopher W. Coates, New York Aquarium, New York Zoological Society.

The Oceanarium: The First Six Months of Marineland of the Pacific.
Kenneth S. Norris, Marineland of the Pacific

The Marine Station Aquarium: T. Wayland Vaughan Aquarium-Museum.
Sam D. Hinton, Scripps Institute of Oceanography.

The Inland Aquarium: The New James R. Record Aquarium.
Lawrence Curtis, Fort Worth Zoo and Aquarium.

The Use of Plastics in the Aquarium.
Ross McBride, Ocean Aquarium, Hermosa Beach, California.

General Discussion of Materials: Pumps, Pipes, Valves, Tanks, etc.
Leader: Earl S. Herald, Steinhart Aquarium, California Academy of Sciences.

BEHAVIOR OF FISH AND OTHER AQUATIC VERTEBRATES

Special Problems in the Maintenance of an Oceanarium Exhibit.
F. G. Wood, Jr., Marine Studios, Florida.

Behavior of Temperate Marine Fishes.
Earl S. Herald, Steinhart Aquarium, California Academy of Sciences.

Behavior of Tropical Freshwater Fishes.
George S. Myers, Stanford University.

Behavior of Trout and Salmon.
Murry A. Newman, University of British Columbia.

Collection and Confinement in Captivity of Two Species of Pacific Dolphins, *Delphinus bairdi* and *Lagenorhynchus obliquidens*.
David Brown, Marineland of the Pacific.

Arrival of Tursiops from Florida.
David Brown, Marineland of the Pacific."

The evening session was moderated by Dr. Christopher W. Coates.

"Porpoises of the Atlantic Coast.
F. G. Wood, Jr., Marine Studios, Florida.

WATER IN THE AQUARIUM WORLD

Portable Salt Brine for Dilution by Inland Aquariums.
Maurice Rakowicz, San Francisco Aquarium Society.

DISEASES AND DISEASE CONTROL

Epizootics in California Freshwater Fishes.
Joseph H. Wales, California Department of Fish and Game.

Trout Hatchery Diseases vs. Hatchery Practice and Design.
Harold Wolf, California Department of Fish and Game.

Demonstration of Control for *Oodinium ocellatum*.
Robert P. Demster, Steinhart Aquarium, California Academy of Sciences.

FOOD AND NUTRITION

Problems in the Use of Brine Shrimp and a Fish Food.
Maurice Rakowicz, San Francisco Aquarium Society.

COLLECTION AND TRANSPORTATION OF FISHES

Salt-Water Fish Transportation Equipment.
Kenneth S. Norris, Marineland of the Pacific.

Demonstration of the Venturi Principle as Applied to the Circulation System of a Fish-Planting Truck.
Personnel of the California Department of Fish and Game.

The Use of Metabolic Inhibitors in Collecting and Transporting Fishes.
Wm. McFarland, University of California, Los Angeles.

The Aqualung in the Collection of Living Materials for Aquarium Display.
Conrad Limbaugh, Scripps Institution of Oceanography.

THE PHILOSOPHY AND PRACTICE OF AQUARIUM EXHIBITION

Aquascaping Small Display Tanks in Public Aquaria.
Donald A. Simpson, Steinhart Aquarium, California Academy of Sciences.

Legends and Labels.
Sam D. Hinton, Scripps Institution of Oceanography."

Evening "Smoker" *(defined as an informal gathering)* on June 29

"In the evening there was a most enjoyable Smoker at the Steinhart Aquarium. In addition to the excellent display of fishes, the members and their guests were treated to an exhibit of living reptiles and amphibians from the western United States. Special demonstrations of the feeding

of Archer fish and Angler fish plus examples of experiments to test responses of fishes to electrical fields and temperature were of unusual interest. The appropriate surroundings, the pleasure of speaking to colleagues, and the diverse refreshments all contributes to a most convivial atmosphere."

BUOYANCY COMPENSATION DEVICE FOR A LARGE MOUTH BASS, *Micropterus salmoides*, TO HELP ALLEVIATE NEGATIVE BUOYANCY DISORDER

Melissa Morrow, Aquarist II mcmorrow@wondersofwildlife.org

Wonders of Wildlife National Museum and Aquarium, 500 West Sunshine Street, Springfield, MO

Introduction

Negative Buoyancy Disorder is a common affliction for fish in the aquarium industry. In most cases it occurs spontaneously with no predisposing causes and has a poor prognosis for long term survival (Wildgoose, 2000). Most commonly Negative Buoyancy Disorder is caused by one of multiple factors such as fluid accumulation in the gas bladder, gas bladder rupture, displacement, infection, poor nutrition, egg bound, or bacterial infection (Wildgoose, 2007). Although Negative Buoyancy Disorder and a possible treatment has not been significantly researched or recorded in scientific literature, it has been reported in hobbyist press and briefly in the Fish Veterinary Journal. Using that collective information, the Wonders of Wildlife team was able to create a Buoyancy Compensation Device to help alleviate the Negative Buoyancy Disorder effecting a Large Mouth Bass, *Micropterus salmoides*.

Situation

On the evening of 6/22/19 a female Large Mouth Bass (LMB) was found on the bottom of her exhibit ventral side up and wedged under a log. She was recovered and transferred to the quarantine facility at that time. Observations were made on 6/23/19, and after no change in physical condition, the decision was made to perform surgery on the LMB to assess and possibly fix the swollen coelom. On 6/24/19 surgery was performed. The ovaries were egg bound and obstructing the other organs, so a bilateral salpingo-oophorectomy was performed. The gas bladder was also examined and excess air was removed, no fluid was found within it. The veterinarian did notate that all the organs were covered in fat indicating fat necrosis, and that the liver was pale yellow. After surgery and post-op recovery the LMB was placed back in the quarantine holding facility. On 6/25/19 the LMB was still lying upside down on the bottom of the exhibit. This was causing lesions to form on the face from constant rubbing on the bottom of the exhibit. To aid the LMB a buoyancy compensation device (BCD) was created.

Six BCD designs were created during the duration of the LMB quarantine. Each design had benefits and complications. All designs had complications with inhibiting the slime coat. The slime coat supports the fish in drag resistance when swimming, protects fish from parasites and infection, as well as promotes healing when injured. Creating a BCD that would not inhibit the slime coat in this particular instance was the most difficult obstacle to overcome.

Design #1

This design was created with a dive weight belt tethered by 10-lb test fishing line to a central point on a pool noodle with a 1 lb dive ankle weight attached to the bottom (Figure 1). This design was not practical and its use is not recommended. The belt would slip off the LMB whenever the animal would try to move (Figure 2). Design #1 only worked for a very finite amount of time, and then was replaced by Design #2 on the same day.

Figure 1.

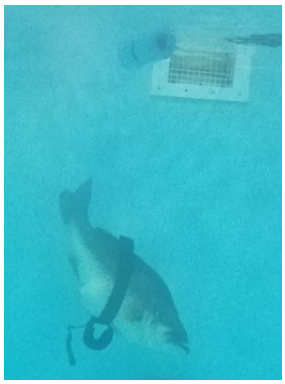


Figure 2.

Design #2

This design was created with a rigid nylon micron filter bag tethered by 10-lb test fishing line to a central point on a pool noodle with a 1 lb dive ankle weight (Figure 5). Later modified to eight 0.5 oz fishing egg weights attached to the bottom (Figure 3). The nylon bag was zip tied snugly around the LMB and was able to hold the LMB in an upright position and permitted the LMB to freely move her pectoral fins and swim (Figure 4). It also did not interfere with the sutures from surgery. The weights on the bottom of the design were used to keep the LMB from rotating ventral side up, as it was when the animal was on the bottom of the exhibit. Later we found that the addition of the weight was unnecessary and that the LMB could remain upright without them. Design #2 worked from 6/25/19 until 7/1/19. At that point the LMB's abdominal swelling had gone down with treatment and the BCD did not fit anymore. This caused the LMB to slip from the BCD repeatedly. We also found that the nylon material of the bag was creating abrasions on the LMB's body due to it rubbing off the slime coat (Figure 6).

Figure 3.

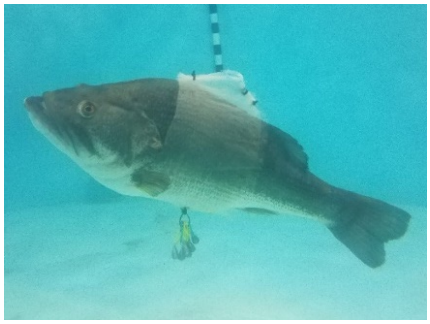


Figure 4.

Figure 5.

Figure 6.

Design #3

This design was created with a 1/8" mesh netting bag with a large zip tie support "belt" tethered by 10-lb test fishing line to a central point on a pool noodle with no weights (Figure 7). This mesh material was chosen due to the 1/8" hole diameter allowing the slime coat to be less inhibited. It also did not interfere with the sutures from surgery. Unfortunately design #3 only worked for approximately 3 hours. It had multiple design flaws. When attempting to zip tie the mesh bag snugly around the LMB the material would bunch up when the pool float was tethered to it, making the LMB fall out of the apparatus. When trying to compensate for the bunching, the use of the belt attached to the float created balancing issues and would make the animal pitch forward and fall out (Figure 8). The LMB was placed into a floating isolation basket on the night of 7/1/19, then on 7/2/19 design #4 was created.

Figure 7.

Figure 8.

Design #4

This design was created with a Spanx® material supported by a ridged airline tethered by 10-lb test fishing line to a pool noodle on a loop with no weights (Figure 9). The Spanx® material was chosen due to its conformity ability to the changing body condition of the LMB (Figure 11). By adding a rigid airline on a loop through the pool noodle solved the pitching problem from design #3 and allowed the LMB to freely swim around the exhibit and didn't interfere with the sutures from surgery (Figure 10 and Figure 12). It was thought that this material would not inhibit the slime coat due to its water absorbency capability keeping it wet, however we later learned that this material when wet does not allow free movement of the LMB slime coat, but sticking (Figure

13). Ultimately causing pressure necrosis (Figure 14). Design #4 was used from 7/2/19 to 7/10/19, then was removed due to pressure necrosis complications.

Figure 9.

Figure 10.

Figure 11.

Figure 12.

Figure 13.

Figure 14.

Design #5 and #6

Two more designs were created with the 1/8" mesh netting bag supported by a ridged airline tethered by 10-lb test fishing line to a pool noodle on a loop with no weights. Design #5 would have been sewn around the LMB to create a custom fit to fix the slipping problem (Figure 15). Design #6 would have used zip ties and mimicked design #4 just with a different material to possibly fix the pressure necrosis problem (Figure 16). Unfortunately, the LMB passed away before these two designs could be tested.

Figure 15.

Figure 16.

Alternative Design

After the design problems associated with using bags to create a BCD, the Wonders of Wildlife team decided to go a different design direction when faced with another case of negative buoyancy disorder in an Orbicular Batfish, *Platax orbicularis*. This design was created using ¼" flexible airline looped around the body of the Batfish, through a small pool noodle, and tied at the top (Figure 17). This design worked well, and it did solve the slime coat inhibiting problem for the most part. The only area effected by the BCD was at the base of the dorsal fin and under the pectoral fins where the airlines came together. Slight rubbing was seen at these pressure points, but nowhere else.

Figure 17.

Conclusion

In studying different materials to create BCD's, any type of bag material is not ideal due to its abrasiveness. When working with aquatic animals their slime coat is extremely important. The slime coat is the glycoprotein barrier that protects the fish from bacteria, physical matter in the water, diseases and parasites. It reduces surface resistance and drag and allows the fish to glide easily through the water. This barrier also works to keep essential fluids and electrolytes in the fish (Sharpe, 2020). Thus, it is essential to the survival of the aquatic animal that this slime coat is not damaged. Each bag design in this study unfortunately inhibited or damaged the LMB slime coat in some way, therefore these previous designs are not recommended.

The alternative design created with the flexible airline was a more ideal material when creating a BCD. Its smooth surface and minimalistic contact point on the animal did not inhibit the production of the slime coat. Although some rubbing occurred on the contact points, in comparison to the bag materials and design it was superior. When creating future BCD designs, investigation into using flexible airline or another type of small smooth line material would be advantageous.

Acknowledgments

Mike Daniel – Curator, Wonders of Wildlife
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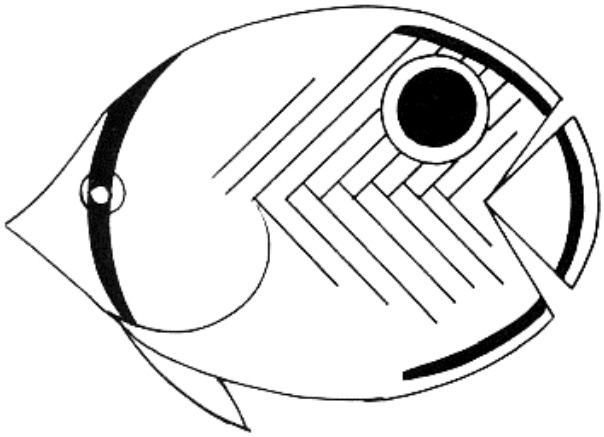
Jami Asher – Aquarist I, Wonders of Wildlife
Jordan Kukal – Aquarist I, Wonders of Wildlife
Dr. Michael Stafford – Veterinarian

References

Sharpe, S. 2020. How to preserve the slime coat on fish. The Spruce Pets. https://www.thesprucepets.com/fish-slime-coat-1379911

Wildgoose, W. H. 2007. Buoyancy disorders in ornamental fish: a review of cases seen in veterinary practice. Fish Veterinary Journal. (9): 22-37.

Wildgoose, W. H. 2000. Fish surgery: an overview. Fish Veterinary Journal. (5): 22-36.



A SEA TURTLE WETSUIT AS A THERAPEUTIC FOR BUOYANCY ISSUES

Aiyana Reissman, Animal Care Specialist- Trainer aiyana@thelivingcoast.org

Living Coast Discovery Center, San Diego, CA

Abstract

A rescued Loggerhead Sea Turtle experienced changes in her buoyancy negatively impacting her quality of life. The novel use of weighted wetsuit therapy was used to correct the problem, improving the sea turtle's ability to swim, eat, and breathe.

Sapphire's Background

The story of Sapphire the Loggerhead Sea Turtle began in the Florida Keys. She presented to The Turtle Hospital in Marathon, Florida with injuries consistent with a boat propeller strike including a large crack across the posterior carapace, blindness in her left eye, digestive disruption, and buoyancy imbalance. Sapphire was deemed non-releasable and found her home across the country in Chula Vista, California at the Living Coast Discovery Center arriving in 2014. Here Sapphire is able to serve as an ambassador for her species helping us educate the public about conserving our natural spaces and coexisting with our wild neighbors. Sapphire is clearly recognized by our guests as she has a tendency to do a "turtle headstand" as she swims and sleeps due to her buoyancy injury.

Our team continues to ensure Sapphire is able to thrive with her injuries. Weights epoxied to her caudal carapace keep her in a delicate balance allowing her to dive to the bottom of her enclosure in order to sleep and eat but not weigh her down as much to prevent her from surfacing to breathe. Sapphire receives a specially prepared diet of deboned fish, de-shelled shrimp, and de-penned squid to help prevent digestive upset and intestinal gas accumulation. She is also target trained to allow aquarists to get a close look at her and feed her any medications.

The Problem

In April 2020 Sapphire's buoyancy worsened and impacted her ability to eat and sleep at the bottom of the pool. As her weights had not been adjusted since 2012, we made a plan to add additional weights. Due to the COVID-19 pandemic, we were closed to the public and were able to move Sapphire over to the Ray Touch Pool. This shallower pool allowed us to work with her in a safer, more accessible environment and to affix the new weights while still allowing her ample space to exercise and explore.

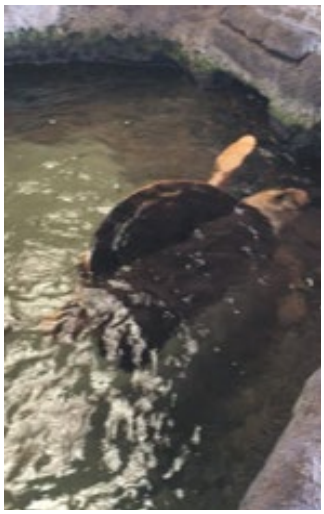


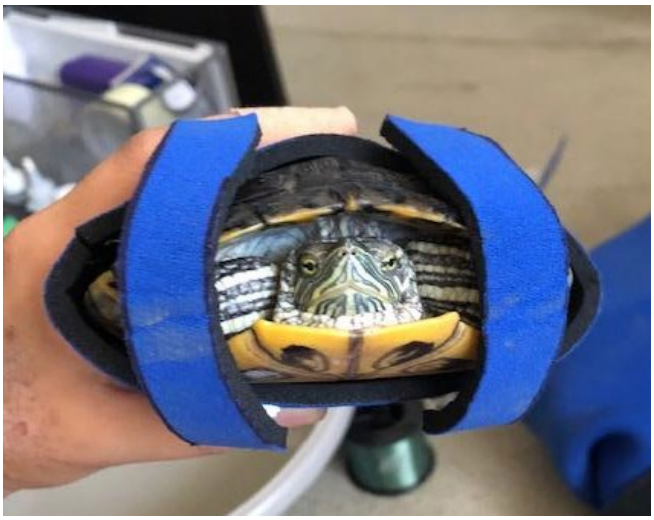
Figure 1. Sapphire pictured at the peak of the positive buoyancy on her left side.

During the first two weeks in the new enclosure, we observed the location of the buoyant portion of her shell seemed to shift, sometimes changing on a daily basis. Hesitant to add new weights in the wrong spot, we began to brainstorm possible causes and treatments for her buoyancy issues and over the next few months we tried diet changes, medications, enemas, temporary weights, and even massages. Due to Sapphire's size, we were unable to find or access x-

ray or a computerized tomography (CT) machines large enough for us to view what may be going on internally. In August, Sapphire's buoyancy shifted further to her left side at its most drastic shift as seen in Figure 1. After several months, attempts, and consultations with other facilities and veterinary specialists we were running out of traditional options as we began to have serious conversations regarding Sapphire's quality of life in her current state.

The Solution

Our team agreed the best idea would be to try different locations for weights before permanently securing them. The first attempt was a standard SCUBA weight belt and weights held in place by zip ties marine epoxied to her carapace. We realized we needed something that could hold closer and tighter to her shell, like neoprene. We began to play with the idea of a wetsuit with pockets to hold the weights in different locations. We first tried it on a smaller scale first using supplies found around the facility. An old wetsuit and Velcro were utilized to make the prototypes for our willing volunteer, a red-eared slider named Michaelangelo.



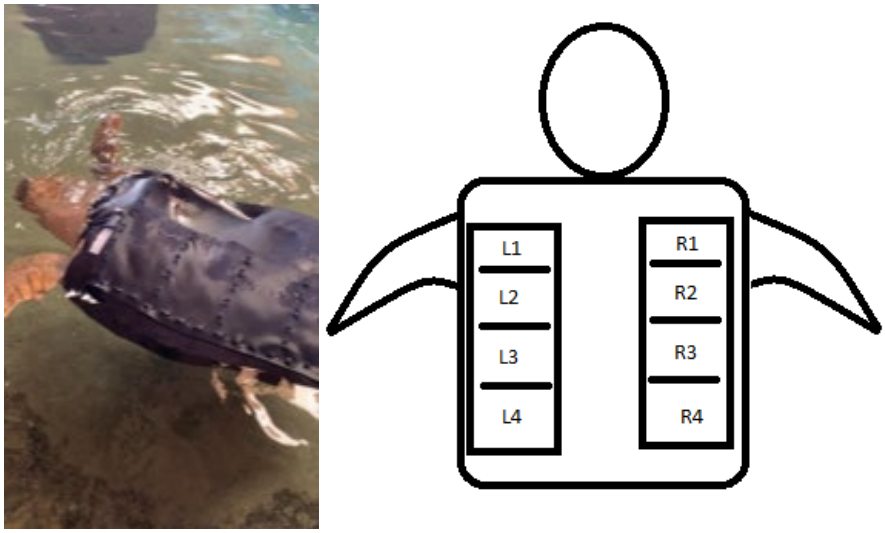
Figures 2 and 3. Michaelangelo the red-eared slider played guinea pig for the day as we tried out different styles for turtle wetsuits.

Once we settled on a design, we up-scaled the design and made the first prototype for Sapphire consisting of an old wetsuit and a dive weight belt. We were able to stabilize her in the water but the weights and suit would fall off throughout the day.

During this time, I also reached out for some outside support at O'Neill Wetsuits and they offered to help us with supplies and a custom sea turtle wetsuit. With guidance and a nice new sheet of neoprene, we created the second prototype complete with Velcro fasteners and zip tie pockets.

The final design was sent off to our new friends at O'Neill Wetsuits and they began building a sturdier piece.

Figures 4 and 5. Sapphire immediately after fitting her with prototype 1.



Figures 6 and 7. Sapphire immediately after fitting her with prototype 2 and a diagram of the weight pockets.

Figures 8 and 9. Sapphire immediately after being fit with the final wetsuit design from O'Neill Wetsuits.

Results

Trial 1

Trial 1 took place from October 1st to October 28th. Changes were made in the morning to allow aquarists to monitor her behavior throughout the day as seen in Table 1. Two prototypes were tried and improved upon. The second prototype had pockets to allow us to pinpoint the weights more accurately. We also switched from using metal dive weights to cloth drive weights which helped prevent them from sliding out. The weight was varied from 5 lbs (2.27 kg) to 2 lbs (0.91 kg) over the course of the trial.

Table 1. Observations and actions outlined over the course of Trial 1 with prototypes one and 2.

Date	Pre-Observation	Action	Post-Observation
Oct 1	Orientation is perpendicular to pool bottom with left side positively buoyant.	Secured prototype 1 with 5 lbs of weigh.t	Orientation is parallel to the pool bottom, she is active, able to surface well for breath.
Oct 5	Prototype 1 is too loose in front and in back causing drag.	Secured prototype 2 with 5 lbs of weight in L4 pocket.	Orientation is parallel to the pool bottom, she is active.
Oct 18	Left side is tilting lower in the water column.	Decreased weight down to 4 lbs in L4.	Orientation returned to parallel, she remains active and interested in food.
Oct 25	Back end is lower in the water.	Shifted weights to 2 lbs in L3 and 2 lbs in L2.	Stable, active, eating.
Oct 26	Sitting lower in the water overall.	Reduced weight to 1 lb in L3 and L2.	Surfacing well for breath, active eating.
Oct 28	Sitting lower in the water overall.	Removed wetsuit.	Returned to original buoyancy.

Trial 2

Having noticed only a slight shift in her buoyancy again on the left hand side, Trial 2 only lasted from December 10th to December 21st before the problem was corrected as seen in Table 2. Now having a better understanding of how her body would react, fewer changes needed to be made throughout the trial. The weights varied between 1 lbs (0.45 kg) and 2 lbs (0.91 kg).

Table 2. Observations and actions outlined using wetsuit prototype 2 during Trial 2

Date	Pre-Observation	Action	Post-Observation
Dec 10	Orientation is slightly shift with some positive buoyancy on the left side.	Secured prototype 2 with 1 lbs in L3.	Stable, good respiration, good appetite.
Dec 13	Orientation is still slightly buoyant on left hand side.	Added 1 lbs for a total of 2 lbs in L3.	Stable, good respiration, good appetite.
Dec 21	Rear of carapace is lowered.	Removed wetsuit.	Returned to neutral buoyancy.

Analysis

The success or failure of the wetsuit was determined through observation of Sapphire's orientation in the water and behavioral analysis. Sapphire's orientation was determined "stable" if she was able to swim with her body parallel to the bottom of the pool. An improvement to behavior was determined based on her ability to surface and breathe, her appetite, her activity level.

In Trial 1 we saw an immediate change from positive buoyancy on the left side to a stable orientation as soon as the wetsuit was put on and weights were adjusted. The appropriate weight and location were determined based on this observation as well and adjusted throughout both trials based on daily changes in her orientation. Weights were always adjusted in the morning to allow for observations throughout the day before leaving her with the suit on overnight.

Because of the drastic tilt Sapphire experienced initially, it made sense that this buoyancy disruption and intestinal gas distension would inhibit her ability to eat. During Trial 1 we saw an increase of consumed food from 200 grams consumed to 600 grams consumed and a continued increase after the wetsuit therapy. In early December, we began to notice only a slight degradation in her buoyancy orientation and a decrease in her appetite which initiated Trial 2. During Trial 2 we saw an increase in her appetite as well as seen in Figure 9.

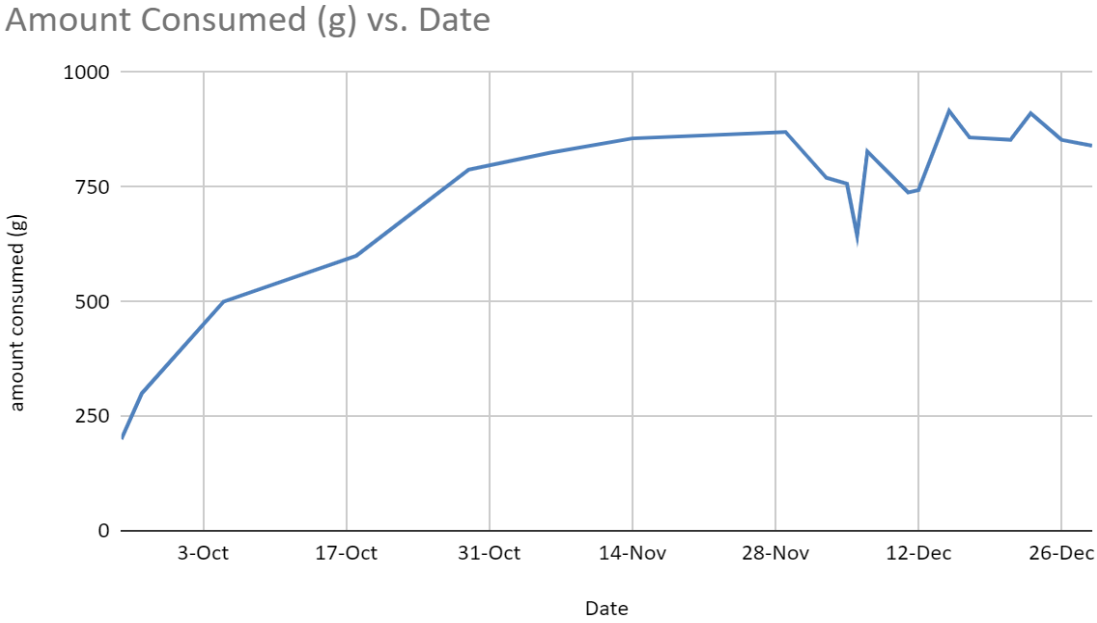


Figure 9. Overall appetite was one of our main indicators for the success of the wetsuit. This graph shows the change in appetite before, during, and after the trials with the wetsuits.

Medications were being administered at the start of Trial 1 from October 1st through October 12th. This was part of the same medication regimen implemented on July 9th. Since we historically saw improvements for Sapphire's appetite while on this medication, we cannot rule out the impact this may have had on our results in the first few weeks of the trial.

Future Trials

Sapphire has always had a buildup of gases in her coelom that have resulted in her rear end to float towards the surface. Now that we have our custom wetsuit from O'Neill Wetsuits, we will continue an attempt to correct her buoyancy further if possible through this wetsuit therapy.

We will continue to search for a permanent solution to Sapphire's buoyancy issues while keeping her comfortable through the use of this wetsuit therapy. With how often boat strikes and injuries of this nature occur with sea turtles, we hope this information can aid others or inspire ideas for innovative care.

Thank you

We would like to extend a very special thank you to our veterinarian, Dr. Todd Cecil at the Western Aquatic Animal Veterinary Services along with Greg Clarke and the rest of the team at O'Neill Wetsuits.

A BRIEF GUIDE TO AUTHORS
Updated 2021

This guide is intended for those not accustomed to using a "Guide to Authors", as provided by more formal periodicals. Historically only about 5% of *D&C* authors get this correct ☺. Please help me out, folks!

The approximate deadline for submissions is December 21st.

As always, typical Drum & Croaker articles are not peer-reviewed and content will not be edited, other than to correct obvious errors, clarify translations into English, modify incorrect or cumbersome formatting, or delete superfluous material. Other types of contributions (announcements, etc.) may be edited to meet space limitations.

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All Articles Must Adhere to the Following Basic Format:

- Use justified, single-spaced, Times New Roman 12-point font throughout (except for the title section, and figure and table legends as noted below).
- A4 users please reformat to 8 ½ x 11-inch documents (North American "letter" size).
- Keep the resolution of photographs LOW. High resolution photos make the final PDF file huge and I always compress them anyway.
- **Format the title section with the line spacing set on 1.5 lines (not another method) and using centered, boldface font. Only the title should be CAPITALIZED (except italicized *Scientific namesii*)**. When using MS Word, go to the "Home" tab, open the detail on the "Paragraph" section, and choose "1.5 lines" under spacing and make sure the before and after spacing settings are at "zero". For these settings, see "Other Things I Whine About" below.
- Double-space after your "institution name" to begin the body of your text. When correct, the title and headings formatting should look like this:

USE OF DUCT TAPE IN THE HUSBANDRY OF *Genus species* AT FISHLAND

Jill Fishhead, Senior Aquarist jfishhead@fishstinking.com

Fishland of South Dakota, 1 Stinking Desert Highway, Badlands, SD, USA

Text Format

Headings and text should look like this heading and paragraph. Use single spacing with 1" (2.54 cm) margins on ALL sides. Please indent/tab 0.5 inch (1.3 cm) at the beginning of each

paragraph (not using the space bar!) and leave a single space between paragraphs. Justify the text (see toolbar options and note how pretty the right margin of this paragraph lines up!). Section headings should be in bold (as above) at the left margin.

Please use the following format for figure legends:

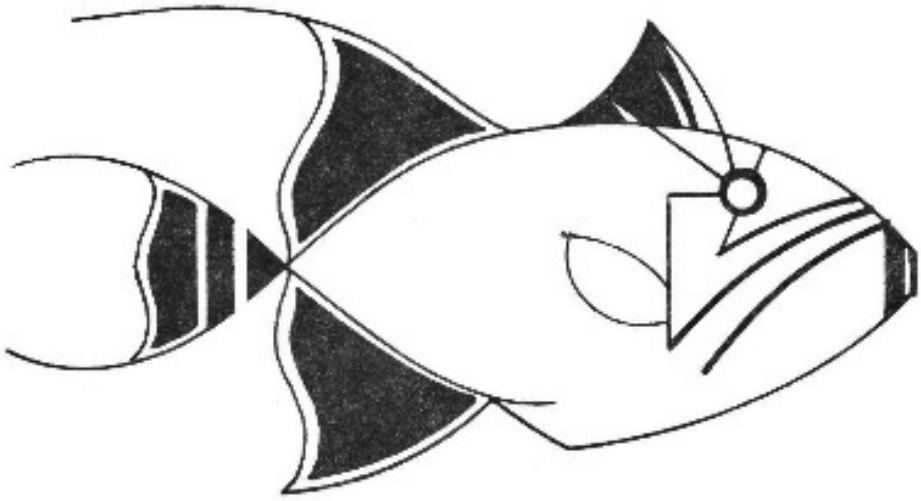


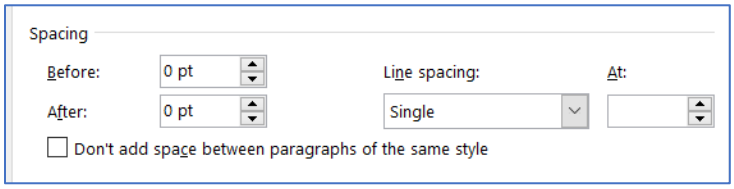
Figure 1. Legends should appear under the photo (such as this drawing by Craig Phillips) or graph in this format in 10-point font, aligned with the sides of the image or figure (center or justify). Very short legends can be centered. Photographs should be pasted into the document in the proper location by the author. All photos MUST be formatted as low-resolution files, ideally no 'larger' than approximately 300 – 500 KB. I may reduce the size (appearance on the page) of figures and photographs to save space. Photos, tables, and figures not referred to in the text may be omitted for the same reason.

Table Legends

Table legends go above the table. Otherwise, formatting is as above for figures.

Other Things I Whine About

- Please don't use Paragraph formatting to add spacing above or below lines. I have to remove all of these. Start with a single-spaced Word template, with NO before or after spacing. You will likely need to select this from the paragraph section on the home tab of Word, as the normal default template may contain unwanted 'before' or 'after' spacing.



- Use the "enter" key for all line spacings ("carriage return" for those who remember typewriters with a slidey thing on top).

- If you submit a table, put the data IN an actual table. Don't use the space bar or tabs to "line up stuff." This formatting can be lost if I have to change margins or otherwise reformat.
- Use the "tab" key to set your 0.5" indent at the start of each paragraph. It's likely your default. Don't use the space bar.
- Use bullets or numbers to make lists. It is easier to reformat these later if needed.

Short Contributions ("Ichthyological Notes")

These include any articles, observations, or points of interest that are about a page or less in length. A brief bold faced and capitalized title should be centered, the body text should be formatted as above, and **author and affiliation should be placed at the end of the piece** with the left end of each bolded line right of the center of the page. Reformatting that must be done by the editor may reduce a shorter "main" article to a note, or may bump a note up to main article status.

Reviews, abstracts, translations (with proper permissions) and bibliographies are welcome. Humor, editorial pieces, apocrypha, and serious technical articles are equally appreciated.

Literature Cited

In the body of the paper, use this method to cite authors: (Phishmonger et al., 2008; Laurel and Hardy, 2009; Frazma, 1992).

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Carrotfish. Bruce Koike