DRUM and CROAKER

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Cover Photo: Bigfin Reef Squid - Alicia Bitondo
Interior Gyotaku: Bruce Koike
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DRUM AND CROAKER ~50 YEARS AGO

Richard M. Segedi

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From: AQUARIUM DESIGN CRITERIA, Drum and Croaker Special Edition (#1), and PLANNING THE PUBLIC AQUARIUM, Drum and Croaker, September 1970, Wm. Hagen et al.

We have lived in an era when even a mediocre fish menagerie could be a box-office success. We are approaching an era when we will be expected to teach and to explain biological concepts rather than to merely exhibit specimens.

An aquarium built almost anywhere will prove to be a popular attraction. Nevertheless, to be successful, whether financially or in terms of education or recreation, it must be sited where a real need exists.

It is also a clear warning that the public will not long continue to accept the standard practice of displaying a fish in a transparent cage with a little note giving its country of origin and its "scientific" name.

Today's youth wants to know about adaptation, behavior, physiology, convergent and divergent evolution and, since it is already aware of continental drift, about speciation through isolation. Accounts of Darwin's voyages are now popular reading and the concept of evolution through natural selection and mutation is discussed at the junior high school level.

An attempt should be made to arrange exhibits in an interesting manner, avoiding the monotony of straight lines of square panes of glass. The sizes of the tanks shown are really not pertinent but it is desirable to have at least one large tank {…} in which a large community of local fishes or reef fishes, or a few porpoises can be displayed. Tanks should be arranged to avoid reflections in tank fronts.

The alignment of display tanks is intended to provide variety and to lead the public along a routine pathway, and provides considerably more display frontage than would a rectangular straight-line arrangement, and it is much more interesting. However, this plan for display tanks is more expensive to install than a straight-line arrangement, because water lines, trough drains, and the raised service platform must follow the irregular route.

As Earl Herald says, "An aquarium is much like an iceberg, 7/8ths of which is hidden from view under the water."

THE CULTURE OF *Sepioteuthis lessoniana* (Bigfin Reef Squid) AT THE MONTEREY BAY AQUARIUM

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Abstract

The Monterey Bay Aquarium has kept *S. lessoniana* continuously since June 2013. This study contains data recorded from January 2017 to January 2019, tracking 23 cohorts from four genetic lines. Official hatch date of each cohort was approximated using the average hatch date. Parameters tracked included temperature, size, dietary milestones, tank transfers, and egg laying, all recorded with respect to days post hatch (dph). New genetic lines are started with wild-caught eggs, and historically our animals have been bred up to eight generations. The eggs hatch after about three weeks, and hatchlings will take adult mysids within the first two days, followed by very small grass shrimp. It is best to shift them to a higher protein food source like live fish as early as possible, eventually transitioning to a diet of frozen fish. As the animals grow, they need to be moved to successively larger enclosures. Our animals are exhibited in an 1875-gallon tank along with coral, small rock structures and tall plastic grasses. Mature females use these grasses to lay their eggs, which are then collected, treated and incubated until hatching. Growth data show a sexual dimorphism where the male growth rate is higher after maturation. *Note that animals grow at different rates and all measurements and observations should be treated as guidelines rather than absolutes.

Background

Sepioteuthis lessoniana (commonly known as Bigfin Reef Squid, Oval Squid or BFRS) are a neritic, schooling squid favoring shallower nearshore habitats (Nabhitabhata, 1996). This species is widely distributed in the Indian Ocean, found as far east as Hawaii and as far north as Hokkaido, Japan (Segawa, 1995). The most extreme estimation of their temperature range comes from Segawa (1995), in which a minimum of 60 and maximum of 82 degrees Fahrenheit (ºF) is described. Metabolic rate, growth and behavior differ depending on temperature in both the wild and in captivity, though maximum age does not seem to be affected (Segawa, 1995; Forsythe, Walsh, Turk, & Lee, 2001; Jackson & Moltschaniwskyj, 2002). In Japan the squid tend to spawn seasonally in the spring, such that their hatchlings develop during the warmest summer months (Segawa, 1995). Growth rate is high even when compared to other cephalopods, averaging an increase of 5-10% wet body weight per day depending mainly on temperature and nutritional intake (Forsythe, Walsh, Turk, & Lee, 2001). Feeding rate peaks at 30% of wet body weight per day, a challenge for captive rearing because squid typically handle one food item at a time, and so must be fed frequently (Lee, Turk, Yang, & Hanlon, 1994; Forsythe, Walsh, Turk, & Lee, 2001). Wild caught adults have been exhibited at several aquariums, but the in-house culturing of squid for exhibit at public aquariums is relatively new.

Egg Care

The process of culturing BFRS begins with egg acquisition, whether wild caught or cultured (wild caught eggs are used to begin new genetic lines). Our captive-raised females will lay on fake grass bunches with blades of 1/4-inch width and of lengths varying from 1-3 feet (Fig.

1A). They prefer grass that already has eggs on it but will lay on bare grass if that is all that is available. The males clean the eggs, so it is ok to leave them undisturbed for about a week to encourage more egg laying. After a week the eggs need to be removed, cleaned and separated.

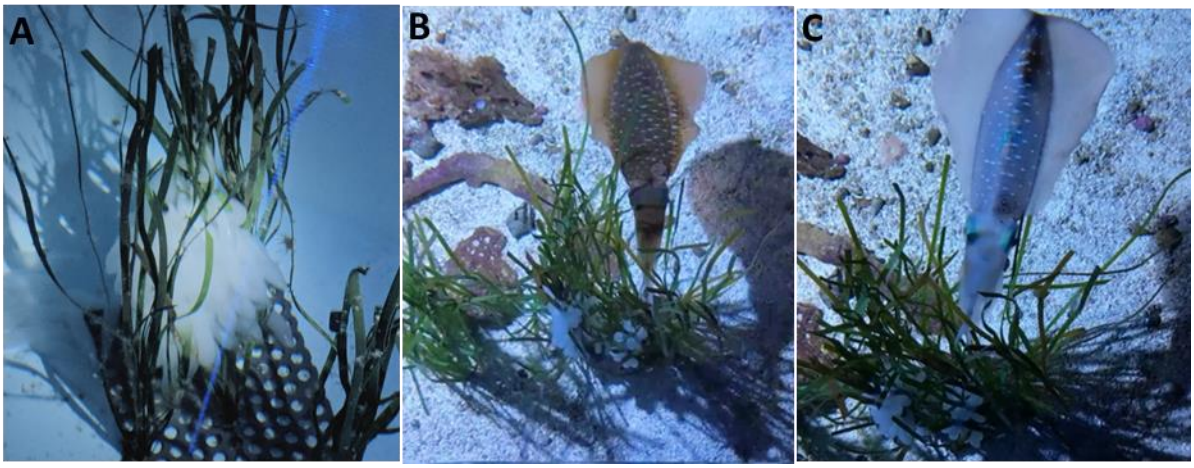

Figure 1. Egg laying A) Eggs on grass B) Female laying eggs C) Male cleaning eggs. Split color display is a common method of communication in coleoids. The pale side faces a possible aggressor and signifies submission to reduce the likelihood of an interaction.

The egg cases or "fingers" are attached to the grass in bunches by a tough, fibrous material sometimes referred to as the "cuticle". Remove bunches from the grass by hand, dislodging the cuticle at its attachment point and sliding individual blades of grass out while the eggs remain submerged. Place in a container of water dosed with Revive coral cleaner at four capfuls per gallon for ten minutes. This process removes parasitic copepods that eat the egg casing. Then place the eggs in clean water to trim off the cuticle, which is extraneous and prone to decay. Use a sharp pair of small dissecting scissors to separate each egg case from the main bunch, but do not penetrate the egg casing.

Once the eggs are trimmed, they can go into a mesh basket in the hatch tank (See Appendix A for dimensions) at 76-78ºF. Baskets should be large enough that the eggs spread into a layer no thicker than two or three egg cases in height. We typically use polyethelyne mesh with a quarter-inch (6.4 mm) opening for our baskets. This size mesh is small enough to cushion the eggs but large enough to let hatchlings escape. The basket should be suspended at the top of the hatch tank, with the supply provided by an upward facing spray bar positioned under the basket (see Figure 3A). Flow rate should be enough that the eggs are moving slightly. Check flow daily as too much motion can trigger premature hatching, and too little will quickly lead to decay in dead spots.

The eggs will begin to swell about 10 days after laying. At this point the eggs should get their first of two Betadine dips. The entire basket can be lifted from the tank and placed in a bucket premixed with 1ml Betadine per liter water for 10 minutes. Bacterial decay of the egg casing causes a mottled appearance Fig. 2D). If egg casing is showing excessive bacterial decay, a second Betadine dip can be done. This must be before the embryo is fully formed as in Figure 2D, or it will incur premature hatching.

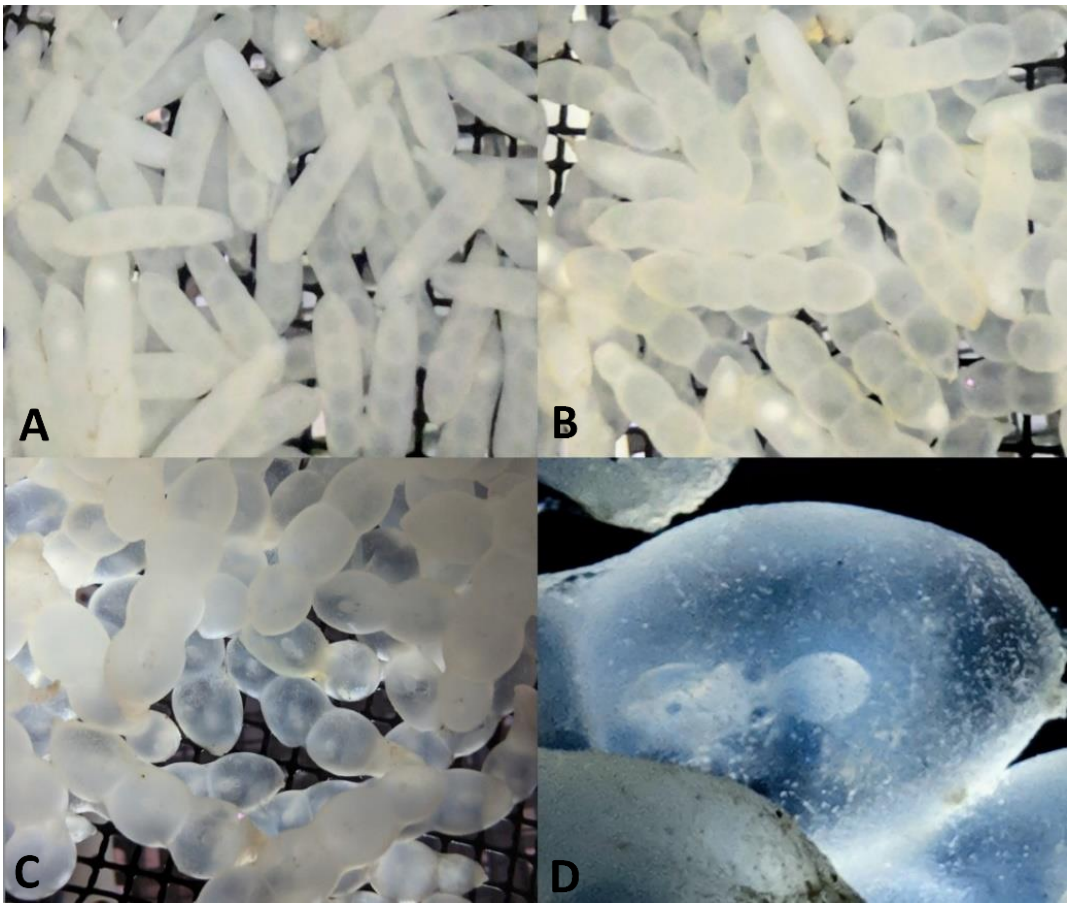

Figure 2. Egg development. Mesh size is ¼". A) 12 days: individual eggs are defined and slightly transparent. B) 20 days: eggs are elongate, swollen, embryo is just visible. C) 24 days: embryo is well defined. D) 25 days: embryo slightly larger than yolk, egg is about 3cm along its longest axis (mottled appearance of egg casing shows moderate bacterial decay).

Eggs begin hatching at an average of 25 days post laying with a range of five to six days at 78ºF. While eggs are hatching, remove spent or rotten egg cases, fallen yolks, and dead hatchlings, but avoid touching viable unhatched eggs. At this stage it is very important to disturb them as little as possible, as pressure triggers premature hatching. Premature hatchlings are noticeably smaller and have a lower chance of survival, especially if they still have yolks.

Even fully developed hatchlings have a high mortality rate during the first 30 days, ranging from 50-100% depending on nutrition, flow, temperature, and the impact of external stimuli such as light. Initial cohort size should ideally be at least three times the target number of adults. Fortunately, the hatchlings are not sensitive to high densities during the first 30 days. After the first month the weak hatchlings will have mostly died out. Mortality after this point is mainly due to aggression, stress and cannibalism. These factors increase with age and stocking density (see Appendix A).

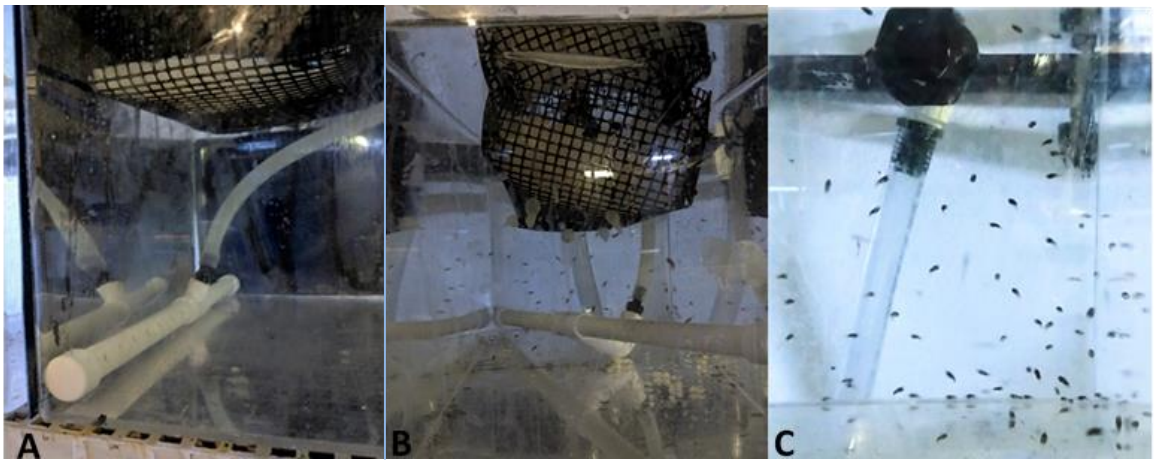

Figure 3. A) Hatch Tank, B) Eggs at 30 days: peak of hatching, C) 7 dph.

Hatchlings will initially be pale (Fig. 3B), but robust hatchlings should exhibit a dark coloration within the first 3-5 days (Fig. 3C). Paleness is common in the youngest hatchlings, but prolonged paleness indicates weakness and is often accompanied by a difficulty in swimming or catching food. After a few weeks the hatchlings will start to develop a few distinct behaviors and patterns (see Figure 4). Arms up in two forks (Figure 4B, 4C) usually indicates defensive behavior, while hanging arms and especially tentacles (Figure 4A, 4D) is a more relaxed posture. Once they begin to school, they will often exhibit identical behaviors. It is at this point that density becomes a concern (see Appendix A).

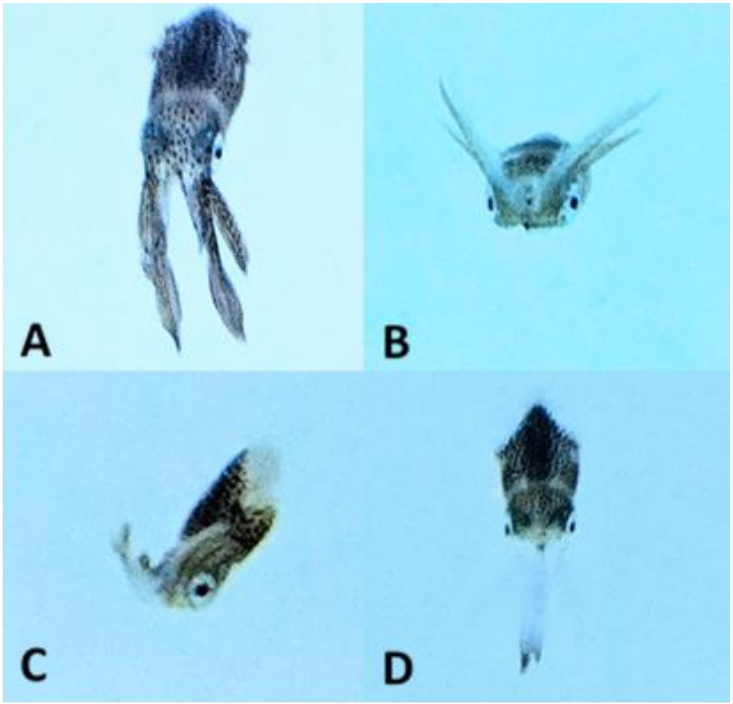

Figure 4. Behavior at 31 dph A) hanging tentacles down, B) arms up in two forks with beak open, C) Clear posterior, arms in V, D) Clear arms/tentacles with dark tentacle tips hanging down.

In general, our squid are kept between 74-78ºF. It is best to keep juveniles at the higher end of this range so they have a healthy appetite (Segawa, 1995). This relationship can be exploited if you want to speed up or slow down growth if holding space is limited, or a greater gap in size of cohorts is desired. Though growth rate will change, maximum size and age at maturity do not seem to be affected by temperature (Nabhitabhata, 1996). In one of our trials, two populations from the same clutch were kept at two different temperatures, 74ºF and 78ºF beginning at three months of age. The warmer cohort grew more quickly, but both groups laid eggs within one day of each other though they had been separated for over two months.

Diet

Sepioteuthis lessoniana has an extremely high metabolism. Jackson & Moltschaniwskyj (2002) emphasize that nutritional intake is just as important as temperature in influencing growth rates. BFRS are highly cannibalistic even when adequately fed, and frequent feedings mitigate the occurrence of aggressive interactions. To keep the animals satiated they must be fed as often and as large a food item as possible. Spread the feeds out, ideally making them part of opening and closing rounds, to mitigate overnight cannibalism. A good metric for item size is to never feed items that are longer than the total body length of the animal (Fig. 5). Pacing of feeds, especially once the animals start becoming more competitive around 30 dph, is also key. Too slow and they will fight over the food items, but if more than one piece is added at a time it causes confusion and most of the food will end up on the bottom uneaten.

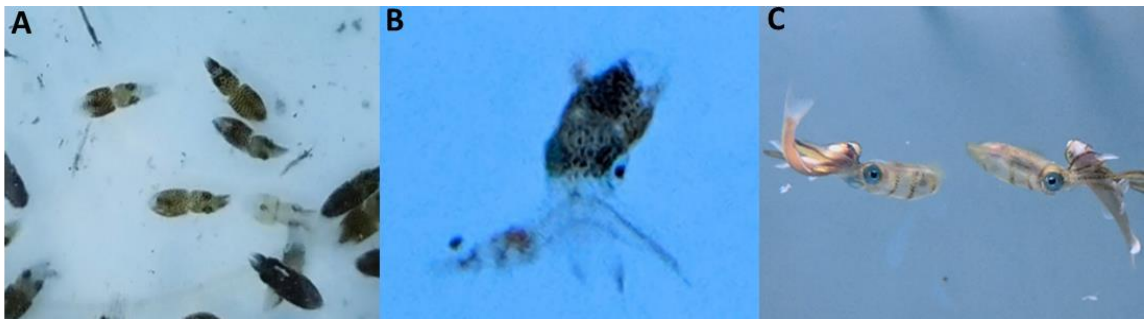

Figure 5. Food item to body size ratio. A) 12 dph with adult mysids, B) 31 dph with grass shrimp, C) 53 dph with rosy red minnow.

BFRS will not take dead food during the first few months, and so the additional logistics and labor involved in housing and maintaining live prey items should be considered. They will also prefer food that is in the water column rather than on the bottom or floating at the top. At 0 dph, gently add a few live mysids (ex: *Americamysis bahia*, Fig 5A), but the squid may not feed for the first couple of days. Try to gauge whether they are eating by watching for the buildup of mysids on the bottom, which should be avoided as they will pester and stress the squid. The hatchlings will not take mysids off the bottom during the first week, so many smaller, spread out feeds increase the amount of time food items are suspended. If there are excess mysids on the bottom, they can be resuspended using a turkey baster or with the spray bar rather than adding additional mysids. Hunting off the bottom starts to occur around 10-12 dph, and at this point excess mysids are not as detrimental. Once the animals are eating reliably, they should receive six feeds a day regardless of age, size or food item.

Hatchlings will start taking tiny grass shrimp or larval fish at any age as long as the items are not bigger than they are (Fig. 6B). Squid need a high amount of protein and do not have large fat stores, so fish are an ideal food source (Jackson & Moltschaniwskyj, 2001). Because finding a large and steady supply of larval fish can be a challenge, we typically use live grass shrimp of progressively larger sizes that have been enriched with krill (*Euphausia pacifica*) until the squid are large enough to take live rosy red minnows (*Pimephales promelas*, Fig. 5C). This happens around 40 dph, and grass shrimp are then alternated with fish throughout the day.

It is important that the squid eventually move to a diet of lean baitfish like silversides (*Menidia menidia*) or whitebait (*Spirinchus starksi*), that are available frozen in bulk. This allows for an overall greater quantity of protein to be ingested without having to maintain populations of large feeder fish. Once the squid are getting dead food the tanks will get dirty very quickly and should be siphoned at least once a day, in addition to being monitored for ammonia. Our squid start taking thawed silversides at an average of 104 days, and whitebait at about 122 days, so attempts can start a week or two before those dates, especially since it will take a while for them to all take it.

Whole fish should be tossed into the tank, but do not reach over the tank as overhead motion will spook the squid and they may not notice the food. If an individual is interested in a food item it will point its arms towards it and the patch above its eyes will pulse between light and dark. They will either "attack" it using their tentacles, or "grab" it more gently with their arms. Start the transition to frozen fish by offering it exclusively at the first feed of the day, when the animals are hungriest. Over the course of a few weeks the pacing of the frozen feed will increase as more of them start competing. When more than half of the squid are taking frozen, increase frequency to the first two feeds of the day. Once the majority are taking it, feed exclusively frozen except for the last feed. The final feed should always be live because live food will always be accepted, whereas not every animal will eat the frozen at every feed, and they should be as full as possible overnight to reduce the likelihood of aggression.

Handling

BFRS should be handled as little as possible, however some handling is necessary as they will need to be transferred into successively larger tanks as they grow. This must be done incrementally because too much space is detrimental (for further discussion on density see Appendix A). It is important when moving the squid to minimize stress, and to prevent air from entering the mantle cavity.

To capture the squid, corral them using nets one at a time and transfer into an intermediate vessel. Brine shrimp nets work well for catching them because their fine mesh gets clogged by the squid's mucus, slowing the drainage of water (Fig. 6B). Cradling the animal with a hand keeps it mostly submerged as it is transferred to the transport vessel, the dimensions of which are determined based on the size of the squid. A sufficient volume of water is necessary to support their high basal metabolic rate, but they are less likely to exhibit stress behaviors such as jetting or inking when in a smaller container. Stressed out BFRS rapidly consume oxygen, and squid ink is highly viscous, increasing the likelihood of asphyxiation. If the squid does ink, the best thing to do is get it into new water as soon as possible, ideally into its new enclosure if close by.

For squid younger than 40 dph a two-liter beaker can be used for transportation of up to ten squid at a time (Fig. 6A), and stress behaviors are not a big concern because the animals are so small. After 40 dph, an appropriately sized transport vessel will greatly reduce the chances of inducing stress behaviors. At 40-90 dph two or three animals can be transported together in a three-gallon bucket. At 90-150 dph, move one squid per three-gallon bucket. Larger (greater than 150 dph) squid stay the calmest when moved individually in a container whose diameter is close to their total body length.

To release a squid into its new enclosure, dip the transport vessel into the water deep enough that it can swim out without contacting the air, and use a gloved hand under the ventral mantle to guide it. Position the animal with its posterior down so that the initial mantle contraction will expel any entrained air upward. We call this method "burping", and it is a common practice when handling cephalopods.

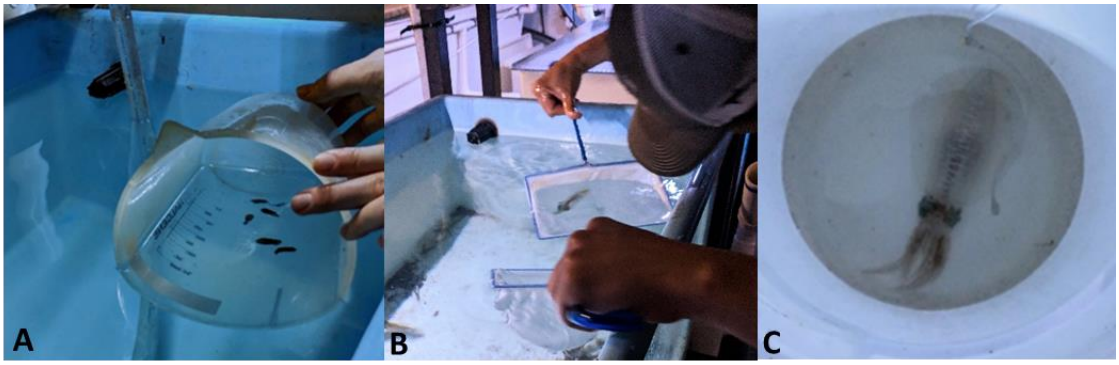

Figure 6. A) 31 dph squid are added to a 2x3 using a beaker. B) 90 dph capture with brine net C) Adult squid calm in bucket.

Exhibition

When adding a new cohort of squid to exhibit, several factors should be considered. Younger animals will likely have a longer stay on exhibit (our minimum age on exhibit was 79 dph), however age is not the only deciding factor. Before being moved to exhibit the animals must be routinely taking frozen food, or else they will have difficulty feeding in their new enclosure. They should also be adapted to the exhibit environment, as described in Appendix A.

Due to the short life span and high growth rate of *S. lessoniana*, the progeny of the exhibit adults will likely not be ready for exhibit before the adults die. The ideal grow-out process propagates two genetic lines at approximately a three-month offset, with several cohorts of different ages. The ability to keep several cohorts of different sizes will be limited by the number of large holding tanks available. w

Aquascaping of the exhibit can vary. We use a combination of rocky reef structure, corals and fake grass. BFRS are capable of navigating around hard structures, but the surface area of vertical structures should be kept to a minimum. BFRS will tolerate the relatively high light and flow levels neeed to support photosynthetic invertebrates as long as they are acclimated during development (Appendix A). The animals will tend to orient facing the window, often chasing the reflection of food.

Mitigate aggression by spreading feeds throughout the day, however overnight cannibalism is likely to occur occasionally and so the population will naturally dwindle over time. Larger males tend to attack females and smaller males, leaving bite marks or eating part of the body (usually the head). This becomes more common as animals mature and begin mating. If there is one obvious aggressor, it can be removed to elongate a cohort's stay on exhibit. The swapping out of entire exhibit populations should allow enough time for the new population to be developed and on frozen food. Because squid do not tolerate divers, the swap should be coordinated with any necessary deep cleaning that requires removing the animals, such as bleaching or diving.

Figure 7. November 2019 BFRS Exhibit at MBA. Photo by Catherine Traub

Growth Study

It is widely agreed that growth rate is affected by both termperature and nutrition, but age at maturity and maximum adult size are similar between wild-caught and laboratory cultured *S. lessoniana* (Ikeda, Anderson, & Matsumoto, 2009). For this growth study 78 individuals were measured post mortem, and size was plotted vs. age (Fig. 8). Undifferentiated BFRS ("U") seem to grow linearly up to about 150 dph. At this point the it appears the males grow much more quickly than the females, resulting in a sexual dimorphism that is usually obvious by 150 dph (Fig. 9A). Jackson (1989) noticed a similar dimorphism in wild caught individuals.

Reproduction:

Males will begin guarding females as demonstrated in Figure 9A. To mate, the male will swim directly above the female, rotate upside down and then flip itself over and grab the female's arms with its own as it inserts a spermatophore into her mantle (Figure 9B). Eggs are laid beginning anywhere from 118 to 171 dph and eggs may be laid over a period of up to three months. Increased aggression causes mortality especially in females as size dimorphism between males and females as well as between dominant and subordinate males widens.

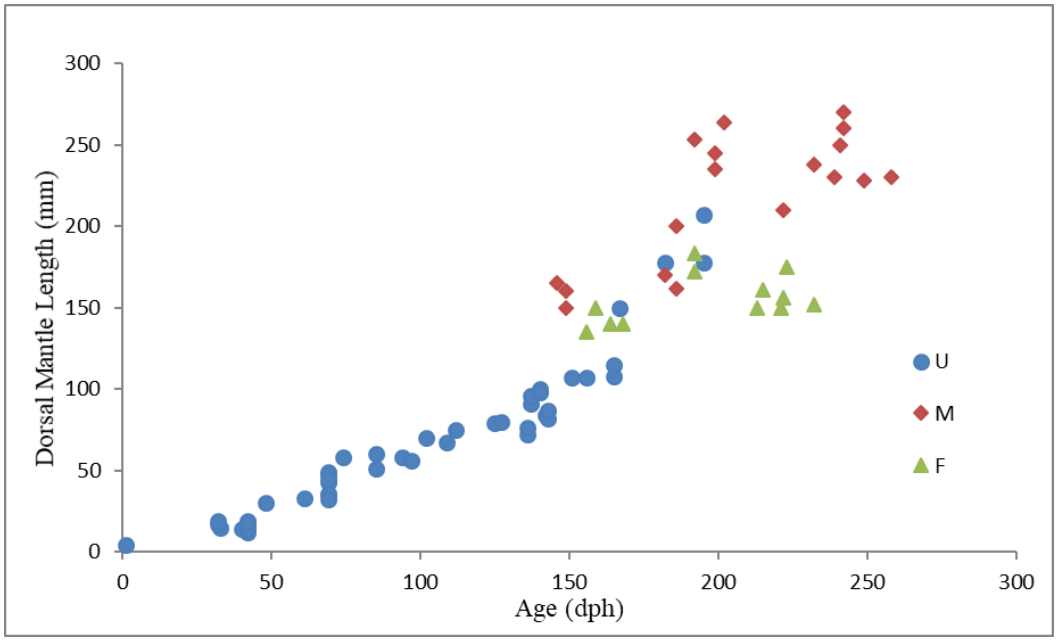

Figure 8. DML (mm) vs Age (dph). U=unknown M=male F=female.

Figure 9. A) Sexual dimorphism/mate guarding, male on left. B) Mating, female on left. Note horizontal color bands on male. C) Female BFRS with eggs visible as the yellow mass inside her posterior mantle cavity.

Appendices

Appendix A: Enclosures

BFRS are well adapted for captivity because they are not highly mobile unless stressed. This allows for the use of rectangular tanks, which tend to be more space efficient than circular tanks. This species is not sensitive to density until about 30 dph, and so the hatch tank density can be high as long as good water quality is maintained. Around 40 dph they will need enough room to spread, but if the tank is too large, they will have difficulty schooling and finding their food. Our target cohort size of 15-20 squid works well with our standard holding tank dimensions, and we typically do two animal transfers. The first transfer is from the hatch tank to a "2x3," then from a 2x3 to a "4x8" or the exhibit. Table 1 outlines the dimensions of these tanks, and the age range of squid kept in that size tank based on the average.

Table 1. Holding tank dimensions in inches.

Outer dimensions (LxWxH)	House Name	Days Post Hatch
24"x12"x24"	Hatch tank	0-34
36x"24"x30"	2x3	34-78
96"x48"x36"	4x8	78-

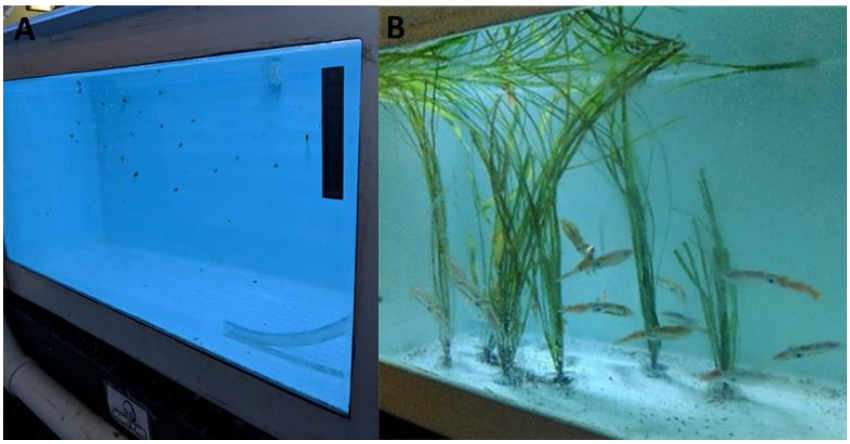

Figure 10. Squid in "2x3" at both ends of range A) 31dph B) 88 dph.

The hatch tank should be kept in a low light area. All other holding tank setups should prepare the squid for exhibit conditions, most importantly higher intensity light and the activity of humans. To address the former, make sure there is a light over the tank bright enough that all corners are illuminated. Refuge is provided by habitat such as grasses (Fig. 10B), and the squid tend to prefer the space where the shadow and the light meet (Lee, Turk, Yang, & Hanlon, 1994). To habituate the squid to human activity, their holding tank should have a window. This is important not only for the squid to get used to seeing movement outside their tank, but also to facing a reflective surface.

Collisions and abrasions will occur as animals contact the sides of any tank. Hard collisions can break the squid's pen, and even small abrasions in their thin epidermis can quickly worsen. These animals are very good at navigating enclosures, so any instances of jetting that result in a collision are likely stress-induced. Rubbing on the sides of the tank is more common than jetting, so to mitigate the occurrence of abrasions keep the tank sides as smooth as possible. To address upward jetting, lids and jump guards will be needed. Lids are necessary starting around 40 dph and are sufficient up to 60-70 dph (Fig. 11A), by which time the animals should be moving to a larger holding tank. Larger holding tanks should be equipped for the largest squid, with jump guards at least three feet above water level. A solid frame consisting of modular units wrapped in mesh (Fig. 11B) is a lightweight design that provides a flexible, soft surface to reduce contact damage.

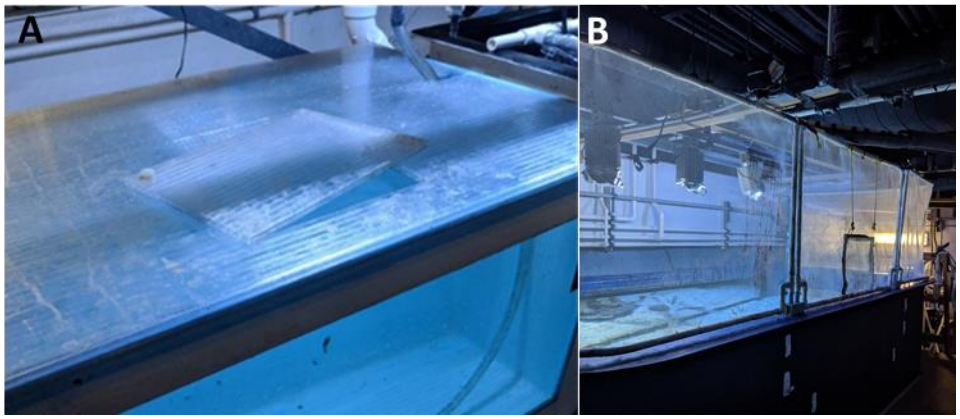

Figure 11. A) Lid on "2x3" with feeding port B) Modular PVC frame and mesh jump guards on exhibit tank, including feeding port.

Appendix B: Euthanasia

Cephalopod euthanasia can be tricky because these animals are highly reactive, and their viscous ink will suffocate them in a closed container. Past methods, using chemicals including ethanol, MS222 and Magnesium Chloride, have all induced jetting and inking in our BFRS. Dr. Mike Murray introduced a new two-step protocol for cephalopod euthanasia to our team in December of 2018. Step One is the dosing of a Magnesium Chloride solution at 200ml/minute from an IV bag. The solution is composed of Magnesium Chloride Hexahydrate and deionized water to a dilution of 7%. A 1:1 ratio with saltwater is used so that after the entire dose is delivered the total volume will have doubled and the concentration of the Magnesium Chloride will be halved to 3.5%. It appears that as long as the animals do not ink initially, they are calmed by placing a lid over their container and begin relaxing visibly within about five minutes of initiating the dosing. The end goal of this step is to depolarize the nerves of the animal, effectively killing it. Step Two is decerebration, an incision along the anterio-posterior plane between the eyes.

This method has been a huge improvement, greatly reducing stress for both animals and aquarists. It has been used on over 20 BFRS of various ages ranging from hatching to large adult, with no jetting or inking observed.

Acknowledgements

I would like to thank the Monterey Bay Aquarium for supporting our continuing work with this and other cephalopod species. A particularly huge thanks as well to the *Tentacles* team, past and present, for countless tanks siphoned and squid fed. To Christina Biggs and Catherine Traub, thank you for your support and invaluable input on this paper.

References

Forsythe, J. W., Walsh, L. S., Turk, P. E., & Lee, P. G. (2001). Impact of temperature on juvenile growth and age at first egg laying of the Pacific Reef Squid *Sepioteuthis lessoniana* reared in captivity. *Marine Biology,* 103-112.

Ikeda, Y., Anderson, F. E., & Matsumoto, G. (2009). Reproduction and life span of the oval squid *Sepioteuthis lessoniana* (Cephalopoda, Loliginidae): Comparison between laboratory-cultured and wild-caught squid. *Marine Biodiversity Records*, 1-7.

Jackson, G. D. (1989). Age and Growth of the Tropical Nearshore Loliginid Squid *Sepioteuthis lessoniana* Determined from Statolith Growth-ring analysis. *Fishery Bulletin*, 113-118.

Jackson, G. D., & Moltschaniwskyj. (2002). Spatial and Temporal Variation in Growth Rates and Maturity in the Indo-Pacific squid *Sepioteuthis lessoniana* (Cephalopoda: Loliginidae). *Marine Biology*, 747-754.

Lee, P. G., Turk, P. E., Yang, W. T., & Hanlon, R. T. (1994). Biological Characteristics and Biomedical Applications of the Squid *Sepioteuthis lessoniana* Cultured Through Multiple Generations. *Biological Bulletin*, 328-341.

Nabhitabhata, J. (1996). Life Cycle of Bigfin Squid, *Sepioteuthis lessoniana* Lesson. *Phuket Marine Biological Center Special Publication*, 83-95.

Segawa, S. (1990). Food Consumption, Food Conversion and Growth Rates of the Oval Squid *Sepioteuthis lessoniana*. *Nippon Suisan Gakkasihi*, 217-222.

Segawa, S. (1995). Effect of Temperature on Oxygen Comsumption of Juvenile Oval Squid *Sepioteuthis lessoniana*. *Fisheries Science*, 743-746.

Razor clam *(Siliqua patula)*. Bruce Koike

COMPARISON OF MEAN ABUNDANCES OF ECTOPARASITES FROM NORTH PACIFIC MARINE FISHES

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Abstract

Ectoparasites provoke a variety of medical issues in public aquarium animal collections. The Point Defiance Zoo and Aquarium had an opportunity to study such parasites in their North Pacific Aquarium (1963) systems with the building of the new Pacific Seas Aquarium (2018). This opportunity provided a foothold for understanding variables involved with infection and follow up responsive treatment. Temperate marine fishes were submerged in freshwater to allow collection of detached ectoparasites. Among 23 species (7 families) of marine fishes that were moved and sampled, three parasites were focused on for this study: the hirudinean leech, *Heptacyclus diminutus* (n=1,866, average prevalence 57.6%), the capsalid monogenean, *Neobenedenia melleni* (n=11,804, average prevalence 84.9%), and the lernaeopodid copepod, *Clavella parva* (n=233, average prevalence 18.3%). Comparisons of mean abundance were made between host species, host exhibit and host size. Developing a better understanding of the variables involved between hosts and parasites allows aquarists to improve methods that prophylactically and responsively manage parasitic ailments.

Introduction

Point Defiance Zoo and Aquarium (PDZA) has historically managed chronic parasitism associated with its displays of native fish, often responsively in cases of extreme infection in individual fish. Anecdotally, staff have observed variable sensitivities between individual fish towards generalist parasites, however limitations on time and resources had prevented a more in-depth look at the patterns of infection. Possible reasons for these variations were believed to be associated with host species, exhibit characteristics, and host size/age.

With the construction of the new Pacific Seas Aquarium (PSA) and closure of the North Pacific Aquarium (NPA), an opportunity presented itself to examine some of these hypotheses in a more structured way. As fish were moved out of the NPA, they were put through responsive quarantine treatments in isolated batches specifically targeting those parasites regularly observed. We collected and analyzed samples of external parasites from approximately 60% of the individual fish that went through treatment.

Exhibit Descriptions

Samples were collected from fish housed in 12 exhibits and 2 off-exhibit holding systems. Exhibits could be supplied with filtered and/or raw seawater feeds; raw seawater was added to help supplement the dietary requirements of a variety of filter feeding invertebrates that inhabited the exhibits. Appendix 1 displays dimensions, volumes, and shapes of the exhibits as well as a brief overview of heterospecific species not sampled for this study that were displayed within each. (see Appendix 1) Natural seawater was supplied to the North Pacific Community (NPC) exhibit from a pump station at the nearby shoreline after being filtered through rapid sand filters. All other

smaller NPA exhibits were supplied seawater from the NPC and all overflow returned to the Puget Sound.

Exhibit Fish

The NPA was devoted to the display of native north Pacific invertebrates, teleosts and chondricthyans focusing on ecosystems scattered around the Salish Sea, with emphasis on Puget Sound. The majority of the teleost species consisted of the *Sebastes* genus of rockfish which made up 84% of the fish utilized in this study. The *Sebastes* species displayed included: *S. auriculatus* (brown), *S. caurinus* (copper), *S. diaconus* (deacon), *S. diploproa* (splitnose), *S. emphaeus* (Puget Sound), *S. flavidus* (yellow tail), *S. maliger* (quillback), *S. melanops* (black), *S. miniatus* (vermillion), *S. nebulosus* (china), *S. nigrocinctus* (tiger), *S. pinniger* (canary), *S. ruberrimus* (yellow eye) and various hybrids collectively referred to in this study as *Sebastes* spp. Additionally, we sampled ectoparasites from the following species in the collection: *Hexagrammos lagocephalus* (rock greenling), *Hemilepidotus hemilepidotus* (red Irish lord), *Anarrhichthys ocellatus* (wolf eel), *Hexagrammos decagrammus* (kelp greenling), *Platichthys stellatus* (starry flounder), *Embiotica lateralis* (striped perch), *Acipenser transmontanus* (white sturgeon), and *Ophiodon elongatus* (ling cod).

Parasite Biology

The biology of the ectoparasites found in this study may help explain some of the similarities and variation seen in relative levels of infection across the different hosts sampled, especially in regards to their reproductive strategies, infection, and feeding behaviors (Rhode et al., 1995, Pouin, 2013). This section will briefly compare those aspects of the three major parasite species observed. Similar biological aspects found among the three species include direct life cycles, portions of the life cycle spent away from hosts, ingestion of host tissues or fluids for nutritive purposes, compromise of the host epidermis allowing for possible secondary infections, and temperature dependent reproductive rates capable of resulting in high numbers of viable offspring (Brazenor and Hutson, 2015, Ravi and Yahaya, 2016).

Neobenedenia melleni

The capsalid monogenean, *Neobenedenia melleni* was found on the surface of skin, fins and eyes of host fish. The adults are oviparous and simultaneous hermaphrodites, with the capability of viable self-fertilization across multiple generations, each able to produce up to 200 eggs/day (Hoai and Hutson, 2014). Eggs are produced with long threads that entangle on textured surfaces following release into the water. Free swimming, ciliated larvae called oncomiracidia hatch and begin to use chemicals emitted by potential hosts to rapidly search for infection opportunities (Trujillo-González, 2015). Once successfully attached, they roam over the surface of the host and feed on epithelial cells and mucous, growing from ~50 µm to upwards of 2500 µm in as little as ten days (Brazenor and Hutson, 2015). At this point the cycle begins anew (see Figure 1).

Wild counterparts reproduce aggressively with the hope that a few offspring have the opportunity to achieve sexual reproduction, whereas the same tactics applied in the restricted space of a captive setting result in relatively higher success rates with the potential to overwhelm hosts and deplete physiological resources to the point of morbidity and mortality (Thoney and Hargis, 1991). The attachment mechanisms these parasites use to hold fast to the slick surface of their

hosts include a large posterior disc-shaped sucker with four sharp hooks called hamuli and two anterior suckers lacking hooked structures (Trujillo-González, 2015). As they move over the skin of their hosts, they may create physical punctures through the epidermis, exposing the fish to the potential of secondary infections (Koneko II et al., 1988, Thoney and Hargis, 1991). Variations in host scale size and pattern and other anatomical proportions may impact available attachment and feeding sites for *N. melleni* (Trujillo-González, 2015).

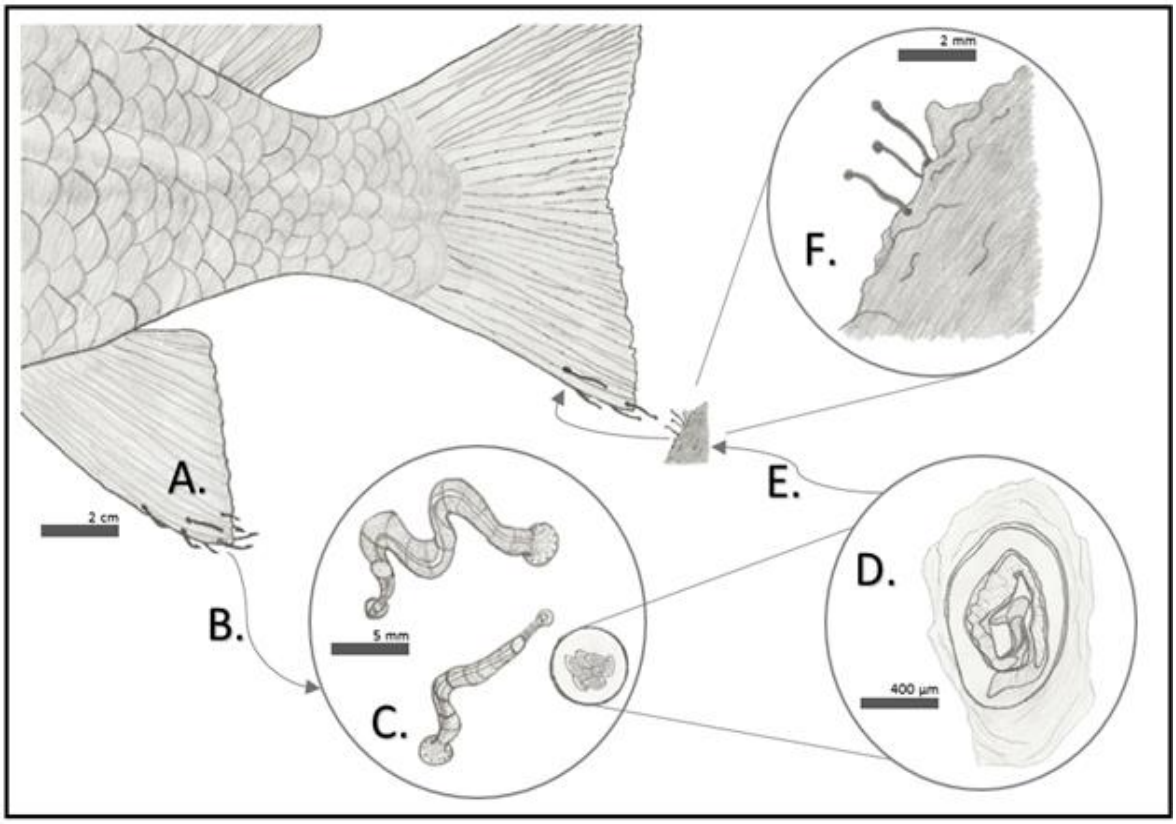

Figure 1. Life cycle of *Neobenedenia melleni*. Adult capsalids live and feed on the outer surfaces of various northeast Pacific fishes (**A**). Cross or self-fertilization (hermaphroditism) results in viable eggs being released into the environment (**B**). Eggs entangle upon surfaces and each other and embryos begin to develop and show eye spots (**C**) before free-swimming oncomiracidia hatch (**D**) and begin to seek out potential hosts (**E**). Oncomiracidia attach to host fish and begin to feed and grow into reproductive adults.

Heptacyclus diminutus

The hirudinean leech, *Heptacyclus diminutus* (previously known as *Malmiana diminuta*) (Williams and Burreson, 2006) was found often on pectoral, pelvic, anal and tail fins and occasionally on the body of host fish. Like *N. melleni*, the adults are oviparous and simultaneous hermaphrodites, however they differ in that adult *H. diminutus* leave their fish host to lay cocoons individually or in small clusters on hard surfaces. It is unknown if copulation takes place on either the host or in the environment (or both). Each cocoon of this species contains a single egg (Burreson, 1975). Once hatched from the cocoon, juvenile leeches begin seeking out locations to attempt attachment to host fish (see Fig. 2). In exhibits that had host fish removed, juvenile leeches

were seen to repeatedly cluster in dense groups in the same locations which may have been given preference by some element (or combination of elements) of the microenvironment in those locations; i.e. water flow, lighting, etc.

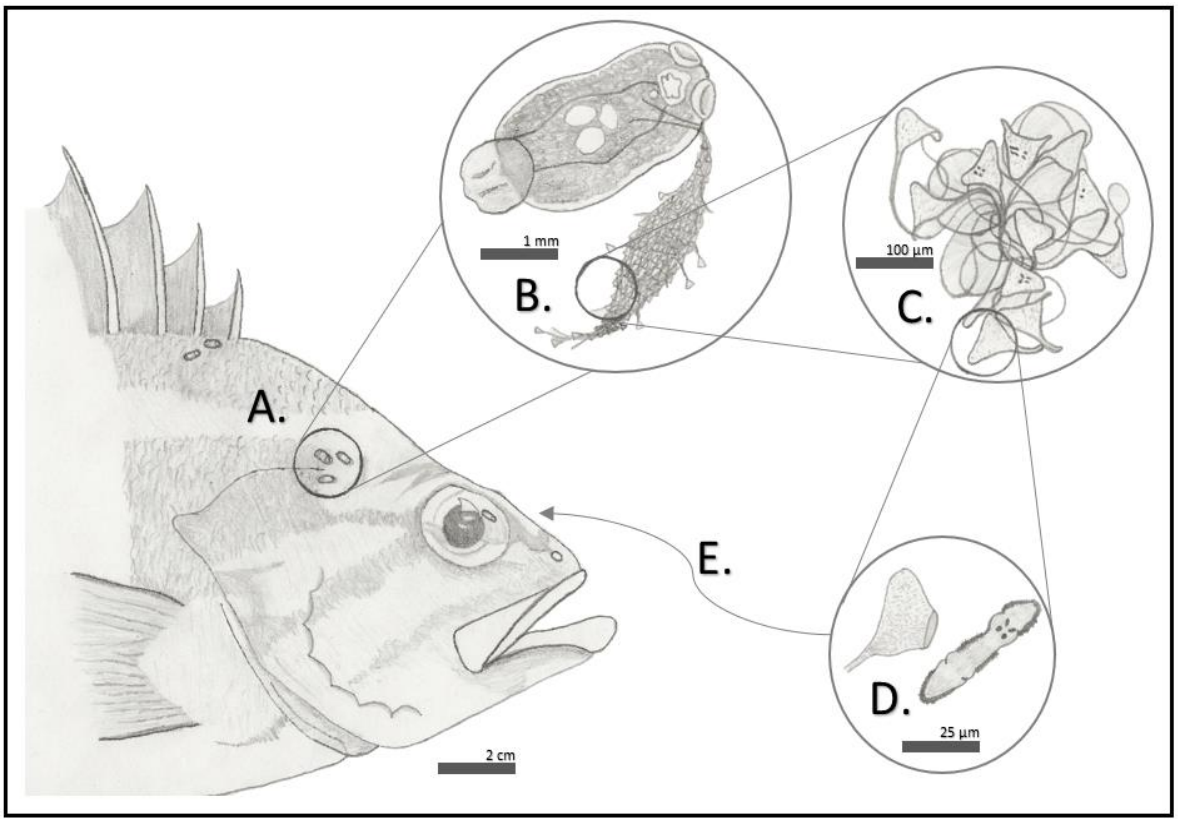

Figure 2. Life cycle of *Heptacyclus diminutus.* Adult and juvenile leeches attach to fins and body of various northeast Pacific fishes (**A**) and feed on host blood. Adults prepare to reproduce and leave the host fish to lay eggs in the environment (**B**). Fertilized eggs are laid individually in cocoons on hard surfaces (**C**). These cocoons are very durable and firmly adhere to rockwork and glass of aquaria. Within the egg, a juvenile leech develops over a few weeks (**D**) before hatching, leaving the cocoon and crawling to find a suitable location to attach to a host fish (**E**). Juvenile *H. diminutus* adhere to surfaces, often with only their posterior sucker disc, leaving the rest of their body to sway outstretched in the water column (**F**). A juvenile leech survives longer than three months waiting for the opportunity to attach to a host fish, and responds to variation in environmental vibrations by flailing outward to improve chances of latching using its anterior sucker. Once attach to a host, the juvenile leech feeds and grows until it is ready to attempt reproduction.

Juvenile *H. diminutus* are independent of hosts for longer than the initial life stages of the other marine parasites addressed in this study. Viable juvenile leeches were observed to persist in exhibits for 120-140 days after the removal of all host fish. Some of this time can be assumed to have been spent with larval leeches still developing in egg capsules before hatching, however identifiable individuals were observed on exhibit walls and rockwork for over 6 weeks before succumbing to presumed starvation. This long independence would benefit wild *H. diminutus*, as juvenile leeches may have relatively few opportunities to attach to a host fish. Host fish also show varying degrees of petechial hemorrhages along the fins and body wall indicative of blood feeding sites and points of potential exposure to secondary infections (Burreson, 1979). Variations in host

scale size and pattern, and fin and epithelial thickness and vascularization may impact attachment and feeding strategies of *H. diminutus*.

Clavella parva

Compared to the number of possible life cycle stages seen in other parasitic copepods, *Clavella parva* has relatively few. Larger females attach to the fins (or gills, though this was not an attachment site observed in this study) of a host fish, harbor a much smaller attached male, and produce a pair of egg sacs from which free swimming nauplii hatch directly into the water column. There is a single stage of nauplius that molts into the infective copepodid stage which is also unusually singular. The copepodid attaches to the host fish with a frontal filament and undergoes a final molt into a unique stage referred to as a pre-adult or "pupa," which then begins a continuous metamorphosis into a reproductive adult (Kabata, 1982) (see Fig. 3).

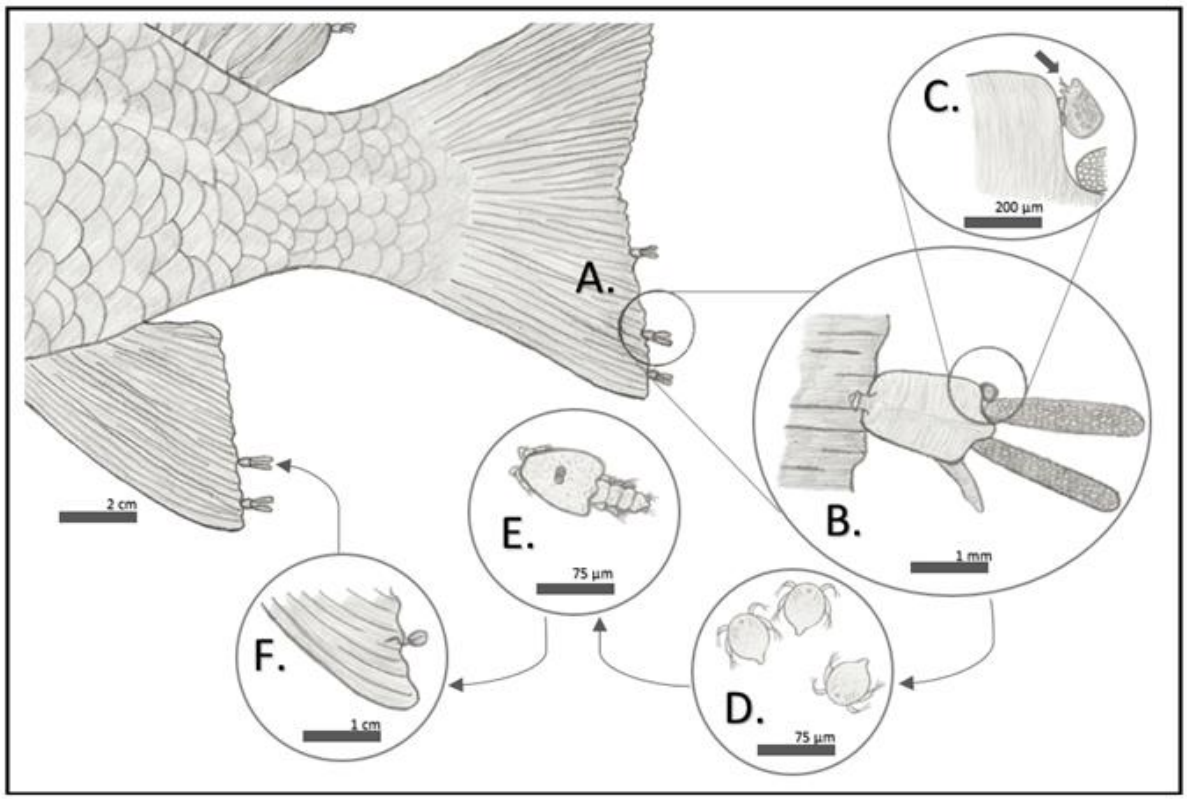

Figure 3: Life cycle of *Clavella parva*. Adult female copepods attach to fins and gills of various northeast Pacific fishes (**A**) and feed on host tissues. The comparatively large females grow paired egg sacs (**B**) and harbor minute reproductive males (**C**). Eggs are retained within the paired sac structures, where they develop before hatching free-swimming nauplii into the water column (**D**). Nauplii undergo a single molt into the infective free-swimming copepodid stage (**E**) which endeavors to attach to a host fish and undergo a final molt into a pre-adult or "pupa" (**F**). The pre-adult then begins a continuous metamorphosis into a reproductive adult.

Attached *C. parva* feed on host epithelial cells. Unlike *H. diminutus* and *N. melleni*, the female copepods remain attached at the same point of fin tissue for the entirety of their sub adult and adult life. Points of attachment and host tissue feeding potentially expose the host fish to secondary infections, as seen in other parasitic copepods (Ahne, 1985, Mulcahy et al, 1990).

Methods

Individual fish were collected from their exhibit and immediately treated with a temperature-matched 3-minute immersion in freshwater (0 ppt). This technique has been used at PDZA historically as a way to manage external parasite infections, as the resultant osmotic shock incapacitates and dislodges many monogeneans and hirudineans from the skin and fins of host fish. Host fish are able to better tolerate the osmotic change for the bath duration than targeted ectoparasites. The freshwater immersion was immediately followed by a 3-minute recovery bath in exhibit matched saltwater (28-32 ppt).

Immersions resulted in incomplete removal of ectoparasites, and were coupled with manual removal over the course of both baths, during which time host fish species were identified and total length to the nearest whole centimeter was noted. Manual removal of parasites focused on copepods that would not detach under freshwater treatments, and secondary efforts were applied to manual removal of leeches and monogeneans. Water from both containers was filtered through 150 µm mesh after each fish was processed, and filtered material was collected and observed under 20x magnification where parasites were identified and counted for each individual fish that underwent treatment.

Figure 4. The combined sample of a majority of the parasites collected and counted for this study preserved in 95% ethanol. The volume of parasites roughly equates to 70 mLs. This sample represents two years of cumulative efforts and currently acts as a paperweight and conversation starter on the authors' desk. Worth it.

Samples of parasites that were kept for counting at later times were moved into vials of 70-95% ethanol (EtOH). This was done most often due to lack of available time following fish processing for parasite counting. There was much to do with a new aquarium to build, after all.

Mean abundance for each of the three parasite species was tested for significant differences in consideration of host fish species, host size ranges and host exhibit. This was accomplished via bootstrap ANOVA tests for mean abundances (Reiczigel et al, 2019), and each test was run on 13,905 resampled points of the data. Abundance was chosen to express parasite quantity in this study as a single value representing both prevalence and intensity, and provides probabilities of observable infection levels in similar situations (ex. the probable level of *N. melleni* infection you could anticipate observing when randomly pulling *S. melanops* from an exhibit like those found in the NPA).

Results

Within this study 23 species (7 families) of fish were sampled with a total sample size of 232 individuals. The total counts of each parasite species observed were 11,804 *N. melleni*, 1,866 *H. diminutus*, 233 *C. parva*, 1 *Microcotyle sebastis*, and *1 Caligus clemensi* (Fig. 4). Voucher specimens of *N. melleni*, *H. diminutus*, and *C. parva* were deposited in the Harold W. Manter Laboratory of Parasitology (HWML) in Lincoln, Nebraska, U.S.A. (HWML 110951, 110952, 110953).

An alpha of <0.10 was chosen to represent statistical significance (Rózsa et al, 2000). Mean abundance variation was found to be statistically significant for *N. melleni* between host fish species (p=0.00036) and host exhibit (p=0.05946), for *H. diminutus* between host fish species (p=0.00036) and host fish size classes (within 2 cm, p=0.06048), and for *C. parva* between host fish species (p=0.09033) and host exhibits (p=0.08637). Mean abundance variation was found to not be statistically significant *for N. melleni* and *C. parva* between host fish size classes (within 2 cm, p=0.33736 and p=0.29694 respectively) and for *H. diminutus* between host exhibits (p=0.18677) (Fig. 5).

N. melleni represents 84.9% of the individual parasites observed in this study. The host species with the lowest prevalence was Puget Sound rockfish, *Sebastes emphaeus*, at 12.5%. Six species had an average abundance of more than 50 individual *N. melleni* per host fish, and in descending order of mean abundance were *S. ruberrimus*, *S. diploproa*, *S. flavidus*, *S. caurinus*, *S. melanops*, and *S. nebulosus*. (see Appendix 2) Of the 14 exhibits sampled, *N. melleni* was found in 11 of them. (see Appendix 3) Four of the exhibits had and average abundance of more than 65 *N. melleni* per fish, and in descending order of mean abundance were NPA19, NPA4, NPA9 and NPC.

alpha<0.10	Species	Exhibit	Size class
N. melleni	0.00036	0.05947	0.33736
H. diminutus	0.00036	0.18677	0.06048
C. parva	0.09033	0.08637	0.29694

Figure 5: Resultant alpha values following bootstrap ANOVA tests comparing mean abundance of three parasites species against three varying factors of their host fish.

Heptacyclus diminutus represents 13.5% of the individual parasites observed in this study. The four fish species found not to harbor *H. diminutus* were *E. bison*, *H. lagocephalus*, *S. diploproa*, and *S. emphaeus*. Six species had an average abundance of more than 9 individual *H. diminutus* per host fish, and in descending order of mean abundance were *O. elongatus*, *S. ruberrimus*, *S. auriculatus*, *H. decagrammus*, *S. nigrocinctus*, and hybrid *Sebastes* spp. (see Appendix 2) Of the host size classes sampled, 5 size classes displayed average abundances at or above 12 *H. diminutus* per host fish; they were comprised of fish measured within 44-49 cm TL, and above 58 cm TL. (see Appendix 4)

C. parva represents just 1.6% of the individual parasites observed in this study. Of the 23 species sampled, 10 were hosts for this copepod. Of the *C. parva* found across all individual hosts, 98.7% were found in 7 species of *Sebastes* rockfish that in descending order of mean abundance were *S. melanops*, *S. flavidus*, *S. maliger*, *S. nigrocinctus*, *S. ruberrimus*, *S. nebulosus*, and hybrid *Sebastes* spp. (see Appendix 2) Of the 14 exhibits sampled, *C. parva* was found in 4 of them, and in descending order of abundance were NPA9, NPA13, NPA10 and NPC. (see Appendix 3)

Discussion

This study was conducted on established exhibits and random stocking of fishes and invertebrates would have resulted in eventual population shifts through competition, aggression and predation between display species. As such, the decision was made to analyze host fish species, exhibit, and host fish size class as variables independent of each other against mean abundance of

each major parasite species to determine where statistically significant variation exists, while acknowledging and identifying functionally incomplete independence between those variables.

Exhibits and host size as variables within this study cannot be considered to have been completely independent of one another as increased exhibit size generally equated to larger fish displayed within them. This is especially interesting when considering that statistically significant variation between exhibits was observed for mean abundances of *N. melleni* and *C. parva*, but not for *H. diminutus*. The opposite was found to be true when testing mean abundances of parasites between fish size classes, with the mean abundance of *H. diminutus* displaying the only statistically significant variation in that line of tests.

Exhibit and host size cannot be considered to have been completely independent of host species, as species with smaller maximum adult sizes were generally displayed in smaller exhibits (ex. *S. emphaeus* attained a maximum adult size of 22 cm in this study, and were displayed in exhibits that were between 271 L and 1974 L). It is possible that the results of this study may have been different were species of varying sizes displayed randomly among all of the NPA exhibits, allowing for smaller species to receive equal chance of parasite infection as larger tank mates.

Host Fish Species as a Variable

Host fish species showed significant variation for mean abundance of each of the three major parasites observed in this study (see Appendix 2). This aligns well with anecdotal observations made by aquarists at PDZA. The fish species with the least cumulative mean abundances in this study was the Puget Sound rockfish, *S. emphaeus*, where of 8 individual host fish that were screened for parasites, only one individual *N. melleni* was found. Also notably low in parasite abundance were *P. stellatus*, *S. diaconus*, *S. miniatus*, *E. bison*, *S. pinniger* and *H. lagocephalus*. Of these, *P. stellatus*, *S. miniatus*, *S. pinniger*, and *H. lagocephalus* had multiple representatives housed within systems of relatively high parasite abundance among tank mates.

The host species with the greatest cumulative mean abundances in this study was *S. ruberrimus*, which also had the individual representative host fish with the greatest number of *N. melleni* (1,118 monogeneans, with an average of nearly 24 monogeneans per centimeter host TL). Other fish with notably high parasite abundance were *S. diploproa*, *S. flavidus*, *O. elongatus*, *S. caurinus*, *S. melanops*, *S. nebulosus*, *S. auriculatus*, and hybrid *Sebastes* spp. Of these, all except *S. diploproa* had multiple representatives housed within systems of relatively high parasite abundance among tank mates.

Ophiodon elongatus had the greatest mean abundance of *H. diminutus* among host fish species at 104.3 per fish, which was more than double the next greatest mean abundance (*S. ruberrimus*, at 47.5 *H. diminutus* per host fish). *S. melanops* and *S. flavidus* each had a relatively high mean abundance of *C. parva*, with 2.9 and 2.8 copepods per host fish respectively.

While not observed in this study, Burreson (1977) reported *E. bison* to host *H. diminutus*, Kabata (1970) reported *S. diploproa* to host *C. parva*, and Margolis and Kabata (1988) reported *E. lateralis* and *S. pinniger* to host *C. parva*. This may suggest that factors other than host fish species affected these parasite-host interactions observed within the sampled exhibits.

Exhibits as a Variable

Exhibits showed significant variation for mean abundance of *N. melleni* and *C. parva*. The exhibits with greatest combined mean abundances of *N. melleni* and *C. parva* were (in descending order) NPA19, NPA4, NPA9, NPC, NPA10, and NPA13. The exhibits with the least combined mean abundances of the same two parasites were (in ascending order) NPA1, NPA18, NPA20, NPA17, NPA14, SPH and NPA3 (see Fig. 6 and Appendix 3).

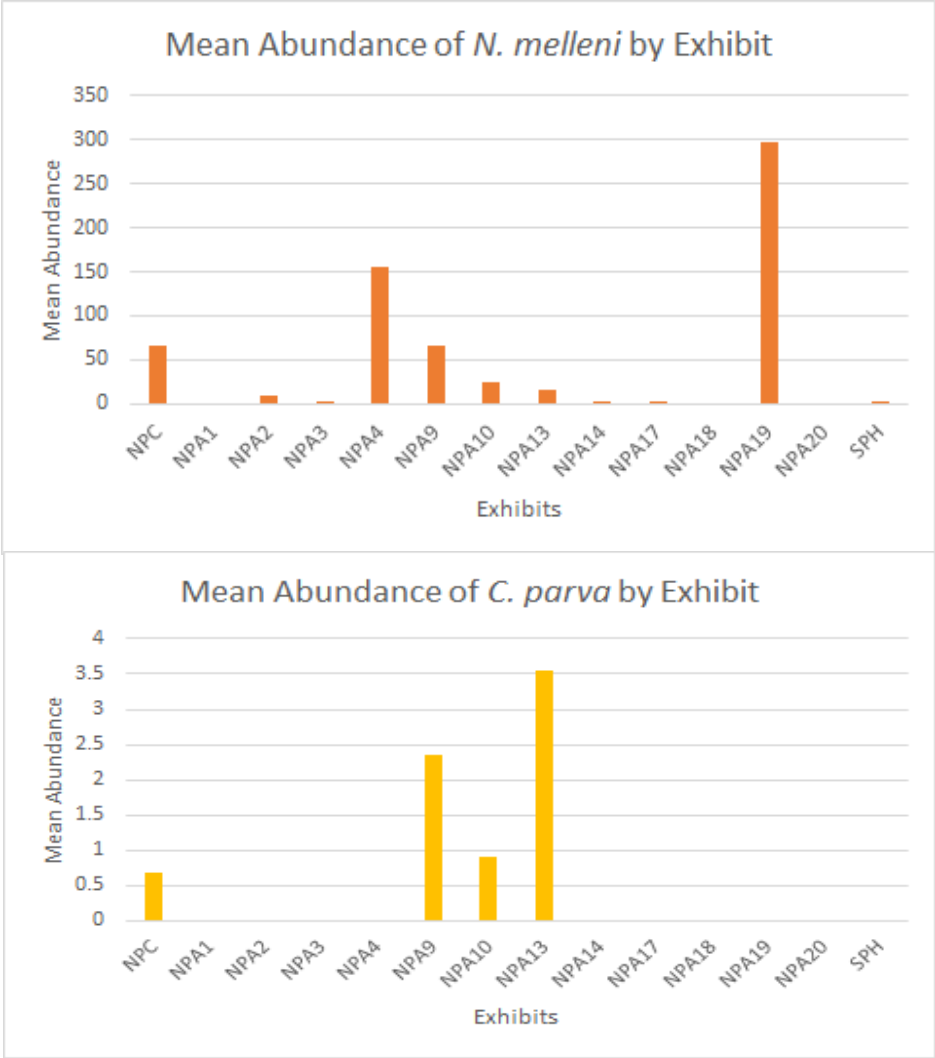

Figure 6. Mean abundance of *N. melleni* and *C. parva* by exhibit.

NPA19 was a holding tank that housed three *S. nebulosus*, which displayed a relatively high mean abundance of *N. melleni* (297.7), when compared to the same value from the 14 other *S. nebulosus* in this study (5.7). This may have been due to some factor associated with NPA19, rather than any potential innate susceptibility of *S. nebulosus* to *N. melleni*.

NPA4 housed a juvenile *S. pinniger* with no observed parasites and a juvenile *S. diploproa* infected with 310 *N. melleni*, which may highlight the relative susceptibility of *S. diploproa* to *N. melleni* or perhaps just the susceptibility of this individual animal, as there were no other examples

of *S. diploproa* to use for comparison. It also caused the mean abundance of that exhibit to appear relatively high, without having a greater number of host fish to potentially offset the average and likely creating an outlier within the data.

While the mean abundances for *H. diminutus* between exhibits were not found to be statistically significant in variation, the exhibits these leeches were found in were (in descending order) NPC, NPA2, NPA9, NPA13, NPA19 and NPA17. This overlaps well with the relatively high abundances of *N. melleni* and *C. parva* in NPC, NPA9, NPA13 and NPA19 which may highlight some of these exhibits as having characteristics more likely to encourage success of the ectoparasites found in this study.

Host Fish Size Class as a Variable

While total lengths of host fish were noted to the nearest whole centimeter, individuals were later organized into size classes of 2 cm, ranging from 10 to 64 cm. A few of the larger fish (8 individuals) were placed into a single group that ranged from 89 to 163 cm. This was done in order to increase the number of representatives within groups to better allow for statistical comparison between them.

Heptacyclus diminutus was the only parasite in this study found to have statistically significant differences between the mean abundances for the size classes observed (see Fig. 7 and Appendix 3). The greatest mean abundance (38.1 *H. diminutus* per host fish) was found in fish 89 cm to 163 cm TL, however 91.8% of the leeches found in that size range were associated with three O. elongatus, which may reflect host species preference rather than host size preference. By comparison, the second highest mean abundance (27.0 *H. diminutus* per host fish) was found in host fish ranging 48-49 cm TL, a size class comprised of 7 different species.

The next five greatest mean abundances were found (in descending order) within groups that were 46-47 cm, 44-45 cm, 58-59 cm, 42-43 cm, and 38-39 cm TL. There were no leeches seen on host fish below 20 cm, and mean abundance was <1 on host fish between the sizes of 20-21 cm and 30-31 cm TL.

Neobenedenia melleni has been observed to display increased prevalence correlated with increased host fish length when infecting Red Snapper, *Lutjanus erythropterus* (Ravi and Yahaya, 2016). *C. parva* was noted to only infect *Sebastes serranoides* that were less than 10 cm in length (Love et al, 1984). Similar trends may become apparent should more abundance observations be made across the different size classes of individual species of host fish included in this study.

Possible Interactions

In situations where more than one parasite of the same or different species inhabits a host, there is the potential for interactions between parasites and/or between parasites and host to be affected (Buchmann et al, 2002, Gotelli et al, 2002, Kotob et al, 2016, Morand et al, 1999, Morand et al, 2002, Poulin, 2013, Salgado-Maldonado et al, 2016, Zolovs et al, 2015) (see Fig. 4). Parasites may display direct or indirect competition for resources such as preferred attachment sites or target feed tissues, or they may benefit other parasites such as by weakening host immune system or

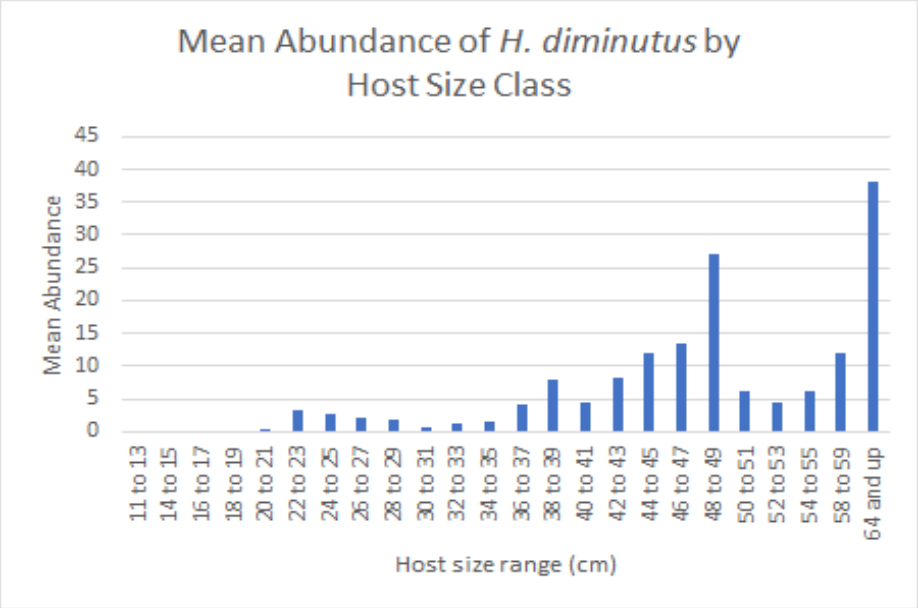

Figure 7. Mean abundance of *H. diminutus* by host size class.

modifying host behavior. *N. melleni* was observed to inhabit more of the host body surface than fins and *H. diminutus* and *C. parva* inhabit fins over other potential sites of attachment and feeding, and such apparent preferences may be due to interspecies competition among the parasites and the physical abilities of each parasite to attach to its host (González, 2015). *N. melleni* is the most abundant parasite observed among the fish in this study, which may be due to the monogenean's ability to compete for resources, the surface area available for them to inhabit, their method(s) of reproduction, their feeding strategies or some combination of the above.

Similarly, host fish social and feeding behavior may affect infection opportunity. Social interaction between conspecifics may force or allow tank mates to inhabit areas of exhibits that allow for more contact with infective stages of parasites, while potentially also increasing stress and reducing immune response.

A polyopisthocotylean monogenean, *Microcotyle sebastis*, was found to infect the gill tissue of some number of the fish tested in this study. While only observed once in the freshwater dip treatments, dozens to hundreds of *M. sebastis* were consistently found in debris rinsed from filter socks and siphoned from the bottom of quarantine tanks after just a few hours of exposure of host fish to 2 mg/L of Praziquantel. Ideally this species would have been included in this study, however quarantine treatments were carried out on groups of multiple species of fish simultaneously for logistical (not to mention financial) reasons, making it impossible to observe which individual fish were definite hosts to this monogenean. *M. sebastis* has been noted to have significant impacts on cultured rockfish (Kim et al, 1998, Chun, 2002), making its presence worthy of consideration when addressing potential parasite interactions on individual hosts.

A caligid copepod, *Caligus clemensi*, was found on one representative of *S. maliger* from this study. The authors have observed this parasite infecting *Aulorhynchus flavidus* (tube-snout), *Gasterosteus aculeatus* (stickleback), and *Clupea pallasii* (Pacific herring). These fish are collected from sea grass or acquired from net pens in Puget Sound for display at PDZA. *C. clemensi* also notably infects net pen salmon and other Puget Sound fish (Kabata, 1988). The *S. maliger* harboring *C. clemensi* was housed with 3 *S. nebulosus*, which had been acquired from net pens approximately six weeks before they were put through the freshwater dips included in this study. It is possible that this copepod came into PDZA on one of the *S. nebulosus* and transferred to the *S. maliger* once they were put on display together.

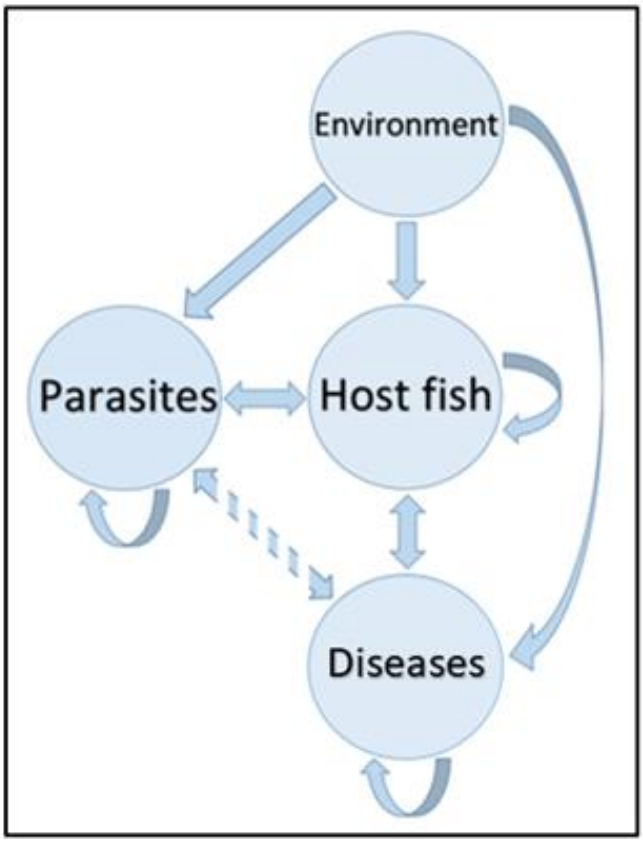

Figure 8. Potential interactions affecting parasite and disease burden upon host fish. Environmental stressors and various biotic interactions centered on the host fish all work towards balance. When imbalances occur that are not corrected, parasite and disease burden can overwhelm the immune responses of host fish to negative consequence.

Other parasites historically noted on teleosts in these exhibits that were not seen during this study include at least one species of *Gyrodactylus* monogenean, at least one species of *Trichodina* ciliate, and an unidentified turbellarian. An unidentified digenean has been observed in the intestinal tract of *Sebastes* rockfish, and a morphologically similar species has been observed within the digestive tract of *O. elongatus*. Again, each of these may have had some level of interaction with the three parasite species focused on in this study, *M. sebastis* and each other where multiple infections were occurring (Morand et al., 1999, Morand et al., 2002, Gotelli and Rhode, 2002, Kotob et al., 2016).

Other diseases (viral, bacterial, protozoal, and fungal) may also have played an indirect role in parasite populations within the exhibits of this study. While potentially benefiting from increased access to susceptible fish tissues created by ectoparasites, these infections could have affected host immune response. *H. diminutus* has been shown to be an important vector for the kinetoplastid, *Trypanosoma beckeri* in *Scorpaenichthys marmoratus* (cabezon) (Burreson, 1979). It also cannot be ruled out that diseases may have competed with one another within hosts, or competed with parasites for available host resources. These pathogens may have acted as primary infections in certain situations, potentially suppressing immune response and allowing ectoparasites the opportunity to infect individual hosts to a degree they otherwise might not have (Ezenwa et al., 2016). (see Fig. 8)

Environmental factors also may have had impacts on parasite populations (Poulin, 2006), and the exhibit parameters were heavily influenced by in-coming seawater. The North Pacific Aquarium lacked temperature control and filtration consisted entirely of rapid sand filters. Constant turnover of natural seawater was the only option for maintaining survivable conditions for the collection. Seasonal variations in temperature and salinity likely contributed to regular, visible increases in parasite populations through affecting physiological processes of the parasites, the hosts or both. Secondary infections may have also varied in pathogenicity across varied environmental conditions. Unsterilized seawater as the primary source of water may have also provided an opportunity for seasonal blooms of pathogens and parasites to enter the aquarium.

Untested Cohabitants

Within the mixed species displays of the NPA, a variety of taxa were exhibited along with the fishes in this study. They were not included here largely due to physiological and biological differences and sensitivities.

None of the displayed invertebrates were put through freshwater dips. The ectoparasites of the fishes this study focused on belong to groups known to display direct life cycles, and thus would not naturally infect crustaceans, mollusks, cnidarians, and echinoderms from these systems. It is noteworthy that any of these groups of animals may act as temporary vectors for the previously described parasites' infective stages without directly contributing to the completion of their life cycles. One *H. diminutus* was observed attached to the outer surface of a *Ctryptochiton stelleri* (gumboot chiton, or the painfully illegitimate "giant western fiery chiton"), but only by its anterior sucker with the rest of the leech swaying in the water and flailing towards passing fish, which suggests the leech was treating the chiton as though it were merely part of the display rockwork.

None of the displayed elasmobranch fishes were put through freshwater dips, based on concerns over how they may have responded to the treatment. *C. parva* was never visually observed to infect these taxa within the NPA. *Squalus acanthias* (spiny dogfish) and *Hydrolagus colliei* (spotted ratfish) were put through praziquantel treatments to determine if they harbored *N. melleni* or *M. sebastis*. The *S. acanthias* were found to carry a host-specific polyopisthocotylean, identified as *Squalonchocotyle squali*. *H. colliei* were observed to harbor *H. diminutus* within their nares and buccal cavity multiple times, though it is unclear if the leeches were successfully parasitizing these animals. *H. colliei* should be considered a potential source of *H. diminutus* infection for known host species.

Teleost fish species that were present in the NPA systems, but not included in this study include *Pholis gunnellus* (rock gunnel), *Chirolophis decoratus* (decorated warbonnet), *Anoplarchus purpurescens* (high cockscomb), *Rhamphocottus richardsonii* (grunt sculpin), *Nautichthys oculofasciatus* (sailfin sculpin), *Chitonotus pugetensis* (roughback sculpin), *Oligocottus maculosus* (tidepool sculpin), *Gasterosteus aculeatus* (stickleback), *Gobiesox maeandricus* (northern clingfish), and *Cymatogaster aggregata* (shiner perch). These fish were omitted from the study due to restrictions on resources and scope, however it is possible that any of them may act as potential hosts or vectors for the parasites listed in this study.

Possible Points of Impact on Results

Ectoparasite censuses taken during this study may have been impacted by physical dislodging prior to sampling due to collection and handling, incomplete parasite removal during baths, and change in exhibit populations over time. While it is almost certain that these had effects on the outcome of measured parasite burden on individual fish, the basis of this study is rooted in the idea that applying the same methods and protocols on every individual fish across random sampling would result in the same effects occurring with proportional impact across the study, allowing the determination of relative averages of parasites sampled from similar categories of hosts. Because of this, it should be noted that the specific values and results of this paper (ex. mean abundance of *N. melleni* observed on *S. melanops* was 64.60 worms/fish) are only useful as relative quantifications within this data set, but that broad results (ex. *N. melleni* displays a greater mean abundance with *S. melanops* over *S. pinniger*) may be applicable in other, similar situations.

Shvydka et al. (2017) stated that accurate and precise estimates of the mean abundance of parasites in fish require a host group of at least 80 individuals. The only group of hosts that meets that standard from this study were the 126 fish pooled together from the NPC exhibit. They go on to say that sample sizes of 25 to 40 had medians with little bias but distributions skewed low, and sample sizes of 10 or less (such as the majority of sample groups utilized in this study) as giving unreliable results. Larger sample sizes will always increase the accuracy and scientific value of a study, which was why effort was put towards collecting data from the majority of individual fish pulled from NPA exhibits. Further studies comparable to this one would provide valuable data to supplement and expand on what is presented here.

Most ecological parasitology is conducted utilizing deceased hosts that researchers can spend time with to ensure that accurate counts of parasite populations can be achieved. Attempting the same level of accuracy with living animals intended for display increases ethical concerns, complexity and resource requirements beyond the capabilities of many public aquariums. It was decided that consistency in sampling a large number of host fish would be the best route to take advantage of a rare situation and gather relative parasite preference information while maintaining a high level of welfare for the entirety of the collection.

This approach eliminated the potential for reliable consideration of host fish gender, which has been shown to impact infection parameters in some parasite-host relationships (Pickering and Christie, 1980, Reimchen and Nosel, 2001).

Possible Avenues of Further Research

Sampling public aquarium exhibits for parasites is a regular part of modern husbandry practices through visual observation, skin scrapes, gill clips, fin clips, necropsy, etc. By standardizing sampling techniques, it would be possible to begin to develop and record preference profiles of generalist parasites commonly seen in these exhibits based on quantitative data. This would be invaluable within the facility it was gathered (where husbandry and medical professionals could utilize trends in the data to better manage aquatic exhibits), and potentially useful within the industry should the trends prove similar across any subset of the wide variety of displays found in public aquaria. Results would also allow for prophylactic quarantine treatments to be better tailored towards specific groups of display animals based on these observations.

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Appendices

Appendix 1. General descriptions of NPA exhibits sampled for this study.

Exhibit	Volume (L)	Shape	Heterospecifics	Description
NPC	516800	Octodecagon	Chondrichthyans, echinoderms, cnidarians	Flow through exhibit, sand filters, source water for other exhibits
NPA1	271	Cylinder faced rectangular box	Cnidarians, crustaceans, mollusks	Rocky subtidal
NPA2	3344	Rectangular box	Cnidarians, crustaceans, mollusks	Rock wall, subtidal
NPA3	2972	Rectangular box	Crustaceans, echinoderms	Sandy, pier pilings
NPA4	114	Rectangular box	Crustaceans, echinoderms	Intertidal
NPA9	895	Cylinder w/ flat face	Crustaceans, cnidarians, echinoderms	Rocky subtidal
NPA10	4687	Rectangular box	Cnidarians, echinoderms	Rocky subtidal
NPA13	4513	Rectangular box	Cnidarians, crustaceans, echinoderms, mollusks	Rocky intertidal
NPA14	406	Rectangular box	Cnidarians, crustaceans	Subtidal
NPA17	1218	Rectangular box	Cnidarians, echinoderms, crustaceans	Deep reef
NPA18	222	Rectangular box	Cnidarians, crustaceans, echinoderms	Subtidal
NPA19	283	Cylinder	None	Holding
NPA20	1974	Cylinder	Cnidarians, crustaceans, mollusks	Rock wall, subtidal
SPH	1794	Rectangular box	Crustaceans, echinoderms	Holding

Appendix 2. Mean parasite abundance observed with groups of host fish species.

Host	n	*N. melleni*	*H. diminutus*	*C. parva*
Acipenser transmontanus	2	12.00	4.00	0
Anarrhichthys ocellatus	3	2.00	6.67	0
Embiotoca lateralis	8	3.38	1.63	0
Enophrys bison	1	6.00	0	0
Hemilepidotus hemilepidotus	2	6.00	8.00	0
Hexagrammos decagrammos	3	20.33	10.00	0
Hexagrammos lagocephalus	5	8.00	0	0.20
Ophiodon elongatus	3	0.33	104.33	0
Platichthys stellatus	10	1.70	0.60	0
Sebastes auriculatus	8	31.13	24.75	0.13
Sebastes caurinus	30	91.87	7.60	0.03
Sebastes diaconus	1	2.00	2.00	0
Sebastes diploproa	1	310.00	0	0
Sebastes emphaeus	8	0.13	0	0
Sebastes flavidus	9	109.78	3.11	2.78
Sebastes maliger	23	32.52	7.83	1.87
Sebastes melanops	40	64.60	7.85	2.90
Sebastes miniatus	5	1.40	3.60	0
Sebastes nebulosus	18	54.39	1.89	1.00
Sebastes nigrocinctus	7	28.14	9.71	1.43
Sebastes pinniger	24	4.96	1.58	0
Sebastes ruberrimus	4	491.25	47.50	1.25
Sebastes spp.	17	41.47	9.53	0.77

Appendix 3. Mean parasite abundance of *N. melleni* and *C. parva* observed within exhibits.

Exhibit	n	*N. melleni*	*C. parva*
NPC	126	65.67	0.69
NPA1	4	0	0
NPA2	4	9.50	0
NPA3	4	3.25	0
NPA4	2	155.00	0
NPA9	17	66.59	2.35
NPA10	21	24.67	0.91
NPA13	24	16.63	3.54
NPA14	1	1.00	0
NPA17	9	0.22	0
NPA18	2	0	0
NPA19	3	297.67	0
NPA20	2	0	0
SPH	10	2.70	0

Appendix 4. Mean abundance of *H. diminutus* observed within host fish class sizes.

Size (cm)	n	*H. diminutus*
11 to 13	2	0
14 to 15	2	0
16 to 17	3	0
18 to 19	3	0
20 to 21	4	0.25
22 to 23	6	3.33
24 to 25	9	2.67
26 to 27	9	2.00
28 to 29	8	1.75
30 to 31	8	0.75
32 to 33	12	1.33
34 to 35	17	1.71
36 to 37	16	4.13
38 to 39	21	7.91
40 to 41	9	4.56
42 to 43	16	8.38
44 to 45	25	12.12
46 to 47	17	13.53
48 to 49	11	27.00
50 to 51	10	6.20
52 to 53	7	4.43
54 to 55	4	6.25
58 to 59	3	12.00
64 and up	9	38.11

Literature Cited

Ahne, W. 1985. *Arugulus foliaceus* L. and *Piscicola gemoetra* L. as mechanical vectors of spring viraemia of carp virus (SVCV). Journal of Fish Diseases. 8(2): 241-242.

Allen, M.J., T. Mikel, D. Cadien, J.E. Kalman, E.T. Jarvis, K.C. Schiff, D.W. Diehl, S.L. Moore, S. Walther, G. Deets, C. Cash, s. Watts, D.J. Pondella II, V. Raco-Rands, C. Thomas, R. Gartman, L. Sabin, W. Power, A.K. Groce, J.L. Armstrong. (2007) VII. Ectoparasitism of Fishes. Southern California Bight 2003 Regional Monitoring Program: IV. Demersal Fishes and Megabenthic Invertebrates. Southern California Coastal Water Research Project. Costa Mesa, CA. P. 231-267.

Arya, P.V., H.S. Singh. 2016. Host specificity and preference assessment for *Mizelleus indicus* (Jain, 1957), Pandey et al. 2003 on experimental hosts. International Journal of Scientific Research. 5(3): 524-525.

Barber, I. 2007. Parasites, behavior and welfare in fish. Applied Animal Bahaviour Science. 104: 251-264.

Barber, I., D. Hoare, J. Krause. 2000. Effects of parasites on fish behavior: a review and evolutionary perspective. Reviews in Fish Biology and Fisheries. 10: 131-165.

Beverly-Burton, M. 1984. Monogenea and Turbellaria. Guide to the parasites of fishes of Canada. Part I. Can. Spec. Publ. Fish. Aquat. Sci. 74: 209.

Brazenor, A. K. 2017. The biology of the cosmopolitan fish parasite *Neobenedenia girellae*. PhD thesis, James Cook University.

Brazenor, A.K., K.S. Hutson. 2015. Effects of temperature and salinity on the life cycle of *Neobenedenia* sp. (Monogenea: Capsalidae) infecting farmed barramundi (*Lates calcarifer*). Parasitol. Res. 114(5): 1875-86.

Brazenor, A.K., T. Bertozzi, T.L. Miller, I.D. Whittington, K.S. Hutson. 2018. DNA profiling reveals *Neobenedenia girellae* as the primary culprit in global fisheries and aquaculture. Mol. Phylogenet. Evol. 129: 130-137.

Buchmann, K., T. Lindenstrøm. 2002. Interactions between monogenean parasites and their fish hosts. International Journal for Parasitology. 32: 309-319.

Bullard, S.A., G. W. Benz, R. M. Overstreet, E. H. Williams Jr., J. Hemdal. 2000. Six New Host Records and an Updated List of Wild Hosts for *Neobenedenia melleni* (MacCallum) (Monogenea: Capsalidae). Comparative Parasitology. 67(2): 190-196.

Burreson, E. M. 1975. Biological Studies on the Hemoflagellates of Oregon Marine Fishes and Their Potential Leech Vectors. Ph.D. Thesis, Oregon State University, Corvallis, Oregon.

Burreson, E. M. 1977. Two New Species of Malmiana (Hirudinea: Piscicolidae) from Oregon Coastal Waters. The Journal of Parasitology. 63(1): 130-136.

Burreson, E. M. 1979. Structure and Life Cycle of *Trypanoplasma beckeri* sp. n. (Kinetoplastida), a Parasite of the Cabezon, *Scorpaenichthys marmoratus*, in Oregon Coastal Waters. The Journal of Protozoology. 26(3): 343-347.

Burreson, E. M. 2007. Hirudinida. The Light and Smith Manual: Intertidal Invertebrates from Central California to Oregon, 4th edition. P. 303-309.

Burreson, E. M., J. K. Passarelli, B. Kim. 2012. A New Species of Marine Leech (Hirudinida: Piscicolidae) from Giant Kelpfish, *Heterostichus rostratus* Girard, in Southern California. The Journal of Parasitology. 98(4): 788-790.

Burreson, E. M., K. S. Reece, F. Dunker. 2019. A New Species of *Ostreobdella* (Hirudinida: Piscicolidae) Infesting Rockfishes in the Genus *Sebastes* (Sebastidae) in Marine Public Aquaria on the Northern California Coast, U.S.A. Comparative Parasitology. 86(1): 30-37.

Castro-Romero, R., H. Baeza-Kuroki. 1985. Two new species and one new record of *Clavella* (Copepoda: Lernaeopodidae) from inshore fishes of Antofagasta, Chile. Systematic Parasitology. 7:103-110.

Catalano, S.R., I.D. Whittington, S. C. Donnellan, B. M. Gillanders. 2014. Parasites as biological tags to assess host population structure: Guidelines, recent genetic advances and comments on a holistic approach. International Journal for Parasitology: Parasites and Wildlife. 3: 220-226.

Chisholm, L.A., I.A. Wittington. 2002. Efficacy of Praziquantel Bath Treatments for Monogenean Infections of the *Rhinobatos typus*. Journal of Aquatic Animal Health. 14: 230-234.

Choudhury, A., T.A. Dick. 1998. Patterns and determinants of helminth communities in the Acipenseridae (Actinopterygii: Chondrostei), with special reference to the lake sturgeon, *Acipenser fulvescens*. Can. J. Zool. 76: 330-349.

Chun, K.S. 2003. Studies on Some Parasites from Aquarium Rockfishes, *Sebastes* spp. Kor. J. Env. Hlth. Soc. 29(4): 17-20.

Chun, K.S. 2003. Infectious Status on Monogenetic Trematodes of *Sebastes* spp. (Family: Scorpaenidae) Including *Microcotyle sebastis*, *Megalocotyle trituba* and *Trochopus australis* in Newport Fish Market. Kor. J. Env. Hlth. Soc. 29(4):55-57.

Chun, K.S. 2002. Studies on the Infestation of Monogenean Helmith, *Microcotyle sebastis*, and Copepod, *Clavalla adunca* from *Sebastes melanops* and *S. maliger*. Kor. J. Env. Hlth. 29(1): 16-18.

Chun, K.S. 2001. Infection of Parasitic *Anisakis* Type Larvae (Nematoda) from Some Rockfishes, *Sebastes* spp. J. Fd. Hyg. Safety. 17(4): 206-209.

Dezfuli, B.S., A. Lui, P. Boldrini, F. Pironi, L. Giari. 2008. The inflammatory response of fish to helminth parasites. Parasite. 15: 426-433.

Ezenwa, V.O., E.A. Archie, M.E. Craft, D.M. Hawley, L.B. Martin, J. Moore, L. White. 2016. Host behaviour–parasite feedback: an essential link between animal behaviour and disease ecology. Proc. R. Soc. B 283: 20153078.http://dx.doi.org/10.1098/rspb.2015.3078

Frankel, V.M., A.P. Hendry, F. Rolshausen, M. E. Torchin. 2015. Host preference of an introduced 'generalist' parasite for a non-native host. 45(11): 703-709.

Gotelli, N.J., K. Rhode. 2002. Co-occurrence of ectoparasites of marine fishes: a null model analysis. Ecology Letters. 5: 86-94.

Guégan J.F., A. Lambert, C. Lévêque, C. Combes, L. Euzet. 1992. Can host body size explain the parasite species richness in tropical freshwater fishes? Oecologia. 90: 197-204.

Hirazawa, N. 2019. Killing effects of various treatments on eggs of the skin-parasitic monogeneans *Neobenedenia girellae* and *Benedenia seriolae*. Aquaculture. 498: 285-288.

Hoai, T.D., K.S. Hutson. Reproductive Strategies of the Insidious Fish Ectoparasite, *Neobenedenia* sp. (Capsalidae: Monogenea). PLoS ONE 9(9): e108801. doi:10.1371/journal.pone.0108801

Huys, R. 2014. Copepoda. Atlas of Crustacean Larvae. P. 144-151.

Kabata, Z. 1970. Some Lernaeopodidae (Copepoda) from fishes of British Columbia. Journal of the Fisheries Research Board of Canada. 27:865-885, figs. 1-83.

Kabata, Z. 1982. Parasitic Copepoda: Problems and Perspectives. Advances in Parasitology. 19: 1-71.

Kabata, Z. 1988. Copepoda and Branchiura. Guide to the parasites of fishes of Canada. Part II — Crustacea. Can. Spec. Publ. Fish. Aquat. Sci. 101: 184.

Kalman, J. E. 2006. Ectoparasites of Demersal Marine Fishes in Santa Monica Bay, California, U.S.A., with 31 New Host Records and Three Range Extensions. Comparative Parasitology. 73: 201-213.

Kaneko II, J.J., R. Yamada, J.A. Brock, R.M. Nakamura. 1988. Infection of tilapia, *Oreochromis mossambicus* (Trewavas), by a marine monogenean, *Neobenedenia melleni* (MacCallum, 1927) Yamaguti, 1963 in Kaneohe Bay, Hawaii, USA, and its treatment. Journal of Fish Diseases. 11(4): 295-300.

Kim, C.S., K.H. Kim. 2001. Correlation between Phamacokinetics of Praziquantel and Extermination of *Microcotyle sebastis* (Monogenea) in Cultured Rockfish *Sebastes schlegeli*. J. Fish. Sci. Tech. 4(4): 197-200.

Kim, K.H., C.S. Kim, J.W. Kim. 2001. Depletion of Praziquantel in plasma and muscle tissue of cultured rockfish *Sebatis schegeli* after oral and bath treatment. Diseases of Aquatic Organisms. 45: 203-207.

Kim, K.H., S. Park, B.Y. Jee. 1998. Efficacy of Oral Administration of Praziquantel and Mebendazole against *Microcotyle sebastis* (Monogenea) Infestation of Cultured Rockfish (*Sebastes schlegeli*). Fish Pathology. 33(5): 467-471.

Kim, K.H., Y.J. Hwang, J.B. Cho, S.I. Park. 2000. Immunization of cultured juvenile rockfish *Sebastes schlegeli* against *Microcotyle sebastis* (Monogenea). Diseases of Aquatic Organisms. 40: 29-32.

Klemm, D.J. 1982. Leeches (Annelida: Hirudinea) of North America. U.S. Environmental Protection Agency, Washington, D.C., EPA/600/3-82/025 (NTIS PB82208679).

Klimpel, S., A. Seehagen, H. W. Palm, H. Rosenthal. 2001. Deep-water Metazoan Fish Parasites of the World. Logos Verlag, Berlin. ISBN 3-89722-681-2. 1316 pp.

Kotob, M.H., S. Menanteau-Ledouble, G. Kumar, M. Abdelzaher, M. El-Matbouli. 2016. The impact of co-infections on fish: a review. Vet. Res. 47: 98.

Kutschera, U. 2016. Sex versus Gender in Sea Urchins and Leeches Two Centuries after Lamarck 1816. Journal of Marine Science: Research and Development. 6(5): 1-3.

Lafferty, K.D. 1997. Evironmental Parasitology: What can Parasites tell us about Human Impacts on the Environment? Parasitology Today. 13(7): 251-255.

Lee, D.L., D.J. Whitaker, R.D. Stanley. 1990. A preliminary examination of the parasite fauna of yellowtail rockfish, *Sebastes flavidus*. Can. Tech. Rep. Fish. Aquat. Sci. 1777:21.

Love, M.S., M. Moser. 1983. A Checklist of Parasites of California, Oregon, and Washington Marine and Estuarine Fishes. Faculty Publications from the Harold W. Manter Laboratory of Parasitology. 750. http://digitalcommons.unl.edu/parasitologyfacpubs/750.

Love, M.S., K. Shriner, P. Morris. 1984. Parasites of olive rockfish, *Sebastes serranoides*, (Scorpaenidae) off central California. Fishery Bulletin, United States National Marine Fisheries Service. 82(3):530-537.

Mackenzie, K. 1999. Parasites as Biological Tags in Population Studies of Marine Organisms. Qatar Univ. Sci. J. 19.

Mackenzie, K. 2002. Parasites as Biological Tags in Population Studies of Marine Organisms: An update. Parasitology. 124(07): 153-63.

Macnab, V., I. Barber. 2011. Some (worms) like it hot: fish parasites grow faster in warmer water, and alter host thermal preferences. Global Change Biology. 18(5): 1540-1548.

Marcogliese, D.J., E. Albert, P. Gagnon, J.M. Sévigny. 2003. Use of parasites in stock identification of the deepwater redfish (*Sebastes mentella*) in the Northwest Atlantic. Fish. Bull. 101: 183-188.

Moles, A., J. Heifetz, D.C. Love. 1998. Metazoan parasites as potential markers for selected Gulf of Alaska rockfishes. Fishery Bulletin, United States National Marine Fisheries Service 96(4):912-916.

Morand, S., K. Rhode, C. Hayward. 2002. Order in ectoparasite communities of marine fish is explained by epidemiological processes. Parasitology. 124: S57-S63.

Morand, S., R. Poulin, K. Rohde, C. Hayward. 1999. Aggregation and species coexistence of ectoparasites of marine fishes. International Journal for Parasitology. 29: 663-672.

Mosquera, J., M. de Castro, M. Gómez-Gesteira. 2003. Parasites as Biological Tags of Fish Populations: Advantages and Limitations. Comments on Theoretical Biology. 8: 69-91.

Mulcahy, D., D. Klaybor, W. N. Batts. 1990. Isolation of infectious hematopoietic necrosis virus from a leech (*Piscicola salmositica*) and a copepod (*Salmincola* sp.), ectoparasites of sockeye salmon *Oncorhynchus nerka*. Diseases of Aquatic Organisms. 8(1): 29-34.

Nagasawa, K., T. Umino, D. Uyeno, S. Ohtsuka, H. Koizumi. 2008. Infection with the Parasitic Copepod *Clavella parva* (Lernaeopodidae) in Gold-eye Rockfish *Sebastes thompsoni* Broodstock in Japan. Fish Pathology. 43(1): 55-60.

Noga, E.J. 2010. Fish Disease: Diagnosis and Treatment. 2nd ed. Wiley-Blackwell. 519 p.

Øines, Ø., J.H. Simonsen, J.A. Knutsen, P.A. Heuch. 2006. Host preference of adult *Caligus elongates* Nordman in the laboratory and its implications for Atlantic cod aquaculture. Journal of Fish Diseases. 29: 167-174.

Ogawa, K. 2015. Diseases of cultured marine fishes caused by Platyhelminthes (Monogenea, Digenea, Cestoda). Parasitology. 142: 178-195.

Olivia, M.E., M.T. Gonzalez. 2004. Metazoan parasites of *Sebastes capensis* from two localities in northern Chile as tools for stock identification. Journal of Fish Biology. 64: 170-175.

Paterson, S., J. Lello. 2003. Mixed models: getting the best use of parasitological data. Trends in Parasitology. 19(8): 370-375.

Pickering, A. D., P. Christie. 1980. Sexual differences in the incidence and severity of ectoparasites infestation of brown trout, *Salmo trutta* L. Journal of Fish Biology. 16(6): 669-683.

Pollitt, L.C., S.E. Reece, N. Mideo, D.H. Nussey, N. Colegrave. 2012. The Problem of Auto-Correlation in Parasitology. PLoS Pathog 8(4): e1002590. doi:10.1371/journal.ppat.1002590

Poulin, R. 2013. Explaining variability in parasite aggregation levels among host samples. Parasitology. 140: 541-546.

Poulin, R. 2006. Variation in infection parameters among populations within parasite species: Intrinsic properties versus local factors. International Journal for Parasitology. 36: 877-885.

Poulin, R. 2004. Macroecological patterns of species richness in parasite assemblages. Basic and Applied Ecology. 5: 423-434.

Poulin, R. 2000. Variation in the intraspecific relationship between fish length and intensity of parasitic infection: biological and statistical causes. Journal of Fish Biology. 56: 123-137.

Poulin, R. 1992. Determinants of Host-Specificity in Parasites of Freshwater Fishes. International Journal for Parasitology. 22(6): 753-758.

Ravi, R., Z. Shariman Yahaya. 2016. *Neobenedenia melleni* Parasite of Red Snapper, *Lutjanus erythropterus*, with Regression Statistical Analysis between Fish Length, Temperature, and

Parasitic Intensity in Infected Fish, Cultured at Jerejak Island, Penang, Malaysia. Journal of Parasitology Research, vol. 2016, Article ID 1946283, 8 p.

Reiczigel, J., M. Marozzi, I. Fábián, L Rósza. 2019. Biostatistics for Parasitologists – A Primer to Quantitative Parasitology. Trends in Parasitology. 1835: 1-4.

Reimchen, T. E., P. Nosil. 2001. Ecological causes of sex-biased parasitism in threespine stickleback. Biological Journal of the Linnean Society. 73: 51-63.

Rodríguez-Santiago, M.A., S. Gómez, J.A. Rosales-Casián, M.I. Grano-Maldonado. 2015. Parasitic Copepods of the Vermillion Rockfish *Sebastes miniatus* (Pisces: Scorpaenidae) from Inshore Waters of Baja California (Eastern Pacific). Neotropical Helminthology. 9(1): 1-12.

Rohde, K., C. Hayward, M. Heap. 1995. Aspects of the Ecology of Metazoan Ectoparasites of Marine Fishes. International Journal for Parasitology. 25(8): 945-970.

Rózsa, L., J Reiczigel, G. Majoros. 2000. Quantifying parasites in samples of hosts. J Parasitol. 86(2): 228-232.

Salgado-Maldonado, G., M.T. Novelo-Turcotte, J.M. Caspeta-Mandujano, G. Vazquez-Hurtado, B. Quiroz-Martínez, N. Mercado-Silva, M. Favila. 2016. Host specificity and the structure of helminth parasite communities of fishes in a Neotropical river in Mexico. Parasite. 23(61).

Sasal, P., S. Trouvé, C. Müller-Graf, S. Morand. 1999. Specificity and host predictability: a comparative analysis among monogenean parasites of fish. Journal of Animal Ecology. 68: 437-444.

Shvydka, S., V. Sarabeev, V.D. Estruch, C. Cadarso-Suárez. 2018. Optimum sample size to estimate mean parasite abundance in fish parasite surveys. Helminthologia. 55(1): 52-59.

Thoney, D. A., W. J. Hargis. 1991. Monogenea (Platyhelminthes) as hazards for fish in confinement. Annual Review of Fish Diseases. 1: 133-153.

Trujillo- González, A. J. 2015. Behaviour, histopathology and immunobiology: interactions between the ectoparasites *Neobenedenia* (Monogenea: Capsalidae) and its host, *Lates calcarifer* (Perciformes: Latidae). MPhil thesis, James Cook University.

Utevsky, S. Y., P. Trotelj. 2004. Phylogenetic relationships of fish leeches (Hirudinea, Piscicolidae) based on mitochondrial DNA sequences and morphological data. Zoologica Scripta. 33(4): 375-385.

Whittington, I. D. 2004. The Capsalidae (Monogenea: Monopisthocotylea): a review of diversity, classification and phylogeny with a note about species complexes. Folia Parasitologica. 51: 109-122.

Williams, J. I., E. M. Burreson. 2006. Phylogeny of the fish leeches (Oligochaeta, Hirudinida, Piscicolidae) based on nuclear and mitochondrial genes and morphology. Zoologica Scripta. 35(6): 627-639.

Zhang, J., X. Wu, Y. Li, M. Zhao, M. Xie, A. Li. 2014. The complete mitochondrial genome of *Neobenedenia melleni* (Platyhelminthes: Monogenea): mitochondrial gene content, arrangement and composition compared with two *Benedenia* species. Mol. Biol. Rep. 41:6583-6589.

Ziętara, M.S., J. Lumme. 2002. Speciation by host switch and adaptive radiation in a fish parasite Genus *Gyrodactylus* (Monogenea, Gyrodactylidae). Evolution. 56(12): 2445-2458.

Zolovs, M., G. Deksne, J. Daukšte, J. Aizups, M. Kirjušina. 2015. Microhabitat preference and relationships between metazoan parasites on the gill apparatus of the European eel (*Anguilla anguilla*) from freshwaters of Latvia. Acta Biol. Univ. Daugavp., 15(1): 241–249.

Blue Crab *(Callinectes sapidus)*. Bruce Koike

A REVIEW OF THE BIOLOGY OF *Neobenedenia melleni* AND *Neobenedenia girellae*, AND ANALYSIS OF CONTROL STRATEGIES IN AQUARIA

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"The ocean is a wilderness reaching around the globe, wilder than a Bengal jungle, and fuller of monsters."

-Henry David Thoreau

Introduction

Perhaps no metazoan parasite is so widely known to aquarists working in public aquaria and zoos as *Neobenedenia melleni* (Monogenea: Capsalidae). As ectoparasites go, this species has been a scourge to those keeping marine fishes in captivity since it was originally described from the New York Aquarium in 1927 (MacCallum). While widely known, the species is also routinely misconstrued in public aquaria, with misidentifications being common. Many aquarists will erroneously declare any capsalid monogene to be *Neobenedenia*, regardless of host identity or identifying features. While there is some scientific confusion between the synonymy (or lack thereof) of *N. melleni* vs. *N. girellae* (or even a broader species complex); the characteristics of *N. melleni*, while subtle, are all too often overlooked by aquarists, leading to some confusion as to the extent of hosts that are susceptible to infection.

The reputation of *N. melleni* in aquariums and mariculture has been well deserved, owing to its high fecundity and, most importantly, lack of host specificity. Most metazoan parasite species display some degree of host specificity, often to extreme examples with each life stage only occurring within a single species. These patterns of host specificity can help us to predict outbreaks in captive environments, as well as manage the quarantine and prophylaxis of animals according to the complement of parasites they are likely to host. However, parasites such as *N. melleni* that will infect a wide array of species defy these norms and their ubiquity demand blanket surveillance and prophylactic treatment when constructing quarantine regimens.

History and Original Description

Neobenedenia melleni was originally described as *Epibdella melleni* in 1927 (MacCallum) possibly from the eye of a spadefish, *Chaetodipterus faber* at the New York Aquarium. The suffix -bdella in the name harkens back to 18th century when Linnaeus grouped the flatworms with the leeches in phylum "Vermes" before they were split in the 19th century. At the time of its description the species would have been classified as a 'monogenetic trematode', and despite the fact that the Trematoda and the Monogenea were split in the 1970's many aquarists and veterinarians still erroneously refer to monogenes as trematodes.

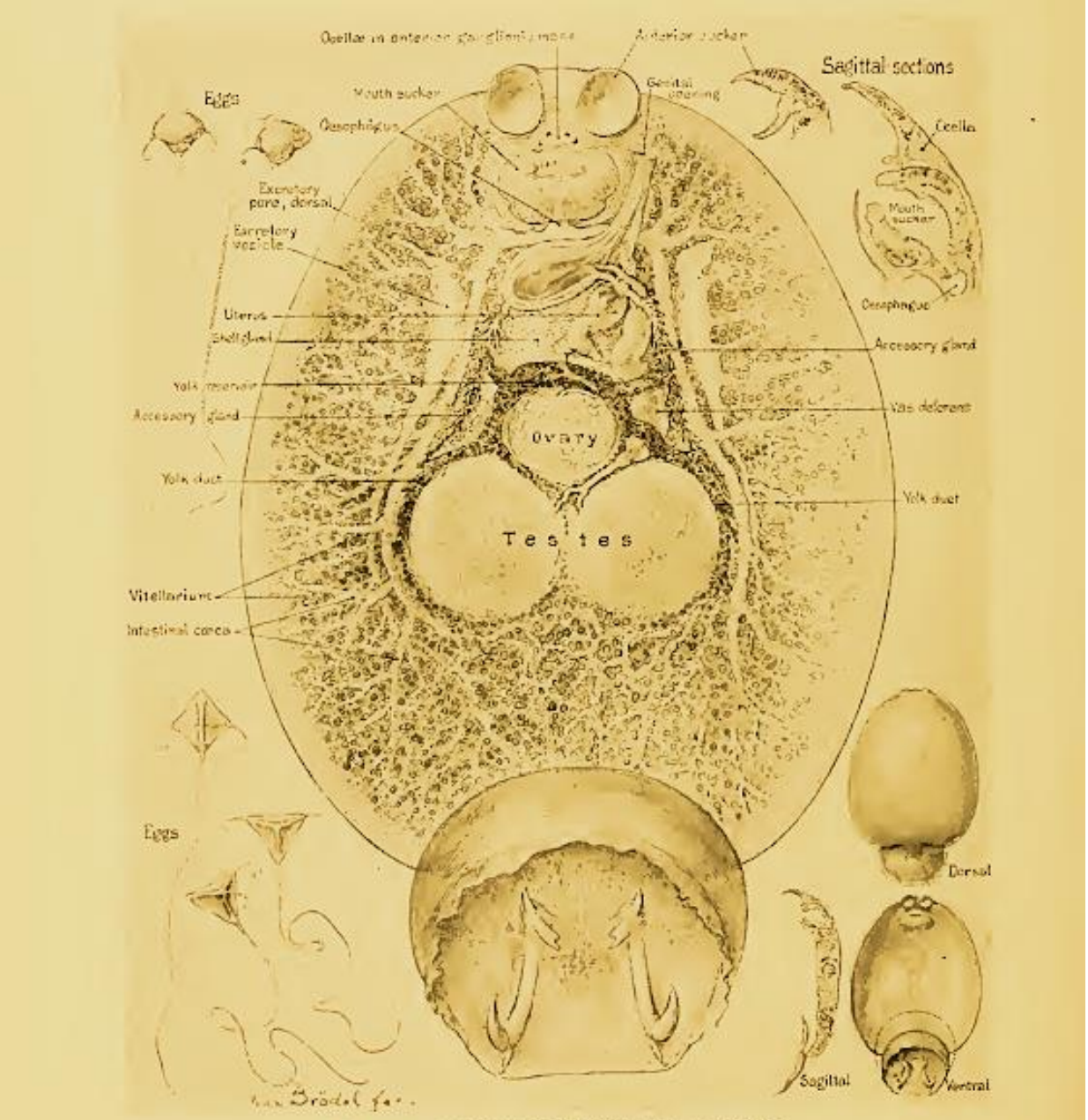

Fig. 151. *EPIBDELLA MELLENI* SP. NOV.

All the anatomical details are indicated by the legends on the drawing. The small figures are intended to show: the structure of the anterior suckers and of the mouth sucker: a longitudinal section of the whole body in the median line and finally the concavo-convex form of the body. In one of the last the dorsal excretory pores are shown in exaggerated form.

Figure 1. Original plate from MacCallum (1927) on the description of *Neobenedenia melleni*. Descriptions of eggs are given in the bottom left corner as well as anatomical features identified, both would be covered in much greater detail by Jahn and Kuhn in 1932. Note the round margin of the testes in MacCallum's drawing as well as the appearance of the haptor having a pronounced 'skirt', giving it a much more concave appearance than living specimens and does not show the 14 marginal hooklets. These two points which would later be addressed and corrected by Jahn and Kuhn (1932) and Whittington and Horton (1996). Image from the public domain.

The species was originally discovered by (and named after) Ida Mellen, a biologist at the aquarium who pioneered many early techniques in fish medicine, and was formally described in partnership with George A. MacCallum in 1927. George MacCallum (1843-1946) and William George MacCallum (1874-1944), were a father and son pair of physicians who had a keen interest in animal parasitology. The elder MacCallum was a noted physician of Canadian birth who took up the position of pathologist with the New York Zoological Society upon his retirement from the faculty of Columbia University, and quickly became such a prolific author that the society established a journal, *Zoopathologica* (1916-1928), entirely for his descriptions of novel parasite fauna from the animal collections of the New York Aquarium and the New York Zoological Park (now the Bronx Zoo). These writings described over 30 new parasite species, including *N. melleni*, and contained redescriptions, taxonomic revisions, notes, host records, and data on over 125 other species. A fascinating history of some of this early American parasitology work is reviewed in Platt (2017). It is also noteworthy that during this time period the day-to-day operations of the New York Aquarium were overseen by Assistant Director Charles M. Breder (1897-1983) who was a prolific author in the area of ichthyology, and later produced a monumental volume on (appropriately enough given his surname) the reproduction of fishes (Breder and Rosen, 1966).

Ida Mellen (1877-1970) authored a number of works, including a dozen scientific papers and even some early volumes on the subject of home fishkeeping, including *Fishes in the Home* (Mellen, 1931), and *1001 Questions Answered about your Aquarium* (Mellen and Lanier, 1948), among many others. In later life, she established herself as an expert on domestic cats and urban gardening. She left the NY Aquarium in 1929 for reasons lost to history (Muka, 2014), but reading the bulletins of the society from those years one gets a sense that her work was marginalized, and that work of equal or lesser significance by male coworkers was greatly celebrated. We can only speculate at the misogyny she must have endured in the early 20th century as one of the first female marine scientists in a male-dominated world. Later in life, she also published works of fiction under the pseudonym Esmerelda de Mar. Her work in *Zoopathologica* (1928) titled *The Treatment of Fish Diseases* is one of the earliest writings on veterinary medicine as applied to fishes, and includes a summary of anecdotes and experimental data on chemotherapeutics from over 50 aquariums and fish hatcheries worldwide.

Epibdella melleni came to be renamed *Benedenia melleni* (Price, 1939), and in 1963 was renamed again by eminent parasitologist Satyu Yamaguti (1894-1976), and placed in the genus *Neobenedenia* with four other species. This represented the most in-depth examination of the anatomy and morphology until the redescription by Whittington and Horton in 1996. In addition to his work with this species, Yamaguti leaves behind perhaps the greatest legacy of any parasitologist; his work spanned 60 papers but his books are monolithic in scope; the five-volume *Systema Helminthium* (Yamaguti, 1963) categorized the entire helminth parasite fauna of the world, and are still standard references today over a half-century later.

Yet another set of characters in this story appear in the mid-twentieth century, and again from a public aquarium. In 1953 R.J. Menzies forwarded some preserved flukes from the aquarium of the Scripps Institute for Oceanography to parasitologist William H. Hargis (1923-2008) at Florida State University for identification. At the time the aquarium was overseen by Curator Sam Hinton (1917-2009), the Texas A&M educated zoologist (and folk singer) perhaps most famous for composing "*It's a Long way from Amphioxus*", and notable figure in aquarium history in his

own right. *{Editor's note: Sam also created the Drum and Croaker logo.}* Hargis described the worms as a new species, *Benedenia girellae* (later *Neobenedenia girellae* Yamaguti, 1963) which differed from *N. melleni* by being larger in size, having minute differences in the reproductive systems (junction of the vitelline reservoir and oviduct), and slight size differences in the hamuli (hooks) of the haptor (Hargis, 1955). This ignited a debate over whether *N. melleni* and *N. girellae* are actually one species, subspecies, or members of a species complex which continues, largely unresolved, to the present day.

It is through these connections, and the many others like them, that the worlds of parasitology and public aquaria are inexorably linked. The propensity of *N. melleni* and *N. girellae* to manifest themselves in aquarium outbreaks among a broad array of fish taxa have ensured that the biology of these species is largely defined by its presence in aquaria. In fact, well over 100 of the 184 currently reported host records are from captive animals, as is discussed in greater detail below.

Figure 2. From left to right: Ida M. Mellen (NY Aquarium), George A. MacCallum (Columbia U., NY Zoological Society), Satyu Yamaguti (Okayama U., U. of Hawaii, Tulane U.), and William H. Hargis (Florida State U., Virginia Institute of Marine Science). Images from public domain or used under Creative Commons Licenses CC-SA 3.0/CC-BY-SA 4.0.

Modern Taxonomy: *N. melleni, N. girellae,* or Both?

With the description of *N. girellae* in 1955 (Hargis) the lines between the capsalid monogenes began to get blurred. The key features between the two species were very subtle, and both had very wide differences in morphology between geographic regions, and even within localized populations. Just as later works would point out that some of the features in MacCallum's original 1927 description of *N. melleni* were skewed (Jahn and Kuhn, 1932; Whittington and Horton, 1996), there were discrepancies noted in Hargis' 1955 description of *N. girellae* by Ogawa et al. (1995) that may have been a result of specimens being insufficiently flattened when mounted.

This confusion has undoubtedly led to erroneous host records in the literature (i.e. host fishes of *N. melleni* being attributed to *N. girellae* and vice-versa); but it gets even more confusing as the original accounts from MacCallum were questionable as to which hosts even originally carried the parasite! The species description of *N. melleni* attributes a Pacific tetraodontiform fish as the type host (MacCallum, 1927) but Jahn and Kuhn (1932) later note that the aquarium's

curator noted that no such fish was present at the time of collection and that the parasites likely came from the Florida Keys, making the spadefish, *Chaetodipterus faber*, or an angelfish species of the genera *Holocanthus* or *Pomacanthus* more likely candidates to be the type host.

In 1995 and 1996 the confusion becomes greater when Ogawa et al. (1995) published a revision of *N. girellae*, followed by a revision of the entire genus *Neobenedenia* in 1996 by Whittington and Horton which declared *N. girellae* a synonym of *N. melleni* and no longer a valid species. Some researchers in Asia rejected this grouping of the two species, and continued to publish numerous works over the years using the name *N. girellae* while other researchers published works on both species as *N. melleni*.

In 2004 Whittington (who was responsible for lumping the two species together eight years prior) published an extensive review of the Capsalidae, and proposed that the huge range of host species for *N. melleni*, coupled with recent studies using molecular methods showed significant differences among populations, which may indicate the existence of a large *N. melleni* species complex. The presence of numerous cryptic species or subspecies within what was considered *N. melleni* would explain the huge diversity of host fishes, and differences in morphology, fecundity, and generation time among the various subpopulations (Whittington, 2004). Later that same year Whittington et al. (2004) published a dataset examining rDNA and concluded that *N. melleni* and *N. girellae* were, in fact, different species after all. The following year Li et al. (2005) published a contradictory opinion using different genetic methods suggesting they were actually a single species. In light of these conflicting opinions many parasitologists stopped assigning a species to their study organisms, and simply referred to them as *Neobenedenia sp.*, leading to even more confusion.

The genome of *N. melleni* was mapped in 2014 (Zhang et al.), and most recently, Brazenor et al. (2018) undertook another wide-ranging genetic study reaffirmed that there are probably two species after all, however, it is likely that most infections world-wide that have been reported as *N. melleni* are actually *N. girellae*. At present, however, there is no practical way to distinguish these species based on morphology, posing a challenge to the clinician and aquarist alike. Additionally, a problem that has plagued researchers working on these two species is the lack of voucher specimens that have been deposited in museum collections to support publications (Whittington, 2004), and many older specimens were fixed in formalin (rather than alcohol) destroying any genetic material within. As such, we may never be able to sort out the tangled mess of which parasites actually belong to which hosts in the hundreds of studies published on the subject.

Nominis Caveat Lector

While the taxonomic debate is an interesting part of the biology of this species, for the purposes of this paper, we will hereafter refer to the capsalid organism in question as *N. melleni*, with the full knowledge that many of the worms we have encountered, and will encounter in aquarium outbreaks may be *N. melleni, N. girellae*, or both. With the exception of the comprehensive list of hosts below, where the authors present fish hosts listed for both species and indicate which is which, the rest of this work will refer to *N. melleni* with the understanding that it is tentative, and the reader should interpret that name to mean "*Neobenedenia cf. melleni* or

possibly *N. cf. girellae*" until the controversy is better resolved and morphological distinctions that are practical to a clinical or aquarium setting are established.

In this review we are most concerned with the biology of these species as it relates to its potential for amplification in closed systems and the control and treatment. After all, the life history and treatment in aquaria is so similar the species distinction is less important to the average aquarist; however, it is interesting to ponder that perhaps the variance in efficacy of treatment methodologies in different aquaria may be in part due to the presence of different organisms within the greater *N. melleni* species complex.

A Primer on Monogene Taxonomy

Many aquarists know a few monogenes grouped most broadly, but in the interest of a complete picture of the place of genus *Neobenedenia* in the family Capsalidae, and Capsalidae within the class Monogenea a cursory view of the overall taxonomy is presented here. Most importantly to note is that the class Monogenea is distinct from the class Trematoda, though confusion still exists and many experienced aquarists and veterinarians still refer to monogenes as "trematodes", despite the differences in taxonomy, life cycles, ecto- vs. endoparasitism, and pathogenicity.

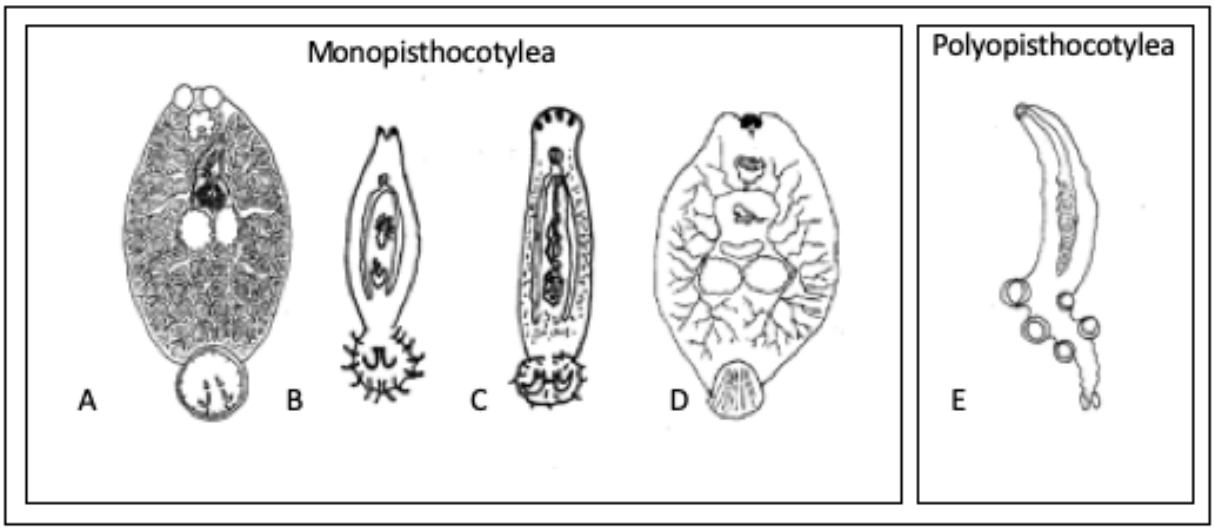

Figure 3. Basic body plans and taxonomic groupings of the Monogenea. Within the Monopisthocotylea: (A) capsalid monogenes, marine species, very large (>1mm), with a round haptor usually armed with hooks. (B) gyrodactylids, freshwater and marine, usually smaller, many spp. prefer gills as infection site. Very prominent haptor armed with multiple hooks. The gyrodactylids are live-bearing species, and juvenile larvae (hooks and all) are usually visible developing within the body of adults. (C) dactylogyrids, freshwater and marine spp., smaller worms with many species preferring gills as infection sites. Egg laying, usually with prominent eyespots, often arranged in a square pattern in marine spp. (D) microbothriids, minimized sucker with no hooks, most species bioadhere to hosts. Mostly marine, some species very large (>1mm). The Polyopisthocotylea, (E) differ greatly in that they have multiple suckers with which to adhere to their hosts, these are encountered less frequently in aquaria but still may be problematic when encountered. Images adapted from Jahn and Kuhn (1932), Yamaguti (1963), Schell (1970), and Benz (1987).

The Monogenea (phylum Platyhelminthes) is subdivided into two subclasses: the Monopisthocotylea and the Polyopisthocotylea. Identification between these two groups is simple,

there is one haptor (sucker) at the posterior end of the worm in the monopisthocotyleans and polyopisthocotyleans have multiple suckers. Polyopisthocotyleans are less common in aquaria, but some common genera such as Erpocotyle, Hexabothrium, or Squalonchocotyle occur on species commonly kept in captivity. The monopisthocotyleans are subdivided into five orders, four of which are common in aquariums: the Gyrodactylidea, Dactylogyridea, Monocotylidea and Capsalidea (Figure 3).

Differentiation of Common Capsalid Genera

There are over 60 genera in the family Capsalidae alone, each with numerous species, so identification of worms to species can present challenges for the aquarist or veterinarian. In general, a much smaller subset of monogenes are regularly encountered in aquaria, and the fact that most species exhibit a high degree of species-specificity in their host infection patterns allows for presumptive identification in many cases. Apart from *Neobenedenia* spp. the capsalid species the aquarist is most likely to encounter is *Benedeniella posterocolpa*, which is at first glance very similar to *Neobenedenia*, but only occurs on elasmobranchs. *Benedenia* spp. are also very common on carangid fishes and others, and *Benedenia seriolae* is a serious pathogen in the mariculture of *Seriola* spp. In general, the genus *Neobenedenia* can be differentiated from others by the absence of a vagina at the urogenital opening (Whittington and Horton, 1996). *Neobenedenia* can also be differentiated from *Benedenia* as the anterior suckers of the former are concave and convex in the latter (Kinami et al., 2005). See Figure 9 for photomicrographs of some of these features.

Traditionally some key features used to differentiate *N. melleni* are the anterior suckers being circular and not bipartite, anterior hamuli being recurved, robust, and non-serrated (Bullard et al., 2000), along with the path of the tendons connecting the haptor, n=14 marginal hooklets on the haptor, the shape and position of the testes, and the morphology of the reproductive tracts, notably the presence of the prostatic reservoir and penis within the cirrus sac (Whittington and Horton, 1996). Whittington and Horton (1996) also note that the presence or absence of a vagina is usually easier to see in living worms, rather than those that have been fixed or stained. However, with the similarities between *N. melleni* and *N. girellae* and the current controversy over taxonomy there are not currently any definitive morphological features that can be used to conclusively differentiate one species from the other in an aquarium setting.

Biology and Ecology

Neobenedenia probably has a worldwide distribution, *N. melleni* is a species whose biology has been largely defined by its presence in captivity, but reports of either *N. melleni* or *N. girellae* in the wild have come from the Caribbean, Gulf of Mexico, western Atlantic, Eastern Pacific, Indo-Pacific, Australia, Hawaii, and the Red Sea (Jahn and Kuhn, 1932; Hargis, 1955; Kaneko et al., 1988; Colorni, 1994; Whittington and Horton, 1996; Bullard et al., 2000; Deveney et al., 2001).

Since the initial finding in 1927 *N. melleni* rapidly established itself in captivity and exhibited little to no preference in fish hosts. Over 40 host fishes were known by 1940 (Yamaguti, 1963), and just 5 years after the initial description outbreaks had occurred at the Shedd Aquarium and Philadelphia Aquarium infecting fish hosts in over 17 different families (Jahn and Kuhn 1932). A current review of the literature for both *Neobenedenia* species in question puts the total number of documented hosts at 184 fishes of 52 families (Tables 1&2).

Neobenedenia has a relatively simple life cycle compared to other platyhelminth parasite groups (i.e. the Trematoda & Cestoda) which allows it to take advantage of the container effect and successfully reproduce in captivity. In addition to aquariums, the species has been problematic in aquaculture, especially in high-density sea-cage farming operations, where it has been introduced and caused mass mortality in several parts of the world (Kaneko et al., 1988; Deveney et al., 2001). In fact, the original outbreak in Australia was responsible for the deaths of over 200,000 barramundi, *Lates calcarifer*, in just two weeks (Deveney, et al., 2001). It would seem that this parasite occurs naturally in very low numbers in reef and near-shore fish populations, especially among certain host fishes known to be 'carriers' (near ubiquitously- infected) such as Florida pompano, *Trachinotus carolinus*, and spadefish, *Chaetodipterus faber*, and only becomes problematic when unnatural conditions such as increased host density or being placed in a closed-system allow for amplification.

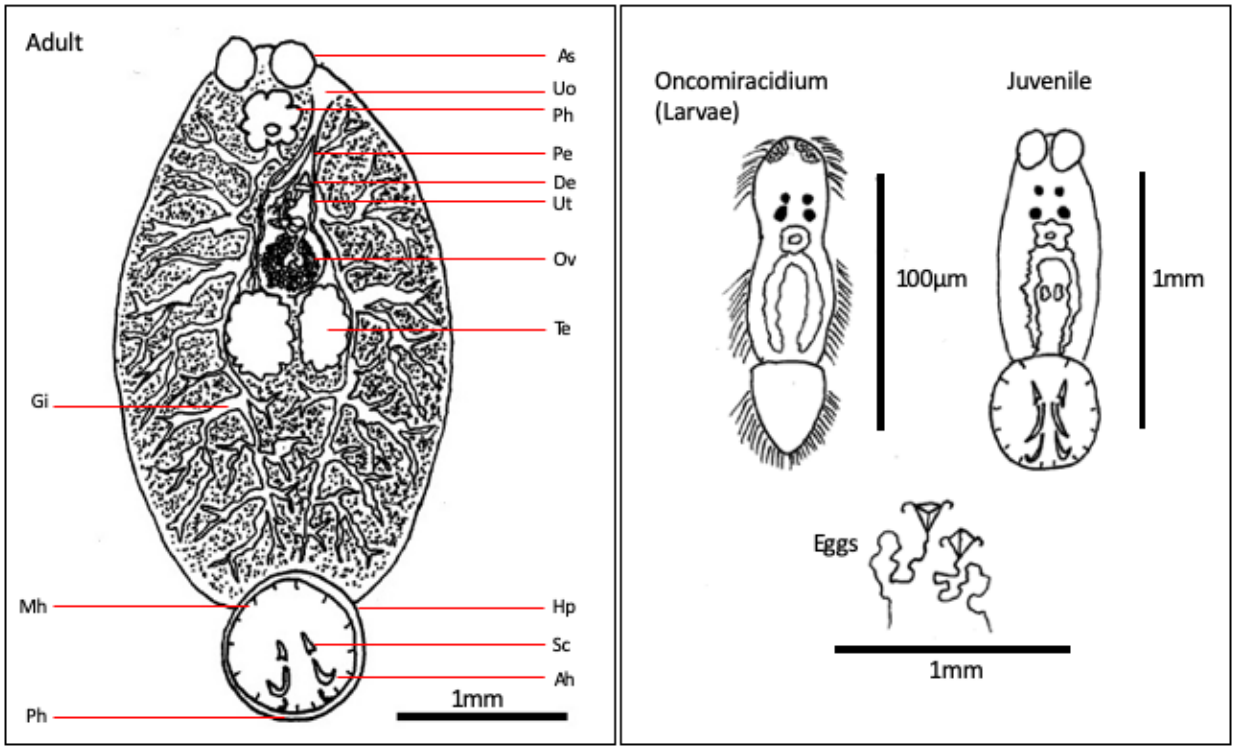

Figure 4. General anatomy of *Neobenedenia melleni* and *N. girellae*, and larval/juvenile stages. All figures oriented anterior end (top) and posterior (bottom). Adult anatomical abbreviations as follows: As=accessory sucker, Uo=urogenital opening, Ph=pharynx, Pe=penis, De=developing egg, Ut= utereus, Ov=ovary, Te=testes(paired), Hp=haptor (sucker), Sc=accessory sclerites, Ah=anterior hamuli, Ph= posterior hamuli, Mh=marginal hooklet, Gi=gastrointestinal tract (bilateral and bifurcating). Figures re-drawn and adapted from Jahn and Kuhn (1932) and Yamaguti (1963).

Life Cycle

The life cycle of this parasite has been long known, and detailed descriptions of egg production, larvae, and juvenile forms have existed for over 80 years (i.e. Jahn and Kuhn, 1932), however only recently has key data on the fecundity and life history have been emerging from parasitologists working in Australia (i.e. Hoai and Hutson, 2014; Brazenor and Hutson, 2015),

allowing construction of evidence-based treatment protocols for aquaculture (see http://www.marineparasites.com/paratreatmentcal.html).

Typical of monogenes, and unlike the trematodes, *N. melleni* has a direct life cycle, where intermediate hosts are not required for completion. As such, this characteristic combined with the demonstrated lack of host specificity make this organism a dangerous pathogen for captive fishes.

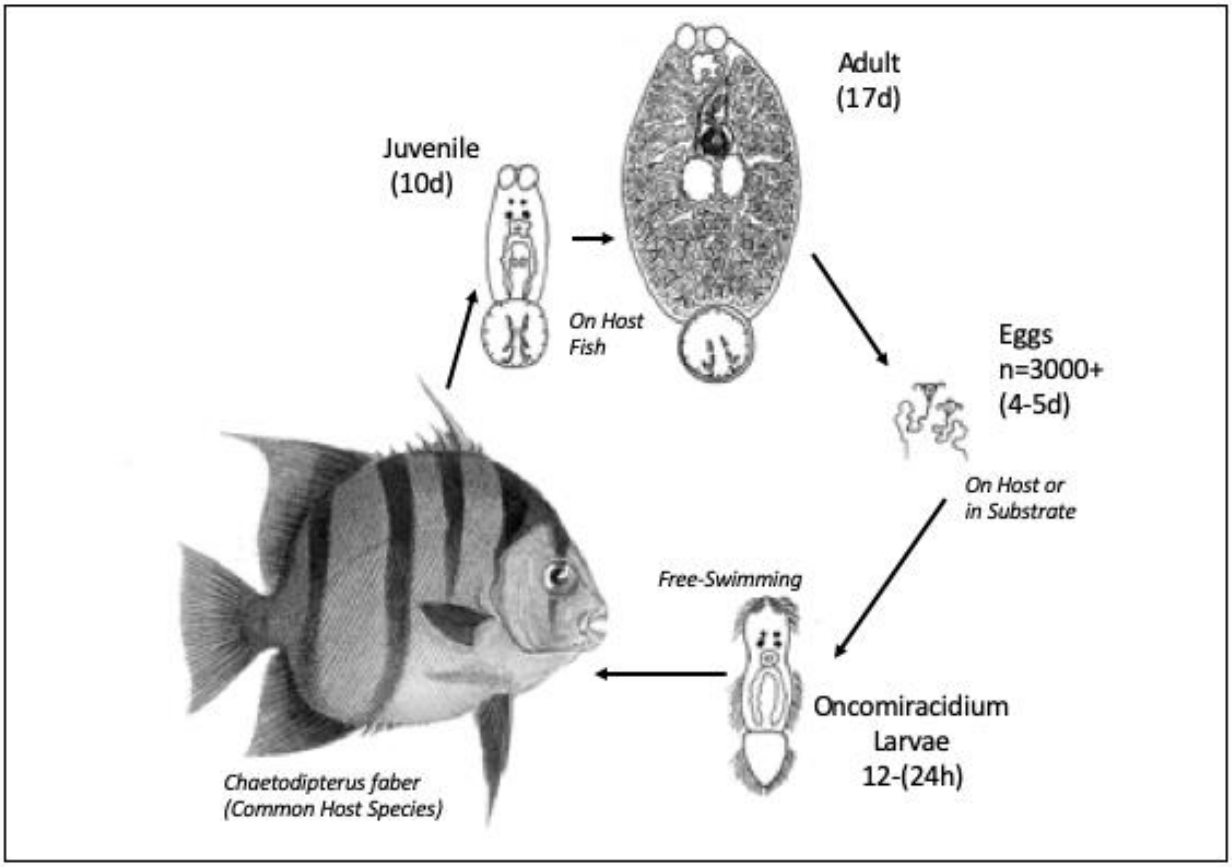

Figure 5. Life cycle of *Neobenedenia cf. melleni* and *N. cf. girellae*. Approximate durations of life stages from Brazenor and Hutson (2015) given as ranges at a salinity of 35ppt and temperatures ranging from 32-22ºC. The worm lives on the host up to 24d, being reproductively active from days 10-24 after infection. Eggs may adhere to the host or fall to the bottom of the tank and develop on the bottom or in substrate. The oncomiracidia larvae emerge from viable eggs within 3 hours of first light after 4-5d of egg development and are most viable for 12-15h after emergence but may persist for up to 24h (or 48h in cooler water). Parasite illustrations re-drawn or adapted from Jahn and Kuhn (1932) and Yamaguti (1963). *Chaetodipterus* illustration from an 1836 plate by Cuvier, from the public domain.

As mentioned above, the duration of the life cycle is highly dependent on the ambient salinity and temperature. As with any poikilothermic organism, one would expect warmer temperatures to speed development and thus shorten life spans, however the effects of salinity provide an interesting new dimension to the biology of *N. melleni*. Brazenor and Hutson (2015) found that the period from first to last hatch of eggs at 35g/l salinity varied for 6-7d at 22ºC to 4-8d at 32ºC, and that in general higher salinities increased infection success rates. Extrapolating from these published data (Hoai and Hutson 2014; Brazenor and Hutson, 2015) *Neobenedenia* can

be shown to complete its life cycle in roughly 15-19d in warmer water (32°C) and 22-35d in cooler water (22°C). It is also worth noting that self-fertilization has been observed through at least 3 generations (Hoai and Hutson, 2014), *ergo*, a single worm may start an outbreak.

After infection, juvenile worms take about 10 days to mature and become viable, and adult worms live 7-8 days in warmer waters and full-strength salinity (Hoai and Hutson, 2014), though Valles-Vega et al. (2019) found faster development (4-5d) from *Neobenedenia* at 24-30°C in México. After maturity, adult *Neobenedenia* in Australia have been observed to produce as many as 3,229 ± 37 eggs, which have a hatch rate of 78-86%, and infection rate of 35-56% (Hoai and Hutson, 2014), though hatch rates of 60.5-92.2% have been observed in the Caribbean (Ellis and Wantanabe, 1993); based on models of these data and observations from aquaria the authors would place the infection rate in public aquariums much lower, at 1% or less (Christie, unpublished data).

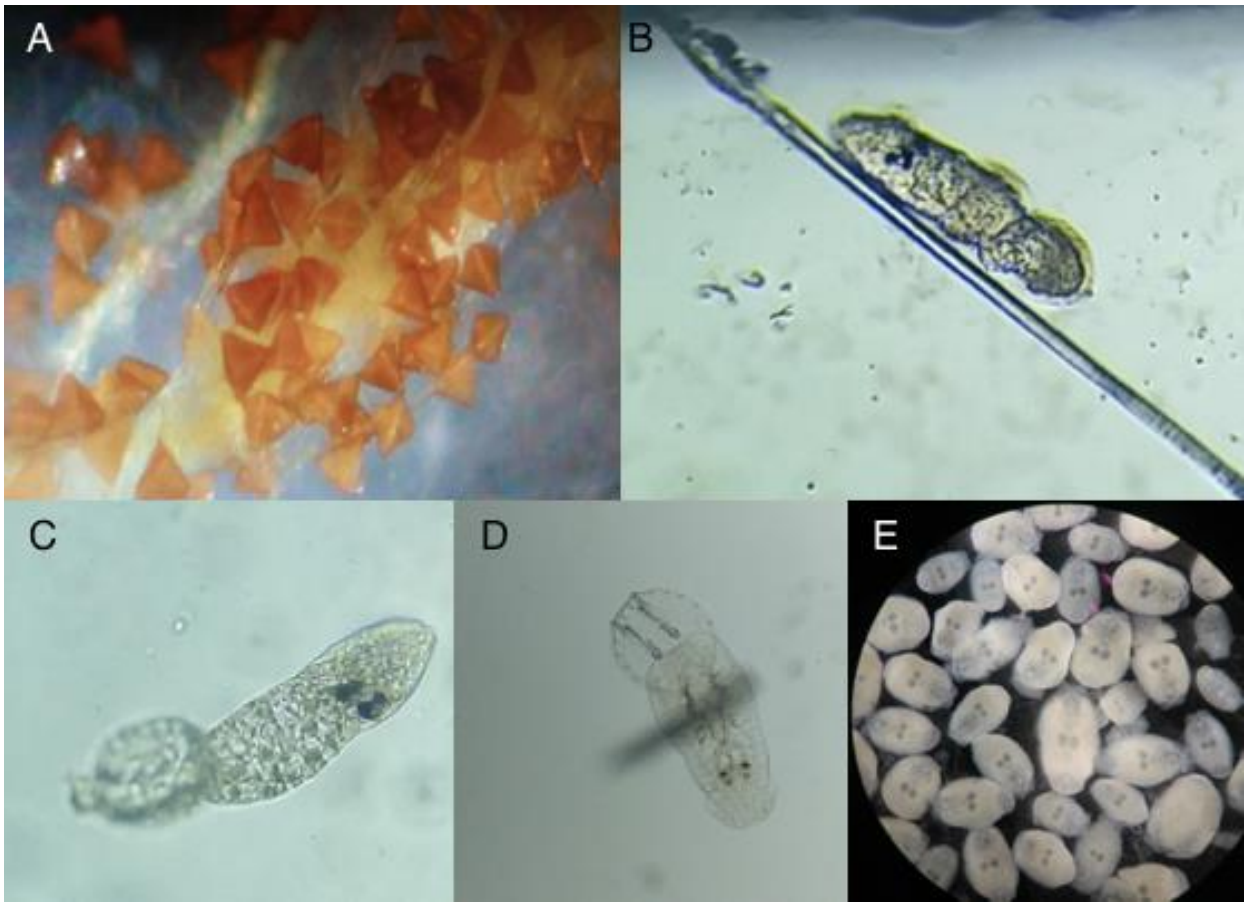

Figure 6. Photomicrographs of various life stages of *Neobenedenia cf. melleni* from living specimens. A. Eggs in a cluster, a single adult worm may lay over 3000 egg capsules in as little as 10-17d. B. Oncomiracidium, the infective larval stage; photos of oncomiracidia are notoriously difficult as they are quite rapid swimmers. Note the cilia and eyespots. C. Oncomiracidium metamorphosing into a juvenile. Note that the haptor is discernable now and eyespots still present. D. Juvenile worm, the haptor with all hooks (2 sclerites, 4 hamuli, and 14 marginal hooklets) well developed, and the larval eyespots are still present. E. Adult worms, with well-developed haptors and visible paired testes and ovaries. All photos taken at the Point Defiance Zoo and Aquarium by J.W. Foster IV.

Reproductive Modelling – The Perils of Exponential Population Growth

As with any r-selected species, and particularly parasite species that are placed in unnatural conditions such as captivity one can expect the container effect to play a significant role in the size of the population. To a monogene that exhibits high fecundity and low host specificity the captive environment of the aquarium and abundance of potential hosts provides an optimal set of circumstances to maximize the probability of explosive population growth. Given that recent studies (e.g. Hoai and Hutson, 2014, Brazenor and Hutson, 2015) have begun to elucidate some fundamental aspects of the reproductive biology of *N. melleni* and *N. girellae* it is now possible to use data to make some assumptions about the potential for population growth and spread of infection in a closed system. Using the basic inputs of life span, duration of larval development, adult lifespan, egg incubation, infection rate, and fecundity a simple model to predict population growth can be constructed using a Microsoft Excel™ spreadsheet, and plotted graphically (Fig. 7).

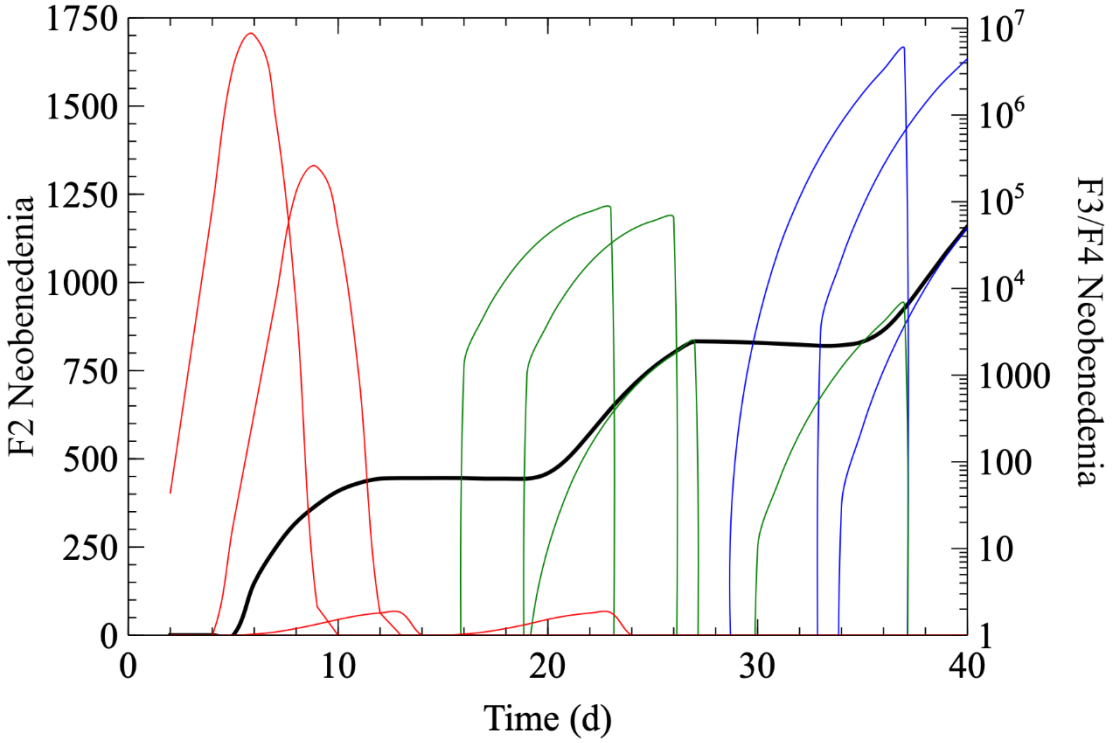

Figure 7. *Neobenedenia* population growth model from theoretical infection of n=1 worm through four generations. Model uses fecundity and life history data from Hoai and Hutson (2014) and makes the following assumptions: salinity = 35g/l, temperature = 26°C, fecundity (F_x) = 3227 eggs worm^{-1}, egg hatch rate = 78%, infection rate = 1%, juvenile development = 10d, adult lifespan = 10d. F2 parasite generation shown in red, F3 generation shown in green, F4 generation shown in blue. Successive peaks indicate numbers of eggs, oncomiracidia, juveniles, and adults. Note that the F2 and F3 generations are plotted against a different y-axis that is log-transformed, as the parasite numbers grow exponentially with each generation. In this estimate, from a single infecting founder the total theoretical parasite population grows to over 2,400 by day 25, and over 96,000 by day 40, as shown by the black line.

As with most r-selected organisms the potential for population growth in *N. melleni* (or *N. girellae*) is exponential, and this portends an extreme threat to fishes maintained by the aquarist or aquaculturist. Figure 7 above represents the population growth of *N. melleni* through three

generations assuming that fecundity is 3,227 and other population characteristics follow Hoai and Hutson (2014), with the exception that adult lifespan is 10d based on observations by one of the authors (BLC) on *N. cf. melleni* from the Gulf of México, and infection rate is set at 1%.

Many of the investigations published on *Neobenedenia* spp. have reported much higher infection rates, as much as 33-56% (Hoi and Hutson, 2014), or greater than 90% (Valles-Vega et al., 2019), though these laboratory trials used much smaller tanks to house specimens, and higher densities of fishes than are typical in public aquaria. As such, extrapolation of these infection rates to larger closed systems in incongruent with observations of the authors. Using unpublished data (B. Christie) on parasite abundance in groups of *T. carolinus* and *A. coeruleus* from 2500gal. quarantine tanks and the one-million-gallon Caribbean exhibit at the Moody Gardens Aquarium, the infection success rate is assumed to be much lower in aquaria than aquaculture (based on 30-day infection increases of 112-459 *N. melleni* host^{-1}). These increases in parasite abundance suggest that *N. melleni* is not nearly as successful in large aquaria (<1% infection), likely due to factors such as lower stocking densities, diversity of host species, higher LSS turnover, ozone sterilization, et cetera. However, the reader should remember that the very definition of species within what we consider to be *N. melleni, N. girellae,* and/or the greater *Neobenedenia* species complex is in flux (e.g. Whittington, 2004; Whittington et al., 2004.; Brazenor et al., 2018), and infection rates and virulence could very well vary widely between populations and/or species.

Figure 8. *Do you even fecund, bro*? An adult *Neobenedenia cf. melleni* next to a massive aggregation of eggs. Adult *Neobenedenia* are known to produce as many as 3229 ± 37 eggs per worm during their lifespan (Hoai and Hutson, 2014). Photo from the Point Defiance Zoo and Aquarium by J.W. Foster, IV.

The Red Queen Theory and Host-Parasite Dynamics

In evolutionary terms, hosts and parasites are known to be locked in an 'arms race' of sorts, with parasites constantly adapting to their host's defenses, forcing hosts to constantly adapt better strategies to avoid parasites and selecting for those with better immune responses. This process has been described as Red Queen Theory (RQT) in evolutionary biology (Van Valen; 1973, Bell, 1982; and Bell 1985), and RQT pressure has been theorized to perhaps be the driving force behind the genesis of sexual reproduction, or the persistence thereof, among living organisms early in the history of life on earth (Bell, 1985; Lively, 2010), to enable hosts to more rapidly create genetic recombination and thus variation to better deal with parasites. RQT is named after the character in the Lewis Carroll novel 'Through the Looking Glass', who proclaims "It takes all the running you can do, to stay in the same place".

Application of RQT in the context of parasites in the artificial environment of the aquarium was first proposed by Smith et al. (2018), in regards to the adaptations made by parasites in response to both host immunity and outside pressures such as treatments imposed by aquarists and veterinarians. This is an apt comparison, and a novel outlook of the results of the container effect, because aquaria have struggled since its description to eradicate this worm in captive environments in an ongoing 'arms race' of chemotherapeutics and management strategies. Despite ninety-two years of our best efforts *Neobenedenia* remains *Parasitus Invictus,* an unconquered worm. Public aquaria currently have managed to develop and refine treatment protocols that allow us to keep this parasite at bay, more or less, but no treatment protocol can as of yet guarantee the banishment of *Neobenedenia,* so until a major breakthrough occurs we continue all the running we can do to maintain a precarious détente with this insidious pathogen.

Host Species List

There have been, over time, several comprehensive lists of recorded hosts for *N. melleni*; though as our understanding of the ubiquity of infection of bony fishes grows these lists invariably become outdated and in need of revision. In the spirit of Sisyphus, forever condemned to labor at pushing an immense object up an infinite hill, the authors hereto present a comprehensive, authoritative, and soon-to-be outdated list of the fishes reported to host *N. melleni* and *N. girellae.* We present this with the full knowledge that this list shall likely be outdated by the time this article goes to print, so the reader is advised to consult the parasitology literature from 2019 onwards when looking for the full scope of infection susceptibility. Given the taxonomic confusion between *N. melleni and N. girellae* a complete list of host records is difficult to compile, though understanding the extent of host records for these parasite species collectively is important as it may help differentiate low-risk from high-risk fishes (Bullard et al., 2002).

Species Predisposed/Carriers

The vast majority of monogeneans infect a single species (Brazenor et al., 2018) so despite the taxonomic confusion between *N. melleni* and *N. girellae* the lack of host specificity as demonstrated by the compiled host list above is astounding. While the total list of hosts is broad in taxonomic scope, certain fishes tend to be more prone to *Neobenedenia* infection. Bullard et al. (2002) note that understanding the patterns in the extensive host records may differentiate low-risk from high-risk fish species. There are a number of species which seem, through aggregation of many anecdotal reports, to be especially predisposed to carrying *N. melleni,* and some of this conventional wisdom has been affirmed by published research.

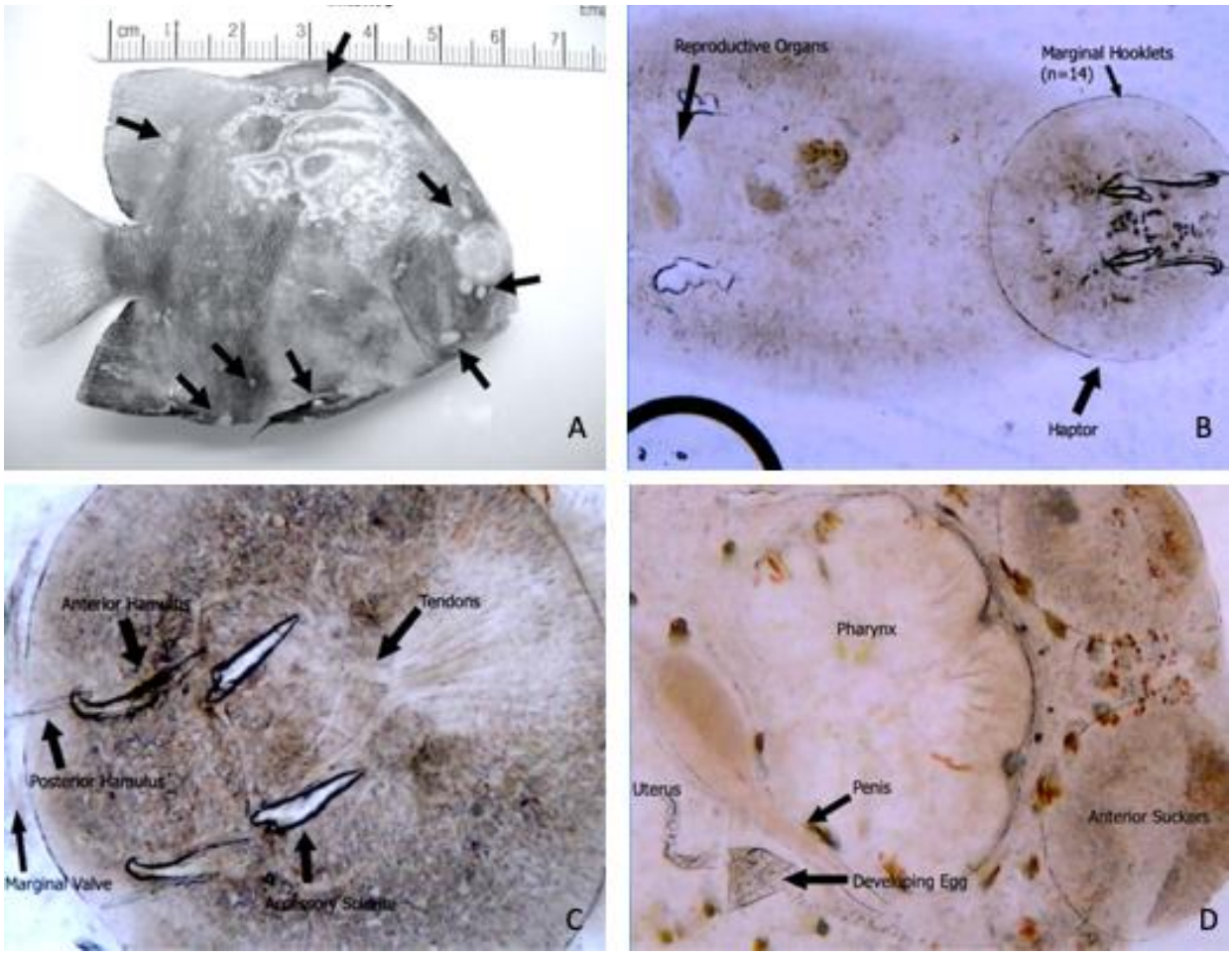

Figure 9. Characteristics of *Neobenedenia melleni*. A) Numerous large adult worms infecting the skin and eyes of *Chaetodipterus faber* (fixed whole in 10% NBF to show monogenes). B) *N. melleni* 50x magnification, note the large haptor with three paired hook structures at posterior, anterior arrangement of the reproductive organs, and marginal hooklets (n=14) encircling the haptor. C) Detail of the posterior end and haptor (100x), note the three major hook structures: anterior and posterior hamule, and accessory sclerites. The path of the tendons can be seen here as well as the notch at the proximal base of the accessory sclerites which tensions the tendons at a near right-angle bend. D) Anterior end (100x), note the anterior suckers and the location of the pharynx. The penis and intersection of the male and female urogenital systems can be seen here. Note the lack of a vagina and the developing egg. Photos from the Aquarium at Moody Gardens by B.L. Christie.

Table 1. Number of Recorded Host Fishes for *Neobenedenia melleni* by Family.

Family	Common	Host spp.
Acipenseridae	Sturgeons	1
Congridae	Conger Eels	1
Ariidae	Sea Catfishes	1
Mugilidae	Mullets	1
Poeciliidae	Livebearers	1
Holocentridae	Squirrelfishes	1
Scorpaenidae	Scorpionfishes	19
Triglidae	Sea Robins	1
Hexagrammidae	Greenlings	3
Cottidae	Sculpins	3
Scatophagidae	Scats	1
Monodactylidae	Monos	1
Lateolabracidae	Asian Seabasses	1
Terapontidae	Tigerperches	1
Latidae	Barramundi	1
Moronidae	Striped Basses	1
Epinephelidae	Groupers	25
Pseudochromidae	Dottybacks	1
Serranidae	Sea Basses	3
Malacanthidae	Tilefishes	1
Pomatomidae	Bluefishes	1
Carangidae	Jacks	17
Coryphaenidae	Dolphinfishes	1
Echeneidae	Remoras	1
Lutjanidae	Snappers	11
Rachycentridae	Cobia	1

Family	Common	Host spp.
Lobotidae	Tripletails	1
Haemulidae	Grunts	2
Sparidae	Porgies	5
Sciaenidae	Drum	4
Kyphosidae	Chub	3
Chaetodontidae	Butterflyfishes	6
Pomacanthidae	Angelfishes	9
Cirrhitidae	Hawkfishes	1
Cichlidae	Cichlids	5
Embiotocidae	Surfperches	2
Labridae	Wrasses	13
Anarhichadididae	Wolfishes	1
Scaridae	Parrotfishes	1
Blenniidae	Blennies	1
Microdesmidae	Wormfishes	2
Ephippidae	Spadefishes	2
Acanthuridae	Surgeonfishes	4
Trichiuridae	Cutlassfishes	1
Scombridae	Mackerals	1
Pleuronectidae	Flounders	2
Paralichthyidae	Sand Flounders	1
Balistidae	Triggerfishes	5
Monocanthidae	Filefishes	3
Ostraciidae	Boxfishes	4
Tetraodontidae	Pufferfishes	7
Diodontidae	Porcupinefishes	2

Table 2. Published Host Records for *Neobenedenia melleni* in Phylogenetic Order

Family	Species	Common	*N. melleni*	*N. girellae*	Reference
Acipenseridae	*Acipencer transmontanus*	White Sturgeon	X		Christie et al., 2020
Congridae	*Heteroconger hassi*	Garden Eel	X		Bullard et al., 2000
Ariidae	*Ariopsis felis*	Hardhead Sea Catfish	X		Whittington and Horton, 1996
Mugilidae	*Mugil curema*	White Mullet	X		Conroy et al., 1986
Poeciliidae	*Gambusia xanthostoma*	Cayman Gambusia	X		Bullard et al., 2000
Holocentridae	*Holocentrus ascensionis*	Squirrelfish	X		Whittington and Horton, 1996
Scorpaenidae	*Pterois antennata*	Antennate Lionfish	X		Christie et al., 2020
	Pterois radiata	Radiated Lionfish	X		Christie et al., 2020
	Pterois volitans	Red Lionfish	X		Christie et al., 2020
	Sebastes auriculatus	Brown Rockfish	X		Christie et al., 2020
	Sebastes capensis	Cape Redfish	X		Gonzalez and Acuna, 1998
	Sebastes caurinus	Copper Rockfish	X		Christie et al., 2020
	Sebastes diaconus	Deacon Rockfish	X		Christie et al., 2020
	Sebastes diploproa	Splitnose Rockfish	X		Christie et al., 2020
	Sebastes emphaeus	Puget Sound Rockfish	X		Christie et al., 2020
	Sebastes flavidus	Yellowtail Rockfish	X		Christie et al., 2020
	Sebastes maliger	Quillback Rockfish	X		Christie et al., 2020
	Sebastes melanops	Black Rockfish	X		Whittington and Horton, 1996
	Sebastes miniatus	Vermillion Rockfish	X		Christie et al., 2020
	Sebastes nebulosus	China Rockfish	X		Christie et al., 2020
	Sebastes nigrocinctus	Tiger Rockfish	X		Christie et al., 2020
	Sebastes pinniger	Canary Rockfish	X		Christie et al., 2020
	Sebastes ruberrimus	Yelloweye Rockfish	X		Christie et al., 2020
	Sebastes rubrivinctus	Flag Rockfish		X	Brazenor et al., 2018
	Sebastes serranoides	Olive Rockfish	X		Love at al., 2002

Family	Species	Common	*N. melleni*	*N. girellae*	Reference
Triglidae	*Prionotus evolans*	Striped Searobin	X		Whittington and Horton, 1996
Hexagrammidae	*Hexagrammos decagrammus*	Kelp Greenling	X		Whittington and Horton, 1996
	Hexagrammos lagocephalus	Rock Greenling	X		Bullard et al., 2003
	Ophiodon elongatus	Lingcod	X		Christie et al., 2020
Cottidae	*Enophrys bison*	Buffalo Sculpin	X		Christie et al., 2020
	Hemilepidotus hemilepidotus	Red Irish Lord	X		Christie et al., 2020
	Leptocottus armatus	Pacific Staghorn Sculpin	X		Whittington and Horton, 1996
Scatophagidae	*Scatophagus argus*	Scat	X		Whittington and Horton, 1996
Monodactylidae	*Monodactylus argentus*	Silver Moony	X		Whittington and Horton, 1996
Lateolabracidae	*Lateolabrax japonicas*	Japanese Seabass		X	Ogawa et al., 1995
Terapontidae	*Terapon jarbua*	Jarbua Terapon	X		Whittington and Horton, 1996
Latidae	*Lates calcarifer*	Barramundi	X		Deveney et al., 2001
Moronidae	*Morone saxatilis*	Striped Bass	X		Whittington and Horton, 1996
Epinephelidae	*Cephalopholis cruentata*	Graysby	X		Whittington and Horton, 1996
	Cephalopholis fulva	Coney	X		Whittington and Horton, 1996
	Dermatolepis inermis	Marbled Grouper	X		Whittington and Horton, 1996
Epinephelidae	*Dermatolepis punctatus*	Leather Bass	X		Whittington and Horton, 1996
	Epinephelus adscensionis	Rock Hind	X		Whittington and Horton, 1996
	Epinephelus akaara	Hong Kong Grouper		X	Ogawa et al., 1995
	Epinephelus awoara	Yellow Grouper	X		Yang et al., 2001
	Epinephelus bleekeri	Duskytail Grouper		X	Dewi et al., 2017
	Epinephelus coioides	Orangespotted Grouper		X	Brazenor et al., 2018
	Epinephelus cyanopodus	Speckled Grouper		X	Ogawa et al., 1995
	Epinephelus fuscoguttatus x lanceolatus	Hybrid Grouper		X	Dewi et al., 2017
	Epinephelus guttatus	Red Hind	X		Whittington and Horton, 1996

Family	Species	Common	*N. melleni*	*N. girellae*	Reference
Epinephelidae	*Epinephelus itaiara*	Goliath Grouper	X		Whittington and Horton, 1996
	Epinephelus malabaricus	Malabar Grouper		X	Ogawa et al., 1995
	Epinephelus marginatus	Dusky Grouper	X		Sanches, 2008
	Epinephelus morio	Red Grouper	X		Whittington and Horton, 1996
	Epinephelus striatus	Nassau Grouper	X		Whittington and Horton, 1996
	Hyporthodus septemfaciatus	Convict Grouper	X		Habu et al., 2009
	Mycteroperca interstitialis	Yellowmouth Grouper	X		Whittington and Horton, 1996
	Mycteroperca microlepis	Gag Grouper	X		Whittington and Horton, 1996
	Mycteroperca rosacea	Leopard Grouper	X		Whittington and Horton, 1996
	Paranthias furcifer	Creole	X		Whittington and Horton, 1996
	Plectropomus leopardus	Leopard Coral Grouper		X	Ogawa et al., 1995
Pseudochromidae	*Pseudochromis fridmani*	Orchid Dottyback		X	Brazenor et al., 2018
Serranidae	*Centropristis striata*	Black Sea Bass	X		Whittington and Horton, 1996
	Cromileptes altivelis	Panther Grouper		X	Keosharyani et al., 1999
	Paralabrax maculatofasciatus	Spotted Sand Bass	X		Whittington and Horton, 1996
Malacanthidae	*Malacanhus plumieri*	Sand Tilefish	X		Whittington and Horton, 1996
Pomatomidae	*Pomatomus salatrix*	Bluefish	X		Whittington and Horton, 1996
Carangidae	*Caranyx hippos*	Crevalle Jack	X		Whittington and Horton, 1996
	Caraynx crysos	Horse Eye Jack	X		Whittington and Horton, 1996
	Elagatis bipinnulata	Rainbow Runner	X		Christie et al., 2020
	Gnathodon speciosus	Golden Trevally	X		Christie et al., 2020
	Naucrates doctor	Pilotfish	X		Whittington and Horton, 1996
	Oligoplites altus	Longjaw Leatherjack		X	Brazenor et al., 2018
	Pseudocaranx dentex	White Trevally		X	Ogawa et al., 1995
Carangidae	*Selene setapinnis*	Atlantic Moonfish	X		Whittington and Horton, 1996
	Seriola dumerili	Greater Amberjack		X	Ogawa et al., 1995

Family	Species	Common	*N. melleni*	*N. girellae*	Reference
Carangidae	*Seriola lalandi*	Yellowtail Amberjack		X	Ogawa et al., 1995
	Seriola quinqueradiata	Japanese Ambarjack		X	Ogawa et al., 1995
	Seriola riviolana	Almaco Jack	X		Whittington and Horton, 1996
	Trachinotis carolinus	Florida Pompano	X		Whittington and Horton, 1996
	Trachinotus falcatus	Permit	X		Whittington and Horton, 1996
	Trachinotus goodei	Palometa	X		Whittington and Horton, 1996
	Trachinotus kennedeyi	Blackblotch Pompano		X	Brazenor et al., 2018
	Trachinotus ovatus	Pompano	X		Whittington and Horton, 1996
Coryphaenidae	*Coryphaena hippurus*	Dorado	X		Whittington and Horton, 1996
Echeneidae	*Echeneis naucrates*	Sharksucker	X		Bullard et al., 2000
Lutjanidae	*Lutjanus analis*	Mutton Snapper	X		Whittington and Horton, 1996
	Lutjanus apodus	Schoolmaster	X		Whittington and Horton, 1996
	Lutjanus argentiventris	Yellow Snapper		X	Brazenor et al., 2018
	Lutjanus campechanus	Northern Red Snapper	X		Bullard et al., 2000
	Lutjanus erythorpterus	Crimson Snapper	X		Ravi and Yahaya, 2016
	Lutjanus griseus	Mangrove Snapper	X		Whittington and Horton, 1996
	Lutjanus jocu	Dog Snapper	X		Whittington and Horton, 1996
	Lutjanus sangeuineus	Humphead Snapper	X		Xuejuan et al., 2000
	Lutjanus synagris	Lane Snapper	X		Whittington and Horton, 1996
	Lutjanus viridis	Blue and Gold Snapper	X		Whittington and Horton, 1996
	Ocyurus chrysurus	Yellowtail Snapper	X		Whittington and Horton, 1996
Rachycentridae	*Rachycentron canadum*	Cobia	X	X	Brazenor et al., 2018
Lobotidae	*Lobotes surinamiensis*	Tripletail	X		Whittington and Horton, 1996
Haemulidae	*Ansiotremus surinamiensis*	Black Margate	X		Whittington and Horton, 1996

Family	Species	Common	*N. melleni*	*N. girellae*	Reference
Haemulidae	*Ansiotremus virginicus*	Porkfish	X		Whittington and Horton, 1996
Sparidae	*Archosargus probatocephalus*	Sheepshead	X		Whittington and Horton, 1996
	Archosargus rhomboidalis	Western Atlantic Seabream	X		Whittington and Horton, 1996
	Calamus calamus	Saucereye Porgy	X		Whittington and Horton, 1996
	Pagrus pagrus	Porgy	X		Christie et al., 2020
	Sparus aurata	Gilthead Seabream	X		Whittington and Horton, 1996
Sciaenidae	*Larimichthys crocea*	Large Yellow Croaker	X		Yang et al., 2001
	Menticirrhus saxatilis	Northern Kingfish	X		Whittington and Horton, 1996
	Micropogon undulatus	Croaker	X		Whittington and Horton, 1996
	Pogonias cromis	Black Drum	X		Whittington and Horton, 1996
Kyphosidae	*Girella nigricans*	Opaleye	X		Whittington and Horton, 1996
	Kyphosus saltatrix	Bermuda Chub	X		Christie et al., 2020
	Medialuna californiensis	Halfmoon	X		Whittington and Horton, 1996
Chaetodontidae	*Chaetodon capistratus*	Foureye Butterflyfish	X		Whittington and Horton, 1996
	Chaetodon collare	Redtail Butterflyfish	X		Whittington and Horton, 1996
	Chaetodon lunula	Racoon Butterflyfish	X		Bullard et al., 2000
	Chaetodon ocellatus	Spotfin Butterflyfish	X		Whittington and Horton, 1996
	Chaetodon semilarvatus	Bluecheek Butterflyfish	X		Cardosa et al., 2018
	Chaetodon striatus	Banded Butterflyfish	X		Whittington and Horton, 1996
	Heniochis acuminatus	Long Finned Bannerfish	X		Whittington and Horton, 1996
Pomacanthidae	*Holocanthus bermudensis*	Blue Angelfish	X		Whittington and Horton, 1996
	Holocanthus ciliarus	Queen Angelfish	X		Whittington and Horton, 1996

Family	Species	Common	*N. melleni*	*N. girellae*	Reference
Pomacanthidae	*Pomacanthus annularis*	Annularis Angelfish	X		Bullard et al., 2003
	Pomacanthus arcuatus	Grey Angelfish	X		Whittington and Horton, 1996
	Pomacanthus asfur	Asfur Angelfish	X		Cardosa et al., 2018
	Pomacanthus maculosus	Yellowbar Angelfish	X		Cardosa et al., 2018
	Pomacanthus paru	French Angelfish	X		Whittington and Horton, 1996
	Pygoplites diacanthus	Regal Angelfish	X		Cardosa et al., 2018
Cirrhitidae	*Neocirrhites armatus*	Flame Hawkfish		X	Brazenor et al., 2018
Cichlidae	*Oreochromis mossambicus*	Mozambique Tilapia	X		Whittington and Horton, 1996
	Oreochromis niloticus	Nile Tilapia		X	Ogawa et al., 1995
	Oreochromis hornorum x mossambicus	Tilapia	X		Whittington and Horton, 1996
	Oreochromis spp.	Tilapia	X		Whittington and Horton, 1996
Embiotocidae	*Embiotica jacksoni*	Black Surfperch	X		Whittington and Horton, 1996
	Embiotica lateralis	Striped Surfperch	X		Whittington and Horton, 1996
Labridae	*Bodianus pulchellus*	Cuban Hogfish	X		Christie et al., 2020
	Bodianus rufus	Spanish Hogfish	X		Whittington and Horton, 1996
	Bodianus scrofa	Barred Hogfish	X		Whittington and Horton, 1996
	Haemullon album	Margate	X		Whittington and Horton, 1996
	Haemulon flavolineatum	French Grunt	X		Whittington and Horton, 1996
	Haemulon macrostomum	Spanish Grunt	X		Whittington and Horton, 1996
	Haemulon plumeri	White Grunt	X		Whittington and Horton, 1996
	Haemulon sciurus	Bluestriped Grunt	X		Whittington and Horton, 1996
	Lachnolaimus maximus	Rooster Hogfish	X		Whittington and Horton, 1996
	Pseudocheilinus hexataenia	Sixline Wrasse		X	Brazenor et al., 2018
	Semicossyphus pulcher	California Sheepshead	X		Whittington and Horton, 1996
	Tautoga onitis	Tautog	X		Whittington and Horton, 1996

Family	Species	Common	*N. melleni*	*N. girellae*	Reference
Labridae	*Thalassoma pavo*	Ornate Wrasse	X		Whittington and Horton, 1996
Anarhichadididae	*Anarrhichthys ocellatus*	Wolf Eel	X		Christie et al., 2020
Scaridae	*Scarus perrico*	Bumphead Parrotfish	X		Whittington and Horton, 1996
Blenniidae	*Scartichthys viridis*	Blenny	X		Diaz and Nascimento, 2002
Microdesmidae	*Nemateleotris decora*	Elegant Firefish		X	Brazenor et al., 2018
	Oxymetopon cyanoctenosum	Blue Barred Ribbon Gogy	X		Bullard et al., 2003
Ephippidae	*Chaetodipterus faber*	Spadefish	X		MacCallum, 1927
	Platax sp.	Batfish	X		Whittington and Horton, 1996
Acanthuridae	*Acanthurus bahianus*	Barber Surgeonfish			Siddel et al., 2009
	Acanthurus chirugas	Doctorfish	X		Whittington and Horton, 1996
	Acanthurus coeruleus	Blue tang	X		Whittington and Horton, 1996
	Paracanthurus hepatus	Hepatus Tang	X		Whittington and Horton, 1996
Trichiuridae	*Trichiurus lepturus*	Atlantic Cutlassfish	X		Carvalho and Luque, 2009
Scombridae	*Scomber japonicas*	Pacific Chub Mackeral	X		Yammamoto et al., 2014
Pleuronectidae	*Platichthys stellatus*	Starry Flounder	X		Christie et al., 2020
	Verasper variegatus	Spotted Halibut		X	Brazenor et al., 2018
Paralichthyidae	*Paralichthys olivaceus*	Olive Halibut		X	Ogawa et al., 1995
Balistidae	*Balistes capriscus*	Grey Triggerfish	X		Whittington and Horton, 1996
	Balistes vetula	Queen Triggerfish	X		Whittington and Horton, 1996
	Canthidermis sufflamen	Ocean Triggerfish	X		Whittington and Horton, 1996
	Melichthys bispinosus	Black Triggerfish	X		Whittington and Horton, 1996
	Melichthys piceus	Black Triggerfish	X		Whittington and Horton, 1996
Monocanthidae	*Aluterus schoepfi*	Orange Filefish	X		Whittington and Horton, 1996
	Aluterus scriptus	Scrawled Filefish	X		Whittington and Horton, 1996
	Stephanolepis hispidus	Planehead Filefish	X		Whittington and Horton, 1996

Family	Species	Common	*N. melleni*	*N. girellae*	Reference
Ostraciidae	*Acanthostracion quadricornis*	Scrawled Cowfish	X		Whittington and Horton, 1996
	Lactophrys bicaudalis	Spotted Trunkfish	X		Whittington and Horton, 1996
	Lactophrys trigonus	Trunkfish	X		Whittington and Horton, 1996
	Lactophrys triqueter	Smooth Trunkfish	X		Whittington and Horton, 1996
Tetraodontidae	*Arothron caercaeruleopunctatus*	Bluespotted Puffer		X	Brazenor et al., 2018
	Canthigaster bennetti	Bennett's Sharpnose Puffer		X	Brazenor et al., 2018
	Spheroides maculatus	Least Puffer	X		Whittington and Horton, 1996
	Sphoeroides annulatus	Bullseye Puffer		X	Brazenor et al., 2018
	Sphoeroides maculatus	Northern Puffer	X		Christie et al., 2020
	Takifugu rubripes	Japanese Pufferfish		X	Ogawa et al., 1995
Diodontidae	*Chilomycterus schoepfi*	Burrfish	X		Christie et al., 2020
	Diodon hystrix	Porcupinefish	X		Whittington and Horton, 1996

Article continued on next page

Spot prawn *(Pandalus platyceros)*. Bruce Koike

Florida pompano, *T. carolinus*, were the first carangid fish documented carrying *N. melleni* in the wild, and they may be a vector for geographic spread in the Caribbean and Gulf of Mexico (Bullard et al., 2002), and wild pompano have been implicated as the source of at least one major public aquarium outbreak (Christie, 2006). When surveyed as to which fishes were most affected by *N. melleni*, the top three species reported were spadefish, *C. faber* (32%), lookdown, *Selene vomer* (24%), and Florida pompano, *T. carolinus* (16%) (Christie, 2015). In fact, two aquaria even reported that spadefish were excluded from their collection plans because of their propensity to carry *N. melleni*, and 15% of institutions report that extra precautions or specialized quarantine prophylaxis were aimed at groups of fishes thought to be more susceptible to harboring *N. melleni* (Christie, 2015).

Characteristics of Aquarium Outbreaks

The following data were collected from survey of public aquaria (n=28) on their experiences with *Neobenedenia* outbreaks (Christie, 2015) and gives some insight into the scope of the problem in captive seawater exhibits. We know from the literature that this parasite has long plagued public aquaria (MacCallum, 1927; Jahn and Kuhn, 1932; Thoney and Hargis, 1991), and 82% of institutions have had recurrence following treatment, 29% more than five times. These outbreaks tend to occur in large (93% over 10,000gal.) tanks with mixed species collections (median 1,200 fishes representing 56 species) resulting in an average of 42 mortalities (7%, range 0-360) per disease event. The majority of identifications as *Neobenedenia cf. melleni* were presumptive, with only 4% of institutions reporting a confirmed species identification. Voucher specimens were deposited in museum collections by 3 institutions (U.S. National Parasite Collection and the Manter Laboratory for Parasitology).

Hyperparasitism

Prophylactic treatment for secondary infections of epidermal lesions and scale loss with antibiotics are often warranted in heavy infections to prevent mortalities, however the possibility of coinfection should not be discounted. When managing a group of fishes with a *Neobenedenia* outbreak coinfection with another parasite is a significant risk. The dinoflagellate parasite *Amyloodinium ocellatum* is known to be a hyperparasite of *N. melleni* itself (Colorni, 1994) and infections commonly manifest during monogene outbreaks. In a survey of over 30 public aquaria in 2015, 11% reported outbreaks of *A. ocellatum*, and interestingly 17% reported coinfection with *Uronema marinum* (Christie, 2015), *A. ocellatum* outbreaks have also been reported during laboratory infection trials of *N. melleni* (Ellis and Wantanabe, 1993). Notably both *A. ocellatum* and *U. marinum* are microparasite species that thrive at lower salinities, such as the conditions typical of hyposalinity treatments. Overall 45% of facilities reported experiencing concurrent infections of a ciliate, scuticociliate, or dinoflagellate during *Neobenedenia* outbreaks (Christie, 2015).

Treatment Strategies

In order to be effective, treatments for *N. melleni* must be applied to take advantage of the life cycle. The eggs of capsalid monogenes in general, and *N. melleni* in particular, are extremely resilient to chemical treatments, and to be effective the adult and oncomiracidia stages need to be targeted. The only treatment that has been shown to be 100% effective in preventing eggs from hatching is hyposalinity, and many other chemotherapeutics, immunostimulants, and biological controls have been tried, summarized below. The literature cited of this paper should serve as a

portal to the literature for those interested in learning more, however as a word of caution treatment strategies employed in the literature intended for aquaculture applications should be taken *cum grano salis*, as the intent of the aquaculturist is to stave off mass outbreak of pathogenic ectoparasites just long enough to allow for outgrowth and harvest of the crop to market. Those in public aquaria are seeking a treatment strategy to allow for long-term control (eradication may be a bit optimistic) of the parasite over years or decades; employing chemotherapeutics with 99% efficacy may offer short-term results in an aquarium, but the potential for developing drug resistance is a very real consequence.

Much of the most promising research into the control of *Neobenedenia* has come from parasitologists in Australia, and a landmark paper (Brazenor and Hutson, 2015) on the life cycle as it relates to salinity and temperature allows one to more accurately construct treatment regimens to target infective stages. This groundbreaking work has been expanded by the authors to a web-based interactive treatment calculator that aquarists and veterinarians combatting *Neobenedenia* are advised to consult: http://www.marineparasites.com/paratreatmentcal.html, with the caveat that treatment in aquaculture and treatment in aquaria are two different beasts, with different goals, and multiple applications of chemotherapeutics are necessary to effectively control this parasite in a closed system.

Praziquantel

Praziquantel (PZQ) first started to see widespread usage in aquaria in the 1990's and early 2000's. The first use of PZQ against a capsalid monogene was reported by Thoney (1990), who noted the failure of copper sulfate and trichlorfon to control *Benedeniella posterocolpa.* Innis (2012) reports that 75% of public aquaria and zoos currently employ PZQ immersion therapy, with a wide range of dosing regimens and only 3% testing PZQ concentrations. Until 2014 there was a lack of basic understanding in analytical chemistry as to how to quantify PZQ in seawater, and for many years it was assumed to be present in therapeutic concentrations in seawater for 20d, and detectable for over 30d based on an HPLC method (Crowder and Charandra, 2003). More recently, it has been discovered that this initial method confused PZQ degradation byproducts with PZQ itself, and furthermore microbial action rapidly reduces PZQ concentrations in water (Marrero and Ellis, 2014, Thomas et al., 2016), rendering it ineffective after a very short time period. Marrero and Ellis (2014) found that most samples degraded within 36h from starting concentrations of 3-6mg/l, and Thomas et al. (2016) expanded these methods to show that PZQ-naïve systems with sterilized seawater would only retain the drug in measurable concentrations for 8d, and repeat exposure to PZQ would show degradation within 48h.

Despite its limitations, PZQ remains a viable drug to control *Neobenedenia*, though the rate of parasite recurrence is higher than some other methods (Christie, 2015), and increasing microbial degradation rates should be considered. This compound may be most useful in a quarantine setting, where small volumes permit more economical dosing of higher concentrations (e.g. 4-5mg/l) more frequently (redosing every 3-4d) as means of antihelminthic prophylaxis.

Oral (*per os*) administration of PZQ has been attempted to control a number of monogenes, including multiple capsalid species including *Neobenedenia* spp. (Janse and Borgsteede, 2003; Hirazawa et al., 2004; Yamamoto et al., 2011), to limited success. In pharmacokinetic studies, the variability of PZQ concentrations in the skin of some species has been determined to be suboptimal

for treatment of monogenes (Tubbs and Tingle, 2006). In an aquaculture setting, administration of oral medications may hold some promise, as it is certainly possible to control the infection long enough for an appropriate drug withdrawal period and harvest, however, as is the case with many other treatment regimens, this is not directly applicable to fishes held in public aquaria. In an aquarium setting there are eggs and larvae well entrenched in the aquarium itself when monogene outbreaks occur, and in order to be effective oral chemotherapeutics would need to reach concentrations in the mucous and epithelium of fishes sufficient to kill 100% of adult worms and prevent 100% of parasite infections, and this level would need to be maintained on every fish in the exhibit for an extended period of time. To date we simply do not have sufficient pharmacokinetic data for the variety of fish taxa we display to create evidence-based oral treatments, and existing trials have shown mixed results.

Thoney and Hargis (1991), recognizing the factors above, cautioned against the use of oral treatment routes to combat monogenes for fear of developing drug resistance. Kim et al. (2001) found that even at extreme doses of PZQ (400mg/kg) the drug was only detectable in plasma for 96h, and tissue/mucous concentrations were even lower. In trials against *N. girellae* with oral PZQ Yamamoto et al. (2001) found only 80% reduction in parasite burden, Hirazawa et al. (2004) tried doses ranging from 40-150 mg/kg P.O. over 3-11d and noted reductions in parasite abundance but not clearance of the infection. In trials against another highly resilient monogene, *Microcotyle sebastis*, PZQ administered at 100-200mg/kg P.O. was more effective when administered with cimetidine at 200mg/kg (Kim et al., 2001b), but still not completely efficacious at clearing monogene infections. Against *Benedenia seriolae* oral PZQ reduced infection rates by 81-99% (Forwood et al., 2016). In a survey of public aquariums, 5 institutions report having tried oral PZQ in doses of 0.5-400mg/l for 14-28d with no reported success (Christie, 2015). In general, the risk of creating PZQ-resistant monogenes is high, and chances of success very low when considering oral administration, and the authors would advise against it unless in combination with another antihelminthic backed by pharmacokinetic data, and/or in conjunction with aggressive waterborne treatment.

Organophosphates

Organophosphate pesticides, especially trichlorfon (Dylox™), have been used in aquariums and aquaculture for over 30 years to combat monogenes, leeches, and crustacean parasites in fishes. Trichlorfon has some advantages as a chemotherapeutic, as it is inexpensive, low concentrations are needed for treatment (<1.0mg/l), and oxidizes rapidly in hard waters. The major disadvantage to trichlorfon use is toxicity to both animals and staff as organophosphates are potent neurotoxins. The use of atropine sulfate as an antidote for organophosphate toxicity is a consideration for aquariums using this class of chemicals, either in response to toxicosis, or prophylactically before and during treatment. It should also be noted that due to the rapid oxidation of organophosphates (<12-18h), treatments should be timed to match the life cycle of the parasite. Additionally, reapplication frequency should be determined by the life history of the worm in relation to salinity and temperature. Eggs of *Neobenedenia* have been found to hatch within the first few hours of light (Hoai and Hutson, 2014) and have a peak viability at 12h (Brazenor and Hutson, 2015) so it is important to apply treatments so that waterborne concentrations overlap with oncomiracidia viability for maximum disruption of the life cycle (Figure 11). In general,

trichlorfon treatments in public aquaria and zoos have only shown mixed results against capsalid monogenes, and have not proven highly effective against *Benedeniella* or *Neobenedenia* (Thoney, 1990, Christie, 2006, Christie, 2015).

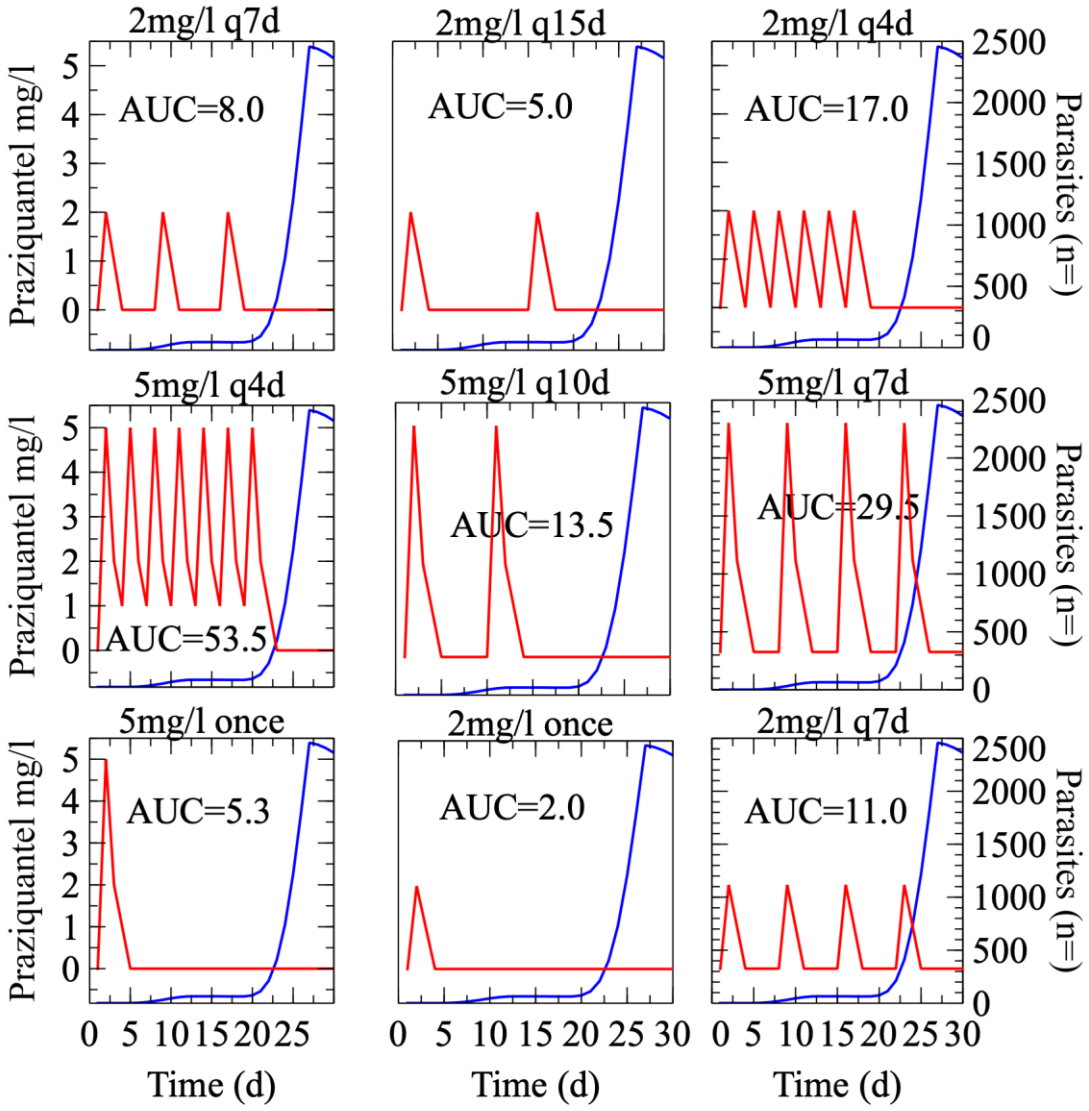

Figure 10. An overview of different praziquantel (PZQ) dosing regimens used at n=28 public aquaria as submitted to a 2015 questionnaire. As has been observed by Innis (2012) there are significant differences in clinical protocols and dosing schemes being used at various facilities. The red line indicates theoretical PZQ concentrations in mg/l assuming that microbial degradation patterns in seawater follow those observed by Marrero and Ellis (2014). The blue line indicates theoretical parasite population growth on a log scale, the red line is PZQ concentration in mg/l. For each treatment protocol, the area under the curve (AUC) is calculated, and expressed numerically as milligram-days (mg d^{-1}) of PZQ.

Table 3. Praziquantel Prolonged Immersion Dosing Regimens Reported in a 2015 Survey of Public Aquaria**.**

Concentration (mg/l)	Duration (d)	Reapplication	Efficacy (Reported)
2.0		q7d	+/-
2.0	28	q7d	-
2.0		q14d	+/-
2.0	30	q10d	n/s
2.0	21	q7d	+
2.0	21-28	q4d	+
2.0	21	q4d	+
2.0	7	-	-
2.0			+/-
2.0	20-30	q10d	+
2.2	21	-	-
3.0	21	q7d	+/-
3.0	21	7	+/-
3.0	20-30	q10d	+
4.0		q7d	+/-
4.0	28	q7d	-
5.0	21	-	+/-
5.0	21	q4d	+
5.0	7	-	-
5.0	30	q4d	+
5.0	20	q4d	+
10.0	7	-	-

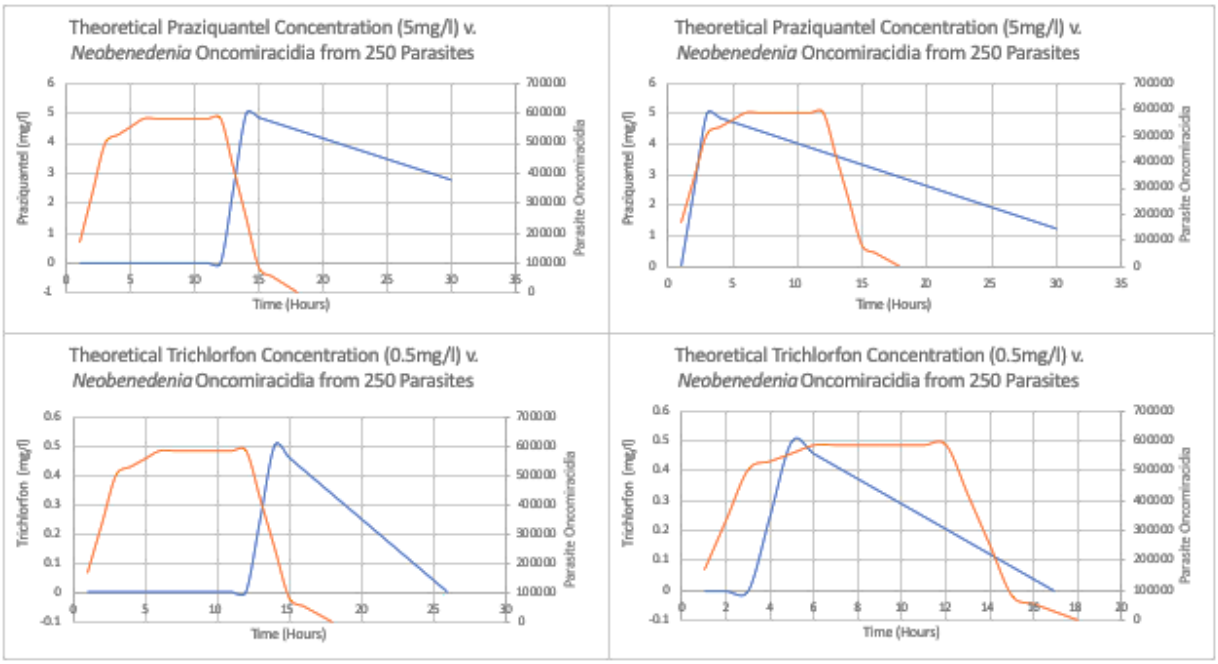

Figure 11. Theoretical plot of two common waterborne antihelminthics (blue lines) applied at mid-day and before first light. Praziquantel (PZQ) is known to degrade in as little as 24-36h (Marrero and Ellis, 2014) through microbial action and trichlorfon (Dylox™) is an organophosphate known to oxidize in 12-18h in high-alkalinity water. As oncomiracidia (orange line) emerge in the first 3h of light and are most viable for 12-15h (Hoai and Hutson, 2014, Brazenor and Hutson, 2015), the effective period for waterborne medications to target the (most vulnerable) infective stages is maximized when drugs are applied just before first light.

Table 4. Trichlorfon (Dylox™) Prolonged Immersion Treatment Regimens Reported in a 2015 Survey of Public Aquaria

Concentration (mg/l)	Duration (d)	Reapplication	Efficacy (Reported)
0.25	21	7	-
0.30	15-20	5	n/s
0.50	21-28	7	n/s
0.70	30-40	10	n/s
0.40	7	-	-
0.63	21	7	-
0.25	21	7	+
0.50	21	7	+
0.25	21-28	7	+/-
0.50	21-28	7	+/-
0.75	21-28	7	+/-

Hyposalinity

The effects of salinity on *N. melleni* were first examined by Mueller et al. (1992) who found that egg hatching success declined substantially below 24g/l. Ellis and Wantanabe (1993) further expanded this knowledge and reported that 18g/l for 7d was effective at preventing *N. melleni* eggs from hatching in the lab during *in vitro* trials, but ineffective during a full-scale aquaculture experiment. However, reducing the salinity to 15g/l for 5d was effective both in the lab and in trials at interrupting the life cycle (Ellis and Wantanabe, 1993). The first application of prolonged, long-term hyposalinity carried out on a large scale was done at Moody Gardens in Galveston, Texas in a million-gallon exhibit (Christie, 2006), and the method has been used by multiple other facilities, such as on the Giant Ocean Tank of the New England Aquarium to good effect (Smith, et al. 2018).

In trials at the New England Aquarium, Smith et al. (2018) reported that reduction in salinity to 15g/l for 60d, then increasing to a maintenance level of 22g/l, and then a second round of 15g/l of 60d was effective at reducing parasite eggs observed effectively to zero, and preventing outbreaks in a large mixed-species exhibit. This method was also unique in that they proactively examined micron-mesh screens placed on the exhibit overflows, and used the frequency of eggs (and especially viable eggs with eyespots) to inform data-driven treatment plans (Smith et al., 2018). The presence of eyed-eggs is important because the percentage of eyespots visible within the egg case is an indirect indicator of viability, Ellis and Wantanabe (1993) found higher hatch rates in groups of eyed-eggs. Interestingly enough, Smith et al. (2018) noted no difference in overall fish mortality during hyposalinity treatments than during non-disease periods at normal salinities, similar to the account of Christie (2006) who only reported animal issues with elasmobranch, but not teleost fishes.

Shorter term freshwater dips have also been found particularly useful as a management strategy to control *Neobenedenia* spp. both in aquaculture and in public aquaria. These hyposaline/hyperosmotic treatments do not always kill the worms, but remove them physically from the host as the osmotic imbalance causes tetany in the worms' musculature and dislodges them. The use of freshwater dips is very common in aquaculture, as the pharmacopeia at the disposal of the aquaculturist is very limited in most countries compared to that of the aquarist in a public aquarium (e.g. aquaculture drugs being restricted by the FDA in the United States). One study of the efficacy of freshwater dips against sea lice and *Neobenedenia* found that parasite reductions of 95% resulted from 20 min dips, and 60 min dips resulted in 99% reduction (Fajer-Ávila et al., 2008).

Freshwater dips suffer the disadvantage of only removing juvenile and adult parasites, with no effect on eggs. This does not interrupt the life cycle, but disrupts it to a degree, and as such is a useful tool in aquaculture where host fishes must only be kept alive long enough to harvest and bring to market, or to temporarily alleviate the complications of parasitism in heavily infected individuals. Freshwater dips of fishes are a common management strategy in public aquaria that have open-system LSS and cannot conduct effective tank-wide treatments, or where incoming seawater will soon re-introduce the parasite. A combination of proactive freshwater dipping, probably bolstered by acquired immunity of the host fishes, has been used as a long-term management strategy in many aquaria. Freshwater dips are also a powerful tool with some potential to disrupt the life cycle when fishes are moved between tanks, such as upon arrival to the

facility before entering quarantine, or after quarantine before being moved to exhibit. However, it is worth noting that not all eggs fall to the substrate, and eggs present on the host will not be affected by a freshwater dip and may lead to reinfection. For maximal effect when constructing a data-driven strategy to employ freshwater dips in ectoparasite management, the reader is advised to consult the treatment calculator specific to *Neobenedenia* based on the life history work of Brazenor and Hutson (2015): http://www.marineparasites.com/paratreatmentcal.html

Table 5. Hyposalinity Treatment Regimens Reported from a 2015 Survey of Public Aquaria.

Salinity (‰)	Duration (d)	Efficacy (Reported)
20	n/s	-
15	24	+
13	45	+
17-24	30-60	+
17	25	+
13-15	16	+
15	30	+
13-15	30	+/-
17	60	+

Ineffective Treatments

A number of treatment strategies have been reported as being ineffective, both in the literature, and in a survey conducted in 2015 of 28 public aquaria. While some of these therapies may have some short-term benefit in reducing adult *N. melleni* populations, over the long-term they fail to control the outbreaks. While the more popular therapies, namely praziquantel and trichlorfon have shown mixed efficacy, this is largely thought to be due to the wide disparity of dosing schemes (as discussed above), and there are some trends which may be useful in developing a strategy to maximize efficacy (discussed below in Analysis of Treatment Strategies section). Other chemical treatments, however, have been found to be either wholly or largely ineffective.

The first (modern) discussions of chemical treatments against *Neobenedenia* in aquaria come from Gallet de Saint Aurin et al. (1991), Thoney and Hargis (1991), and Mueller et al. (1994). These early reports contained notes on the use of copper sulfate, formalin, and trichlorfon. Trichlorfon has had mixed results, but the use of copper and formalin have historically been ineffective, and this was further validated by surveys of public aquaria (Christie, 2006; Christie 2015). Formalin may offer some temporary reprieve by reducing parasite burden, but is overall not an effective strategy for long-term management. Numerous other treatments have been reported in surveys (Christie, 2015) that are puzzling: one aquarium reported treating with diflubenzuron (Dimilin™) unsuccessfully, which is unsurprising as this compound is a chiton synthesis inhibitor. Other facilities reported use of chloroquine phosphate, chloroquine diphosphate, or chelated copper (Cupramine™), with none reporting any success (Christie, 2015). An investigation by Ohno et al. (2009) reported no effect of the antibiotics oxytetracycline,

florfenicol, ampicillin, erythromycin, or sulfamonomethoxine on *Neobenedenia,* though all of these compounds could be useful in combatting secondary infections resulting from monogenean infections.

Oral (*Per Os)* Treatments

Numerous oral treatments have been attempted to control a variety of monogene species, and almost all have proven ineffective in closed systems. Most of the literature concerning treatment strategies for *N. melleni* are focused on aquaculture, so extrapolation of methods for use in closed-system aquaria must be done with caution, as efficacy in aquaculture is often defined as keeping the fish stock alive long enough to bring to market. Eradication or control of *N. melleni* in aquaria (as a function of whether one is an optimist or pessimist, respectively) requires a more long-term approach, and many methods used in aquaculture are as such ill-suited, as they are more likely to breed drug resistance over time. Effectively the use of some methods may buy a temporary reprieve from morbidity and mortality and alleviate acute symptoms exhibited by animals, while still leaving the chronic problems associated with ectoparasite infestations.

Despite their general lack of efficacy against monogenes, there is a fair bit of literature on the various attempts to employ oral antihelminthics (Janse and Borgsteede, 2003; Hirazawa et al., 2004; Christie 2006; Yamamoto et al., 2011; Christie, 2015; and Forwood et al, 2016) and they are generally regarded as ineffective in public aquaria. See the above discussion on the use of praziquantel for more detailed information.

Biological Controls

In taking a more holistic approach to ectoparasite management the use of biological controls has provided some solutions with surprising efficacy. Numerous cleaner organisms have been employed in aquaria to control minor or latent infections in the captive environment. Up to six institutions report using as many as nine species of cleaner fishes and decapods, the most common being neon gobies, *Elacatinus oceanops* (36%), porkfish, *Anisotremus virginicus* (26%), and various labrid fishes, including blueheaded and yellow wrasses, *Thallasoma bifasciatum* and *Halichoeres garnoti,* respectively (18%) (Christie, 2015). Given the problem of *Neobenedenia* spp. in aquaculture there exists a fair amount of literature on the use of cleaner organisms as a control strategy. De Souza et al. (2012) found that gobies could reduce levels of *N. melleni* as much as 90% on groupers, though they disproportionately preferred larger fishes to clean. *Thallasoma bifasciatum* and *Elactinus* spp. gobies have been shown to reduce parasite loads of *N. melleni* by 50% or more in aquacultured fishes (Cowell et al., 1993).

Not all of the shrimps traditionally thought of as cleaner organisms had equal performance on parasite reduction, McCammon et al. (2010) found that *Periclimines pedersoni* was more effective than other species at reducing *N. melleni*, and has a significant effect on oncomiracidium transformation. Overall numerous studies have found that the effect cleaners have is dependent not only on the size of the host fishes (De Souza et al., 2012), and the parasite load (Becker and Grutter, 2005) but also the size of the monogene, with larger parasites being selectively removed (Grutter et al., 2002). More recently, Militz and Hutson (2015) found that not only do cleaner shrimps consume adult worms from fishes, but they actively consume oncomiracidia and even feed on eggs. This finding is especially important because other than desiccation or hyposaline inhibition there are no treatment or control strategies for reducing the viability of *Neobenedenia* ova.

Nontraditional and Emerging Treatments

The severity of the threat posed by *N. melleni* to aquaculture has resulted in a wide range of potential treatments being experimented with and subsequently reported in the literature. Some of the more nontraditional areas of research include exploring natural immunity, application of various herbal and phycological extracts, use of metallic nanoparticles, and the use of newer antihelminthic formulations.

There are a number of newer antihelminthic medications that have yet to see common use in public aquariums against *N. melleni* but may hold promise. As new drugs come to market, the economic considerations of applying them in large quantity in waterborne treatments is often an expensive gamble. Nevertheless, some small-scale experiments from aquaria and reports from the literature provide some examples of drugs which may merit larger-scale experimentation. One example is in the application of metallic nanoparticles. The use of nanoparticles as delivery vehicles for enzymes, drugs, and other bioactive compounds is a rapidly growing area of pharmaceutical research, and there are some very preliminary investigations on the effects on fish parasites. One study has been published on the effect of silver nanoparticles on monogenes, and has shown some promising, if preliminary, effects on gyrodactylid worms *in vitro* (Pimentel-Acosta, et al. 2019). One of the authors (BLC) has conducted small-scale trials with mebendazole dissolved in a methanol/formic acid solution at a 3mg/l concentration and found little effect on *N. melleni* (Christie, unpub. data), but a 5mg/l albendazole (Valbazen™ Zoetis) treatment dissolved in DMSO reapplied every third day for 21d has shown some promise (Christie, unpub. data). In what may seem like the ultimate anthelminthic "shotgun" approach, researchers in México have been investigating the use of a product marketed as Adecto™, a combination of ivermectin, praziquantel, pyrantel pamoate, and fenbendazole with some success against adult monogenes (Morales-Serna et al., 2018). Olivera et al. (2019) provided some data on the hematological changes from levamisole treatment in lutjanid fishes infected with *Neobenedenia*, but failed to note the effect, if any, on parasite burden.

Some botanical/phycological extracts have been examined for their effect on *Neobenedenia*. Extracts of the alga *Asparagopsis* were shown to reduce *Neobenedenia* hatch rates to 3% (versus 99% in control), and delayed the life cycle (14-18d from first to last hatch compared to 5-7d in control) (Hutson et al., 2012). A number of other studies have examined the potential application of garlic, *Allium sativum*, extracts against *Neobenedenia*. Militz et al. (2013) found the infection success of *Neobenedenia* dropped from roughly 25% to <10% when fishes were fed 50ml/kg of a 200mg/ml garlic extract for 30d. No effect on parasitism was seen in this study in fishes fed garlic for 10d, and interestingly effects were not greater with a higher dose (150ml/kg) dose of garlic extract (Militz et al., 2013). The effects of waterborne garlic extract on *Neobenedenia* has also been examined, adult and juvenile worms seem to be unaffected at extract concentrations up to 15.2µl/l, but at this dose hatch success dropped to 5% and oncomiracidium longevity was reduced to <2h (Militz et al., 2014). Lower concentrations of garlic extract showed some effect on hatch rates as well, 25% hatch was observed at 0.76µl/l and 11% hatch rate at 1.52µl/l (Militz et al., 2014). It is important to note regarding these garlic studies that they were conducted with freshly prepared garlic extractions, as many of the bioactive organosulfur or organoselenium compounds are highly volatile, and do not survive preparation or drying (Cai et al., 1994, Amagase, 2006). The findings of these experiments cited should not be confused with the propagation of pseudoscience regarding garlic supplementation and immunity that has plagued

aquarium hobbyist magazines and online forums in recent years in an attempt to market commercial products of questionable efficacy.

The immune response of fishes to *Neobenedenia* infections is another interesting and promising area of study, and immune response or lack thereof has been implicated in some of the disastrous outbreaks in aquaculture. In Hawaii *N. melleni* outbreaks occurred in sea cage tilapia farms, and the intensity of the parasite outbreak was presumably due to the (normally) freshwater species being exposed to a parasite it had not coevolved with, and therefore had no natural immunity (Kaneko et al., 1988). In an investigation of whether tilapia could develop immunity through injections of tissue extract Rubio-Godoy et al. (2011) found no immune effect, but did note that there were differences in susceptibility between natural *Oreochromis mossambicus* and hybrid *Oreochromis* varietals. However, Kishimori et al. (2015) found that after a natural infection, *Oreochromis mossambicus* did retain *N. melleni*-specific antibodies for up to 120d which did confer some degree of protection against reinfection. Buchmann (1999) explored the mechanism of these adaptive immune defenses in fishes against monogenes, and a release of cytokines that leads to a decrease in parasite populations seems to be the primary method of action. These fragmented studies offer a glimpse into the parasite-host interaction and may lead to future strategies for control or prophylaxis.

Analysis of Treatment Strategies

Leading up to a special session on *Neobenedenia* at the AZA Annual Conference in Salt Lake City a survey was conducted on the prevalence and treatment strategies from 28 different institutions (Christie, 2015). Their responses allow some general trends to be identified, though it is important to note that cursory analysis of these data should be interpreted with caution, as self-reported data are not the most robust from which to draw broad conclusions. To identify trends in success or failure of the most common chemotherapeutic regimens a N-1 two-proportion test was applied (assuming Z=2.31) to determine at which point the reported concentrations, durations of treatment, and reapplication frequency favored success. Efficacy was scored as a binary (1=reported effective, 0=reported ineffective or mixed results) for comparison against the other factors in treatment strategies. It is worth noting that an N-1 two-proportion test does not report results with the same certainty as a more rigorous statistical method (i.e. those using an α=0.05 to report within a 95% confidence interval), but rather this test only determines if something is more likely than not to occur from the given dataset. Thus, some trends may be identified which may guide best practices for treatment, but should not be viewed as dogma or concrete fact.

For prolonged immersion praziquantel treatments, this analysis showed that the concentration of PZQ was surprisingly not a major factor in the likelihood of success (p=3.98042); factors favoring successful outcomes were 1) at least 21 days or more of treatment (p=0.14858), and 2) reapplication of drug less than every five days (p=0.39934). As shown above in Figure 10, the area under the curve (AUC) of PZQ concentrations is also a useful metric when comparing various treatment regimens (Table 3). Calculation of AUC gives a single number from which to make comparisons, expressing treatment as milligram-days of praziquantel ($mg \cdot d^{-1}$ PZQ). Treatments with higher $mg \cdot d^{-1}$ PZQ values were more likely to be effective at preventing recurrence of the parasite.

Using the N-1 two-proportion test with Z=2.31 and reported efficacy scored as a binary (1/0) we are also able to draw some conclusions about the likelihood of recurrence of *Neobenedenia* after treatment. Risk factors for recurrence are as follows 1) tank sizes over 100,000 gallons (p=0.10184), 2) lack of antihelminthic prophylaxis in quarantine protocols (p=0.00386), and 3) lack of screening (scrapes/dips) before animals leave quarantine (p=0.28734). Surprisingly, as many as 20% of institutions report having no antihelminthic treatment as part of their normal quarantine, and 44% do not routinely screen fishes for ectoparasites prior to release from quarantine (Christie, 2015), and those facilities were statistically more likely to report issues with recurrence of *Neobenedenia* in their collections.

These data suggest that long-duration praziquantel treatments with frequent re-treatment are more likely to be effective, and also that having strong quarantine protocols in place with proactive parasite screening are key to preventing outbreaks in aquaria.

Quarantine Best Practices

Prophylactic or responsive quarantine can be tailored towards the management or even elimination of monogeneans in general, depending on certain infrastructure aspects of the display facility. Aquariums operating closed systems with filtered, sterilized source water have the greatest potential to actualize control of *Neobenedenia*, especially where effective quarantine practices and prophylactic treatments are applied from the very beginning of acquiring and building the animal collection.

Quarantine systems should be designed to be as simple and effective at holding intended collection species as possible. Tanks, sumps, piping and life support equipment should be installed where quarantine staff are able to access, clean and disinfect the system and its components. No life support component currently in existence will fully eliminate *Neobenedenia* from infected fish, but mechanical filters, fractionators, and sterilizers (UV, ozone) can all help to reduce successful reinfection by oncomiracidia provided adequate flow and turnover are utilized.

If prophylactic treatments are planned, remember that any wetted surface within a quarantine system can harbor viable eggs and oncomiracidia. Isolating life support components during bath treatments will provide a haven for those life stages of *Neobenedenia* and other monogeneans. Reinstating those same components following the completion of treatment without adequate disinfection protocols being observed effectively negates those treatments applied with the goal of eliminating *Neobenedenia*. Long term baths (at least long enough to act on any hatching larvae) that can be tolerated by established biofilters are best candidate treatments for complete elimination. Fractionators and sterilizers should be deactivated for the duration of the bath treatment to prevent removal and degradation of treatment chemicals.

General practices focused on reducing pathogen transference in aquariums absolutely apply to combating *Neobenedenia*. Excellent biosecurity measures need to be enforced for the full course of quarantine, both between quarantine and display systems and among all occupied quarantine systems. Dedicating one or more aquarists to quarantine systems in use can make maintaining these measures easier for the aquarium. Footbaths, gloves, medical aprons or scrubs, and other forms of staff focused PPE and disinfection work to reduce risks of human vector transference, and simultaneously help to keep staff alert regarding proper biosecurity practices

within quarantine spaces. "All in, all out" protocols for moving entire groups of animals in and out of each quarantine system maintains the efficacy of treatments by ensuring each fish receives the same durations and dosages. Dedicated equipment and tools should be disinfected (to act on free living stages) and allowed to completely dry (to desiccate eggs) between uses to reduce risks of fomite transference.

Entry examinations (gross observation, dip sediments, skin scrapes, fin/gill clips, etc.) can provide some initial information on the presence and prevalence of any ectoparasites infecting newly acquired fishes. Exit examinations for fish that have completed their prescribed quarantine should be thorough and involve all or a significant sampling of each system's occupants. Confirmed or reasonably suspected persistence of *Neobenedenia* infection should be considered and addressed before any fishes from the affected system(s) are approved for display.

Sanitization of Tanks and Equipment

The resistance of *Neobenedenia* eggs to chemical therapeutics and sterilants has been problematic for aquaculturists and aquarists alike. The authors have found that exposures of 1-8h to 125-250mg/l Cl^-, or 175mg/l H_2O_2 have been ineffective for killing all eggs (Christie, unpub. data). This stands in stark contrast to studies of other monogenes that have found chlorine concentrations as low as 120mg/l for 3h to be effective against *Haliotrema* and *Euryhaliotrema* sp. (Fajer-Ávila et al., 2007). Hirazawa (2019) found that the eggs of *N. girellae* were resistant to 60mg/l Cl^- and 100mg/l benzalkonium chloride for 24h, but were killed by exposure to 120mg/l Cl^- for 24h. Eggs of *N. girellae* were also inviable after exposure to temperatures of 50^oC for 1min. or by desiccation for 1h (Hirazawa, 2019). Cecchini and Cognetti-Varriale (2003) found that desiccation was more effective than formalin or organophosphate exposure against the eggs and embryos of *Diplectanusm elegans*, another resilient monogene.

Screening for Parasites in the Aquarium

Aside from direct identification of parasites on skin scrapings on infected fishes, there are a few techniques to screen for the harbingers of a parasite outbreak. First and foremost, freshwater dips of fishes entering and leaving quarantine allow dip sediment can be siphoned through a micron sieve and examined microscopically (500-1000μm is sufficient for adult worms, 100μm should suffice for most eggs and oncomiracidia) for direct evidence of infection. Smith et al. (2018) describe a more proactive approach whereby micron sieves were placed in the overflows of a large marine tank, and examined regularly for ova; this was tracked and used to indicate necessity of treatment, and the aggregated data also gives insights into correlations with other stressors such as water chemistry, and with behavioral data such as frequency of scratching/flashing behaviors.

Polyester filter floss may also be used on the outflows of tanks, and periodically examined, for the presence of eggs (Figure 12), though with this method one of the authors (JWF) has found the large amount of additional detritus that collects in the floss disadvantageous for parasite surveillance. Fajer-Ávila et al. (2007) describe a delightfully simple and effective parasite surveillance technique where a cotton string is tied to an airstone; aeration acts as an airlift, moving water (and parasite ova) constantly over the string, which entraps them and can be easily removed and examined microscopically for parasite eggs or cocoons of numerous taxa (copepods, monogenes, leeches, et cetera).

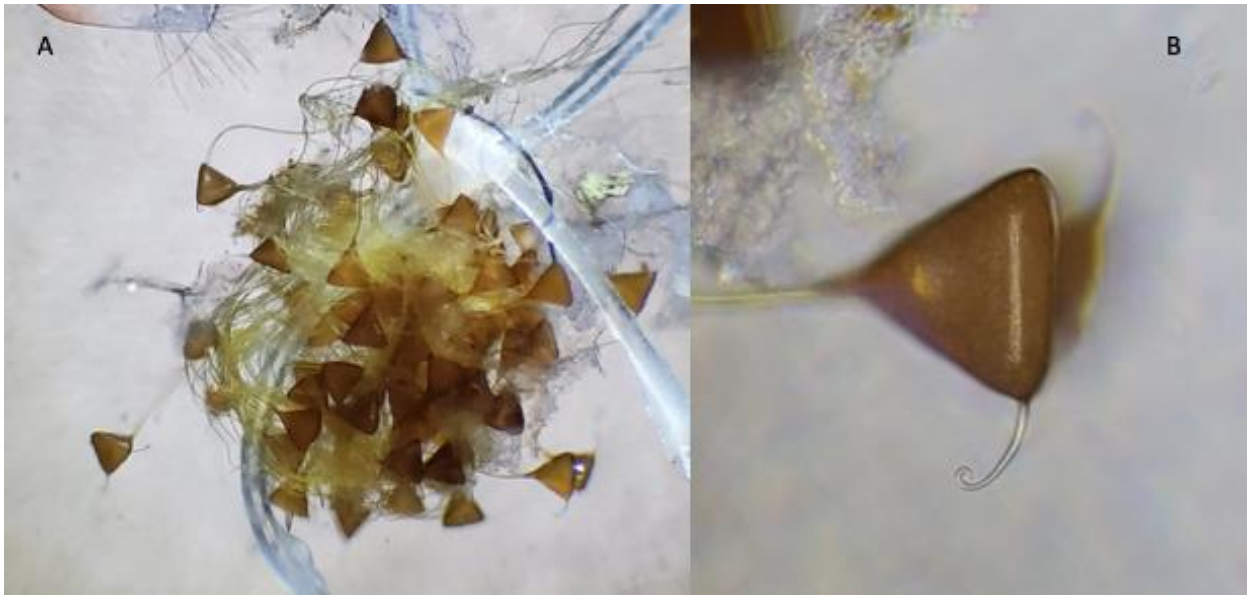

Figure 12. Eggs of *Neobenedenia sp.* from a parasite surveillance program. These eggs were collected in polyester filter floss, but micron mesh sieves or cotton string can also be used in parasite screening. In photomicrograph (B) note the long filament (left) and two shorter filaments (right) that are hooked distally as originally described by Jahn and Kuhn (1932). These filaments act like hook-and-loop fasteners (i.e. Velcro™) and will facilitate attachment to numerous surfaces. Photos from the Point Defiance Zoo and Aquarium by J.W. Foster IV and Tai Fripp.

Preservation of Monogenes

Whenever possible, parasite specimens from skin or gill biopsies should be examined and photographed while living, but often the rigors of the day to day routine of the aquarist or clinician may require specimens to be fixed and examined at a later date. With monogenes, especially specimens which may be of research value, care should be taken in preservation to ensure that key features are not obscured and/or that genetic material is available later. We now know that formalin destroys DNA and RNA (Strona et al., 2008), so specimens preserved for posterity or destined for museum collections as vouchers are better preserved in ethanol (EtOH). An investigation of monogene preservation techniques showed that preservation in 70% EtOH or DMSO are suitable for morphological preservations and DNA amplification, while 95% EtOH was suitable for the latter but not the former (Strona et al., 2008). Conventional methods for preservation pf platyhelminths involve fixation in a formalin-acetic acid, ethanol solution (referred to as FAA or AFA solution in the literature) either directly or after relaxation of the worms in refrigerated seawater or use of an anesthetic (Schell, 1970). AFA solution is typically made of 50% ethanol, 10% formalin, 2% glacial acetic acid, and 38% DI water (Schell, 1970), and was the gold-standard for many years.

Large monogenes such as the Capsalidae are difficult to stain, though for definitive identification (especially of *N. melleni* when attempting to trace the path of haptoral tendons) staining can be useful. Platyhelminths are typically stained with Semichon's Acetocarmine technique (see Hoffman, 1999). Typically, worms are destained in this process until the musculature is a faint pink and internal organs are stained bright red, however, with the key diagnostic features being the haptoral tendons in *N. melleni* the authors have found it useful to have a heavy hand when applying carmine stain, and destain to a lesser degree so these structures are still clearly visible.

Hematoxylin, or hematoxylin and eosin staining techniques are also commonly used on monogenes and are more readily available in most clinical settings, but require a long exposure to penetrate these large worms. Note that the means of collection may impact the specimen(s), worms collected from freshwater dips may show deformities as a result of the osmotic pressure. In redescribing *Neobenedenia* Whittington and Horton (1996) noted that MacCallum's original description (1927) of the testes was round and smooth, but in all the material they examined the margins were more scalloped, and that the marginal skirt of the haptor was more pronounced (see Fig.1). The original description (MacCallum, 1927) does not specify how the worms were collected, but in the authors' experience such deformities are common as in worms that have been collected in a freshwater dip as a result of osmotic pressure on the tissues. It is interesting to note, however, that the collector of these original specimens stated that she had *"no success in the use of fresh water for ocean fishes"* in treating fish ailments (Mellen, 1928), so the exact mechanism of collection of the original specimens remains a mystery.

Summary

This insidious ectoparasite has been with us for nearly a century in public aquaria, and given its resilience to chemical treatment will likely continue to plague aquarists for many years to come. Many advances in management of these parasites have decreased the lethality of outbreaks, and strategies for management have been largely successful at keeping the beast in check, even if never achieving a decisive 'checkmate' resulting in effective eradication. Data-driven treatment strategies should be embraced to maximize efficacy of treatment and control, including parasite surveillance of exhibits, strong antihelminthic prophylaxis in quarantine, screening of fishes prior to release from quarantine, extended duration praziquantel application with frequent re-dosing, use of protracted hyposalinity, strong biosecurity protocols, and consideration of the parasite's life cycle and hatching rhythms in treatment. Further elucidation of the full range of hosts will likely show that *Neobenedenia* is a ubiquitous parasite of bony fishes, though as this list grows and the taxonomic confusion between *N. melleni* and *N. girellae* is further resolved trends may emerge in host preferences. Until such time as complete eradication is possible, we, as an industry, will do all the running we can to stay in the same place.

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Literature Cited

Amagase, H. 2006. Clarifying the Real Bioactive Constituents of Garlic. Journal of Nutrition. 136(3): 716S-725S.

Becker, J.H. and A.S. Grutter. 2005. Client fish ectoparasite loads and cleaner shrimp *Urocaridella sp.* hunger levels affect cleaning behavior. Animal Behaviour. 70: 991-996.

Bell, G. 1982. The Masterpiece of Nature: Evolution and the Genetics of Sexuality. University of California Berkeley Press.

Bell, G. 1985. Two Theories of Sexual Variation. Experimentia. 10: 1235-1245.

Benz, G.W. 1987. *Dermophthirius penneri* sp. n. (Monogenea: Microbothriidae) as Ectoparasite of Carcharhinid Sharks, *Carcharhinus brevipinna* and *Carcharhinus isodon*. Proceedings of the Helminthological Society of Washington. 54(2): 185-190.

Brazenor, A.K. and K.S. Hutson. 2015. Effects of temperature and salinity on the life cycle of *Neobenedenia sp.* (Monogenea: Capsalidae) infecting farmed barrumundi (*Lates calcarifer*). Parasitological Research. 114(5): 1875-1886.

Brazenor, A.K., T. Bertozzi, T.L. Miller, I.D. Whittington, K.S. Hutson. 2018. DNA profiling reveals *Neobenedenia girellae* as the primary culprit in global fisheries and aquaculture. Molecular Phylogenetics and Evolution. 129: 130-137.

Breder C.M. and D.E. Rosen. 1966. Modes of Reproduction in Fishes. American Museum of Natural History. 941p.

Buchmann. K. 1999. Immune mechanisms in fish skin against monogeneans- a model. Folia Parasitologica. 46: 1-9.

Bullard, S.A., G.W. Benz, R.M. Overstreet, E.H. Williams, and J. Hemdal. 2000. Six New Host Records and an Updated List of Wild Hosts for *Neobenedenia melleni* (MacCallum)(Monogenea: Capsalidae). Comparative Parasitology. 67(2): 190-196.

Bullard, S.A., R.J. Goldstein, R. Hocking, and J. Jewell. 2002. A New Geographic Locality and Three New Host Records for *Neobenedenia melleni* (MacCallum) (Monogenea: Capsalidae). Gulf and Caribbean Research. 15: 1-4.

Cai, X., P.C. Uden, E. Block, X. Zhang, B.D. Quimby, J.J. Sullivan. 1994. Allium Chemistry: Identification of Natural Abundance Organoselenium Volatiles from Garlic, Elephant Garlic, Onion, and Chinese Chive using Headspace Gas Chromatography with Atomic Emission Detection. Journal of Agriculture and Food Chemistry. 42(10): 2081-2084.

Carvalho, A.R. and Luque, J.L., 2009. Occurrence of Neobenedenia melleni (Monogenea: Capsalidae) in *Trichiurus lepturus* (Perciformes: Trichiuridae), naturally infested off coastal zone of Rio de Janeiro, Brazil. Revista Brasileira de Parasitologia Veterinária, 18, pp.74-76.

Cecchini, S. and A.M. Cognetti-Varriale. 2003. Dehydration is more effective for control of embryonic development and larval hatching of *Diplectanusm elegans* (Monogenea, Diplectanidae) than formalin and trichlorphon. Aquaculture International. 11: 261-265.

Christie, B.L. 2006. Extreme Duration Hyposalinity in the Control of *Neobenedenia melleni* (Monogenea: Capsalidae), with Notes Regarding the History of Treatment in Public Aquaria. Proceedings of the Regional Aquatics Workshop, Drum and Croaker. 38: 26.

Christie, B.L. 2015. A Review of Treatment Strategies in Public Aquaria for *Neobenedenia melleni* (Monogenea: Capsalidae). Association of Zoos and Aquariums Conference. Hogle Zoo, Salt Lake City, UT.

Christie, B.L., J.W. Foster, T.N. Fripp. 2020. Thirty New Host records for *Neobenedenia melleni* (MacCallum, 1927) (Monogenea: Capsalidae), from Outbreaks in Public Aquaria. *SUBMITTED, UNDER REVIEW.*

Colorni, A. 1994. Hyperparasitism of *Amyloodinium ocellatum* (Dinoflagellida: Oodinidae) on *Neobenedenia melleni* (Monogenea: Capsalidae). Diseases of Aquatic Organisms. 19: 157-159.

Conroy, G., D.A. Conroy, and A. Rodriguez. 1986. First Record of *Neobenedenia* (Monogenea: Monopisthocotylea: Capsalida) as a Parasite of Grey Mullets (Mugilidae) from the Atlantic Coast of South America. Rivisita Italiana Piscicultura e Ittiopatologica. 21(4):157-159.

Cowell, L.E., W.O. Wantanabe, W.D. Head, J.G. Grover, and J.M. Shenker. 1993. Use of tropical cleaner fish to control the ectoparasite *Neobenedenia melleni* (Monogenea: Capsalidae) on seawater-cultured Florida red tilapia. Aquaculture. 113:189-200.

Crowder, J. and Charandra, T. 2003. Development and application of a method to detect and quantify praziquantel in seawater. Aquality I Symposium, Ocenario de Lisboa, Portugal.

De Souza, R.A., W.F. Annunciacão, S.F. Lins, E.G. Sanches, M.L. Martins, and M.Y. Tsuzuki. 2012. Can barber goby *Elacatinus figaro* control *Neobenedenia melleni* infections on dusky grouper *Epinephelus marginatus.* Aquaculture Research. 45(4): 619-628.

Deveney, M.R., L.A. Chisholm, I.D. Whittington. 2001. First Published Record of the Pathogenic Parasite *Neobenedenia melleni* (Capsalidae) from Australia. Diseases of Aquatic Organisms. 46: 79-82.

Ellis. E.P. and W.O. Wantanabe. 1993. The effects of hyposalinity on eggs, juveniles and adults of the marine monogenean *Neobenedenia melleni* treatment of ecto-parasitosis in seawater cultured tilapia. Aquaculture. 117: 15-27.

Fajer-Ávila, E.J., S.P. Velásquez-Medina, M. Betancourt-Lozano. 2007. Effectiveness of treatments against eggs, and adults of *Haliotrema* sp. and *Euryhaliotrema* sp. (Monogenea: Ancyrocephalinae) infecting red snapper *Lutjanus guttattus.* Aquaculture. 264: 66-72.

Fajer-Ávila, E.J., I. Martinez-Rodriguez, M.A.A. de la Parra, L. Alvarez-Lajonchere, M. Betancourt-Lozano. 2008. Effectiveness of freshwater treatment against *Lepeophtheirus simplex* (Copepoda: Caligidae) and *Neobenedenia* sp. (Monogenea: Capsalidae), skin parasites of bullseye puffer fish, *Sphoeroides annulatus* reared in tanks. Aquaculture. 284: 277-280.

Forwood, J.M., E.J. Bubner, M. Landos, M.R. Deveney, T.D. Antignna. 2016. Praziquantel delivery via moist pellets to treat monogenean parasites of yellowtail kingfish *Seriola lalandi*: efficacy and feed acceptance. Diseases of Aquatic Organisms. 121: 201-209.

Innis, C. 2012. Observations on Praziquantel Concentrations During Experimental and Clinical use in Marine Aquaria. Proceedings of the American Association of Zoo Veterinarians Conference 2012: 137-139.

Gallet de Saint Aurin, D., J.C. Raymond, and V. Vianas. 1990. Marine finfish pathology: specific problems and research in the French West Indes. IFREMER Actes de Colloques 9: 143-160.

Gonzalez M.T. and E. Acuna. 1998. Metazoan Parasites of the Red Rockfish *Sebastes capensis* off Northern Chile. Journal of Parasitology. 84(4): 783-788.

Grutter, A.S., M.R. Deveney, I.D. Whittington, and R.J.G. Lester. 2002. The effect of the cleaner fish *Labroides dimidatus* on the capsalid monogenean *Benedenia lolo* parasite of the labrid fish *Hemigymnus melapterus*. Journal of Fish Biology. 61: 1098-1108.

Habu, K., Kuriyama, I. and Tanaka, S., 2009. Influence of immersion into fresh and low salinity waters on survival of young sevenband grouper *Epinephelus septemfasciatus* and removal of monogenean parasites *Benedenia epinepheli* and *Neobenedenia girellae*. Bullletin of the Mie Prefecture Fisheries Research Institute. 17: 45-54.

Hargis W.H. 1955. A New Species of *Benedenia* (Trematoda: Monogenea) from *Girella nigricans*, the Opaleye. Journal of Parasitology. 41(1): 48-50.

Hirazawa, N. 2019. Killing effects of various treatments on eggs of the skin-parasitic monogeneans *Neobenedenia girellae* and *Benedenia seriolae*. Aquaculture. 498: 285-288.

Hirazawa, N., T. Mitsuboshi, T. Hirata, and K. Shirasu. 2004. Susceptibility of spotted halibut *Verasper variegatus* (Pleuronectidae) to infection by the monogenean *Neobenedenia girellae* (Capsalidae) and oral therapy trials using praziquantel. Aquaculture. 238(1-4): 83-95.

Hoai, T.D. and K.S. Hutson. 2014. Reproductive Strategies of the Insidius Fish Ectoparasite, *Neobenedenia sp.* (Capsalidae: Monogenea). PLOS One. 9(9):e108801

Hutson, K.S. L. Mata, N.A. Paul, R. de Nys. 2012. Seaweed extracts as a natural control against the monogenean ectoparasite *Neobenedenia sp.* infecting farmed barrumundi (*Lates calcarifer*). International Journal for Parasitology. 42: 1135-1141.

Hoffman, G.L. 1999. Parasites of North American Freshwater Fishes. Comstock Press. 576p.

Jahn, T.L. and L.R. Kuhn. 1932. The Life History of *Epibdella melleni* MacCallum 1927, A Monogenetic Trematode Parasitic on Marine Fishes. Biological Bulletin. 62: 89-111.

Janse, M. and F.H.M. Borgsteede. 2003. Praziquantel treatment of captive white-spotted eagle rays (*Aetobatis narinari*) infested with monogenean trematodes. Bulletin of European Fish Pathology. 23(4): 152-156.

Kaneko, J.J., R. Yamada, J.A. Brock, and R.M. Nakamura. 1988. Infection of Tilapia, *Oreochromis mossambicus* (Trewavas), by a Marine Monogenean, *Neobenedenia melleni* (MacCallum, 1927) Yamaguti, 1963 in Kaneohe Bay, Hawaii, USA, and its Treatment. Journal of Fish Diseases. 11: 295-300.

Kinami, R., J. Miyamoto, T. Yoshinaga, K. Ogawa, and Y. Nakagura. 2005. A Practical Method to Distinguish Between *Neobenedenia girellae* and *Benedenia seriolae.* Fish Pathology. 40(2): 63-66.

Kishimori, J.M., A. Takemura, and J.A.C. Long. 2015. *Neobenedenia melleni*-Specific Antibodies are Associated with Protection after Continuious Exposure in Mozambique Tilapia. Journal of Immunology Research. 2015: e635387

Kim, K.H., C.S. Kim, and J.W. Kim. 2001. Depletion of praziquantel in plasma and muscle tissue of cultured rockfish *Sebastes schlegeli* after oral and bath treatment. Diseases of Aquatic Organisms. 45: 203-207.

Kim, K.H., E.H. Lee, S.R. Kwon, J.B. Cho. 2001b. Treatment of *Microcotyle sebastis* infestation in cultured rockfish, *Sebastes schlegeli* by oral administration of praziquantel in combination with cimetidine. Diseases of Aquatic Organisms. 44: 133-136.

Keosharyani, I., K. Yuasa, and K. Hatai. 1999. Two species of capsalid monogeneans infecting cultured humpback grouper *Cromileptes altivelis* in Indonesia. Fish Pathology. 34(3): 165-166.

Li AX, Wu XY, Ding XJ, Lin RQ, Xie MQ, Lun ZR, Zhu XQ. 2005. PCR-SSCP as a molecular tool for the identification of Benedeniinae (Monogenea: Capsalidae) from marine fish. Molecular and cellular probes. 19(1):35-9.

Lively, C.M. 2010. A Review of Red Queen Models for the Persistence of Obligate Sexual Reproduction. Journal of Heredity. 101(Sup.1): S13-S20.

Love, M.S., M. Yoklavich, L. Thorsteinson. 2002. The Rockfishes of the Northeast Pacific. U. California Press, Berkeley. 404p.

MacCallum, G.A. 1927. A New Ectoparasitic Trematode. Zoopathologica. 1(8): 291-300.

Marrero, C. and H. Ellis. 2014. Effectively Monitoring Praziquantel in Immersion Baths. Aquality II Symposium, Atlantis, Bahamas.

McCammon A., P.C. Sikkel, and D. Nemeth. 2010. Effects of three Caribbean cleaner shrimps on ectoparasitic monogeneans in a semi-natural environment. Coral Reefs. 29(2): 419-426.

Militz, T. and K.S. Hutson. 2015. Beyond Symbiosis: Cleaner Shrimp Clean up in Culture. PLOS One. DOI:10.1371/journal.pone.0117723

Militz, T.A., P.C. Southgate, A.G. Carton, K.S. Hutson. 2013. Dietary supplementation of garlic (*Allium sativum*) to prevent monogenean infection in aquaculture. Aquaculture. 409: 95-99.

Militz, T.A., P.C. Southgate, A.G. Carton, K.S. Hutson. 2014. Efficacy of garlic (*Allium sativum*) extract applied as a therapeutic immersion treatment for *Neobenedenia sp.* management in aquaculture. Journal of Fish Diseases. 37(5): 451-461.

Mellen, I.M. 1928. The Treatment of Fish Diseases. Zoopathologica. 2(1): 1-31.

Mellen, I.M. 1931. Fishes in the Home. New York Zoological Society. 178p.

Mellen I.M. and R. Lanier. 1948. 1001 Questions Answered about your Aquarium. Dodd, Mead, & Co. 450p.

Morales-Serna, F.N., M. Chapa-López, J.M. Martínez-Brown, L. Ibarra-Castro, R.M. Medina-Guerrero, and E.J. Fajer-Ávila. 2018. Efficacy of praziquantel and a combination anthelmintic (Adecto®) in bath treatments against *Tagia ecuadori* and *Neobenedenia melleni* (Monogenea), parasites of bullseye puffer fish. Aquaculture. 492: 361-368.

Mueller, K.W., W.O. Wantanabe, and W.D. Head. 1992. Effect of Salinity on Hatching in *Neobenedenia melleni*, a Monogenean Parasite of Seawater-Cultured Tilapia. Journal of the World Aquaculture Society. 23(3): 199-204.

Mueller, K.W., W.O. Wantanabe, and W.D. Head. 1994. Occurrence and Control of *Neobenedenia melleni* (Monogenea: Capsalidae) in Cultured Tropical Marine Fish, Including Three New Host Records. The Progressive Fish Culturist. 56: 140-142.

Muka, S.K. 2014. Portrait of an Outsider: Class, Gender, and the Scientific Career of Ida M. Mellen. Journal of the History of Biology. 47: 29-61.

Hirayama, T., Kawano, F. and Hirazawa, N., 2009. Effect of *Neobenedenia girellae* (Monogenea) infection on host amberjack *Seriola dumerili* (Carangidae). Aquaculture, 288(3-4), pp.159-165.

Ohno, Yasuhiro, F. Kawano, and N. Hirazawa. "The effect of oral antibiotic treatment and freshwater bath treatment on susceptibility to *Neobenedenia girellae* (Monogenea) infection of

amberjack (*Seriola dumerili*) and yellowtail (*S. quinqueradiata*) hosts." Aquaculture 292(3-4): 248-251.

Ogawa, K., M.G. Bondad-Reantaso, M. Fukudome, and H. Wakabayashi. 1995. *Neobenedenia girellae* (Hargis, 1955) Yamaguti, 1963 (Monogenea: Capsalidae) from Cultured Marine Fishes of Japan. Journal of Parasitology. 81(2): 223-227.

Oliveira, B.D.L., R.M. de Souza, J.P.D.S. Filho, H. P. Ribeiro, D.B. Fioresi, L.D.C. Gomes, L.F.L. Fernandes. 2019. Levamisole on the biochemical and hematological responses to parasitism by monogenoids in dog snapper. Ciência Rural de Santa Maria. 49(9): e20190013

Pimentel-Acosta, C.A., F.N. Morales-Serna, M.C. Chavez-Sanchez, H.H. Lara, A. Pestrakov. N. Bogdanchikova, E.J. Fajer-Avila. 2019. Efficacy of silver nanoparticles against the adults and eggs of monogenean parasites of fish. Fish Parasitology. 118(6): 1741-1749.

Platt, T.A. 2017. The Genus *Spirochis* MacCallum, 1918 (Digenea: Schistosomatoidea) and the Early History of Parasitology in the United States. Journal of Parasitology. 103(4): 407-420.

Price, E.W. 1939. North American monogenetic trematodes. III. The family Capsalidae (Capsaloidea). Journal of the Washington Academy of Sciences. 29(2): 63-92.

Rubio-Godoy, M., A. Monteil-Leyva, J.A. Martinez-Harnandez. 2011. Comparative susceptibility of two different genetic types of tilapia to *Neobenedenia sp.*(Monogenea). Diseases of Aquatic Organisms. 93: 171-177.

Sanches, E.G., 2008. Control of *Neobenedenia melleni* (MacCallum, 1927)(Monogenea: Capsalidae) in dusky grouper, *Epinephelus marginatus* (Lowe, 1834), reared in floating net cages. Revista Brasileira de Parasitologia Veterinária, 17(3), pp.145-149.

Schell, S.C. 1970. How to Know the Trematodes. William C. Brown Publishers. 355p.

Sikkel, P.C., Nemeth, D., McCammon, A. and Williams Jr, E.H., 2009. Habitat and species differences in prevalence and intensity of *Neobenedenia melleni* (Monogenea: Capsalidae) on sympatric Caribbean surgeonfishes (Acanthuridae). Journal of Parasitology, 95(1), pp.63-69.

Smith, M., C. Innis, N. Fischer, B. Bailey, S. Bailey, C. Fernald, D. Laughlin, and M. O'Neil. 2018. The Red Queen: Using Hyposalinity to Treat Neobenedenia sp. and *Cryptocaryon irritans* in a Large Multi-Taxa Exhibit. Abstracts of the Regional Aquatics Workshop, Drum and Croaker. 50: 88-89

Strona, G., F. Stefani, and P. Galli. 2008. Field preservation of monogenean parasites for molecular and morphological analysis. Parasitology International. 58(1): 51-54.

Thomas, A., M.R. Dawson, H. Ellis, and M.A. Stamper. 2016. Praziquantel degradation in marine aquarium water. PeerJ. 4:e1875

Thoney, D.A. 1990. The effects of trichlorfon, praziquantel and copper sulphate on various stages of the monogenean *Benedeniella posterocolpa*, a skin parasite of the cownose ray, *Rhinoptera bonasus.* Journal of Fish Diseases. 13: 385-389.

Thoney, D.A. and W.J. Hargis. 1991. Monogenea (Platyhelminthes) as hazards for fish in confinement. Annual Review of Fish Diseases. 91: 133-153.

Tubbs, L.A., and M.D. Tingle. 2006. Bioavailability and pharmacokinetics of a praziquantel bolus in kingfish *Seriola lalandi*. Diseases of Aquatic Organisms. 69:233-238.

Valles-Vega, I., F. Ascensio, T. Sicard-Gonzalez, C. Angulo, E.J. Fajer-Avila, R.B. Inohuye-Riviera, J.C. Perez-Uribola. 2019. Effects of temperature on the life cycle of *Neobenedenia* sp. (Monogenea: Capsalidae) from *Seriola riviolana* (Almaco Jack) in Bahia de la Paz, BCS Mexico. Parasitology Research. 118(12): *in press.*

Van Valen, L. 1973. A New Evolutionary Law. Evolutionary Theory. 1: 1-30.

Williams, R.E., I. Ernst, C.B. Chambers, and I.D. Whittington. 2007. Efficacy of orally administered praziquantel against *Zeuxapta seriolae* and *Benedenia seriolae* (Monogenea) in yellowtail kingfish *Seriola laland*i. Diseases of Aquatic Organisms. *77*(3): 199-205.

Whittington, I.D. and M.A. Horton. 1996. A revision of *Neobenedenia* Yamaguti, 1963 (Monogenea: Capsalidae) including a redescription of *Neobenedenia melleni* (MacCallum, 1927) Yamaguti 1963. Journal of Natural History. 30: 1113-1156.

Whittington, I.D. 2004. The Capsalidae (Monogenea: Monopisthocotylea): a review of diversity, classification, and phylogeny with a note about species complexes. Folia Parasitologica. 51: 109-122.

Whittington, I.D., Deveney, M.R., Morgan, J.A.T., Chisholm, L.A. and Adlard, R.D. 2004. A preliminary phylogenetic analysis of the Capsalidae (Platyhelminthes: Monogenea: Monopisthocotylea) inferred from large subunit rDNA sequences. Parasitology, 128(5): 511-519.

Xuejuan, D., L. Anxing, Z. Jianying, and L. Xianghua. 2003. Studies on the ITS1 Sequences of some Benedeniines (Monogenea: Capsalidae) in Chinese Marine Fishes. Journal of South China Normal University. 03(2): 85-89.

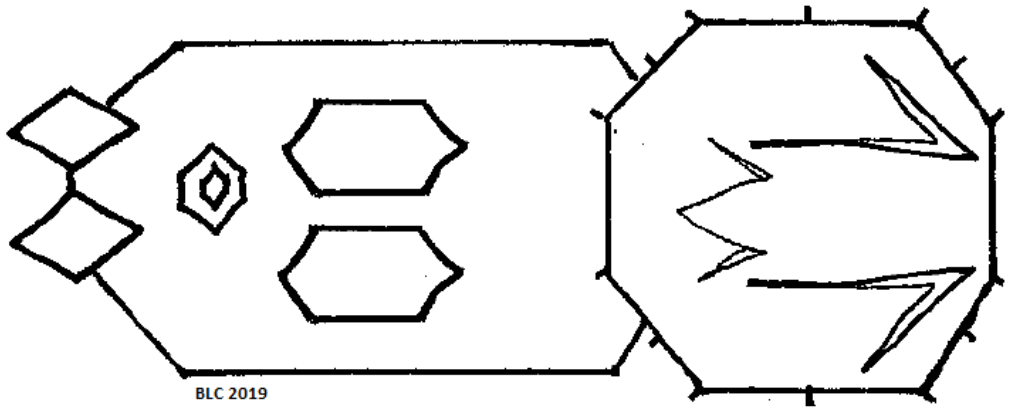
Yamaguti, S. 1963. Systema Helminthium, Volume IV: Monogenea and Aspidocotylea. John Wiley & Sons. 699p.

Yang, L., L. Li-Wei, L. Shi, J Wang, and Y. Su. 2001. Study on aetiology of *Neobenedenia melleni* (Monogenea: Capsalidae) from Marine fishes In Fujian, China. Journal of Oceanography in Taiwan Strait. 01(2): 205-209.

Yamamoto S, S. Shirakashi, S. Morimoto, K. Ishimaru, O. Murata. 2011. Efficacy of oral praziquantel treatment against the skin fluke infection of cultured chub mackerel, *Scomber japonicus*. Aquaculture. 319(1-2):53-7.

Yamamoto, S., Fukushima, A., Ishimaru, K. and Shirakashi, S., 2014. Shading of net cage is an effective control measure against skin fluke *Neobenedenia girellae* infection in chub mackerel *Scomber japonicus*. Fisheries science, 80(5): 1021-1026.

Zhang, J., X. Wu, Y. Li, M. Zhao, M. Xie, A. Li. 2014. The complete mitochondrial genome of *Neobenedenia melleni* (Platyhelminthes: Monogenea): mitochondrial gene content, arrangement and composition compared with two Benedenia species. *Molecular Biology Report 41*(10): 6583-6589.



Avaginate Wonderbeast (a haiku) B.L. Christie

kill fish without heed
Neobenedenia
magnificent beast

The Conqueror Avagniate Worm – J.W. Foster IV (with apologies to Edgar Allan Poe)

Lo! ’t is a capsalid blight
Upon the lonesome fish these years!
An angelfish, befinned and bright
Flashing, and swimming in its tears,
Set in an aquarium, you see
A play of hopes and fears,
While the fishes retire fitfully
In boxes, cylinders and spheres.

Cleaners, in the form of wrasses try,
To pluck while tankmates flow,
And hither and thither do they fly—
Mere platters they, who come and go
At bidding of the nature of things
That shift the balance to and fro,
Suctioning, while their hamuli stings
Nearly Invisible Wo!

That motley drama—oh, be sure
It shall not be forgot!
With its mucous supped upon evermore
By a crowd that it sees not,
Through a cycle of life that ever returneth in
To the self-same teleost,
And much of dermis, and more of fin,
Such great physiological cost.

But see, upon the ocean trout,
A crawling shape intrude!
A colorless thing that writhes from out
The aquatic solitude!
It writhes!—it writhes!—with mortal pangs
The trout become its food,
And aquarists sob at vermin fangs
And fishy gore imbued.

Out—turn out the lights—out all!
Cannot help but to pity,
The hosts, serve yet as mating stall,
Home to great fecundity,
Moribund, the angels, a cure their wish,
Their tissues taken by logarithmic storm
That this exhibit is the tragedy, “Fish,”
Infected by the Conqueror Avaginate Worm.

TRENDS IN AQUARIUM OPENINGS AND CLOSINGS IN NORTH AMERICA: 1856 TO 2020

Pete Mohan, petemohan55@gmail.com

Editor, Drum and Croaker

This article began as a simple search for significant milestones in public aquarium history that were related to the creation of *Drum and Croaker (D&C)* and the Regional Aquatics Workshop (RAW). My goal was to identify potential changes or developments in the community that might have stimulated the need for the periodical in 1957-1958 and the conference in 1989. This led to investigations of other old aquarium meetings and their origins. As I searched for stimuli promoting networking, I needed to examine growth in the public aquarium community. This became a slippery slope leading to an ever-expanding list of public aquarium openings and closings (Appendix 1).

I focused on the public aquariums of North America because of my personal interest in both RAW and *D&C*, which are both based in this region. I was one of the founding members of the former in 1989. I've been editing the latter since 1993, when RAW attendees agreed that it was needed again. *D&C* had been dormant or issued in an abbreviated format for much of the previous decade.

The Need for Communication

In 1992 an Associated Press article described that era as "the spawning of the Age of Aquariums," a riff on a line from the opening song from the 1967 musical, *Hair*. Note the juxtaposition of this date, and those associated with the beginning of RAW, and the relaunch of *D&C*. This period saw the opening of many major modern aquariums. As a result, there was an exodus of experienced staff from older or smaller facilities resulting in a loss of "institutional memory." At the same time there was a new emphasis on ground-breaking husbandry research and breeding programs. A number of retirements and promotions (out of husbandry duties) also deprived the community of the generation that had led the aquarium community in the 1950s and 1960s. All of these phenomena required that younger curators and aquarists communicate more for both professional and technical training. The RAW concept was enthusiastically embraced and *D&C* responded to CPR.

As it turns out, the need for formal communication was nothing new. In the 1800s and early 1900s, curators and directors with scientific interests gathered at various zoological society meetings. They also worked locally with supportive enthusiast societies. Professional organizations for the public aquarium community came later, including the American Association of Zoology Parks and Aquariums (AAZPA), founded in 1924. It appears that there was limited participation by aquariums at these early AAZPA meetings. However, not unlike events of the 1980s and 1990s, a pulse of new construction in the 1950s likely led to the formation of the Annual Aquarium Symposium. This was held in association with the American Association of Ichthyologists and Herpetologists (ASIH) conferences beginning in 1955. The presentation topics and importance of using social events to stimulate the exchange of information foreshadowed RAW, and members of this group also began publishing *D&C* as a mimeographed newsletter in

1958. The Aquarium Symposia met with ASIH through 1970. In 1971 the group gathered at the AAZPA meeting in Salt Lake City and agreed to merge its sessions with future AAZPA meetings. AAZPA was later rebranded as AZA (the Association of Zoos and Aquariums).

While not the focus of this article, it is important to mention that many international groups were continuing to evolve as well. Notably, the first "public aquarium" anywhere was the "Fish House" at the London Zoo, opening in 1853. It was not long before this "spawned" new facilities throughout Europe and North America. The current World Association of Zoos and Aquariums (WAZA) evolved from the International Union of Directors of Zoological Gardens (IUDZG) which was originally founded in 1935, ceased to exist during World War 2, and was reestablished in 1946. *D&C* travel reports from the 1960s and 1970s confirm that mutual cross-pollination was occurring across both the Atlantic and Pacific oceans. International Meetings on Aquariology were held in Monaco in 1960, evidence that the uptick in communication seen in the US was not unique. This gathering would become known as the first International Aquarium Congress (IAC). As AAZPA became an independently chartered organization in 1972, the European Union of Aquarium Curators (EUAC) formed at the Basel Zoo in Switzerland, also the birth place of the original IUDZG. The European Association of Zoos and Aquaria (EAZA), an analog of AAZPA/AZA, was founded in 1992, again paralleling the increased networking in North America. Many other regional zoo associations now exist globally. Most recently, the National Aquarium Workshop (NAW), created along the RAW model by some Brits that had joined us from "across the pond," began meeting in 1999 for facilities in the UK and Ireland.

Trends in Public Aquarium Construction in North America

Data Limitations

While I have cast a wide net, I have likely not created a full list of all aquariums that subsequently opened and closed in the 19th or early 20th centuries. The internet was a major source of data and it has its limits. There are probably a number of zoos that opened, closed or replaced small aquarium exhibits in this era, but not every facility wants to advertise things it has torn down on its home or wiki pages. Some zoos may have had small aquarium features before opening the formal aquariums reflected herein and these are rarely reported in the histories available online. Some aquariums closed and no convenient record exists for that date (such as for the Ocean Life Park Aquarium in San Juan, Puerto Rico).

Some small aquariums are probably just lost to time and will only be found documented in the archives of local historical societies. For example, there was apparently an attraction called "Davey Jones' Locker" on Catalina Island (CA) back in the 1950s or 1960s. There is little evidence online other than a pair of intriguing photos of exhibits facing out of the front windows of the small dockside structure.

A number of aquariums have closed for periods of time because of reconstruction or oversight issues. Recent examples include the Belle Isle Aquarium (2005-2012) and Wonders of Wildlife (2007-2017). Some institutions such as Steinhart, Scripps, and the National Aquarium in DC have occupied multiple structures. Other institutions have phased out old aquarium buildings years after constructing additional new ones on their campuses (Columbus Zoo and Aquarium). Closure of individual buildings at zoos is not considered a "closing" unless no new

true aquarium buildings are present or built later. Gaps in operation or overlaps of new and demolished buildings are generally not reflected in my data for aquarium longevity.

I have also intentionally excluded most facilities that contain aquaria, but were primarily designed for other purposes. Restaurant chains and smaller nature centers largely fill this category. I've attempted to include marine science centers if they have recognized aquarium components, but I'm sure I've missed a few. I used Wikipedia's various lists of aquariums, other "wiki" pages, information from archived *D&C* issues, AZA's member list, historic tourism blogs, etc. to flesh out the list provided in this article. Where conflicting data was found in multiple sources, I made my best educated guess based on the provenance of the data.

With one exception, I have not reflected small aquariums may have been built in some of the numerous amusement parks serviced by early train and trolley lines in many metropolitan areas. The local Silver Lake Amusement Park featured the first aquarium in Ohio (Figure 1). When the park closed in 1917 the building was dismantled, moved about a mile up the road (1920),

Figure 1. The Aquarium at Silver Lake Amusement Park, (now Silver Lake, OH). Photo courtesy of, and with permissions by, the Akron Beacon Journal. This and other photos of the park may be Viewed on the "Summit Memory" website, which is administered by the Akron-Summit County Public Library. (https://www.summitmemory.org/digital/collection/ABJarchives/id/6416/rec/1). The rough similarity in construction to the New York Aquarium in Figure 2 is not a coincidence.

and reassembled as a church. The structure remains in use, so it might technically be the oldest remaining aquarium building in the continental United States, other than Castle Clinton, the site of the original 1896 New York Aquarium in Battery Park. Ironically, the tiny Silver Lake Park Aquarium was said to have been modeled on this much larger round building (Figure 2).

Changing Styles of Aquariums

Aquariums have always attracted visitors interested in oddities. In the early days this meant any fishy menagerie behind glass. It is not surprising that the first American facilities were operated by P. T. Barnum (of circus fame) in New York City and Boston in 1856 and 1859. Both were short-lived. The New York location was part of his American Museum and was destroyed by fire in 1865. Boston's Aquarial Gardens was moved in 1860, sold to Barnum in 1862, and closed the following year. The animals moved to his ill-fated New York location. What could be worse? In France the collection of the Jardin Zoologique d'Acclimatation was rumored to have been eaten during the 1870-71 Siege of Paris.

After the failure of Barnum's operations, the aquariums that opened in major cities over the next 60 years were typically associated with cultural, government, or academic institutions. In 1873 Woodward's Gardens Aquarium (San Francisco, CA) and the first iteration of the National Aquarium both opened. The first Woods Hole Science Aquarium opened in 1885, followed a decade later by the original New York Aquarium (Figure 2). The first edition of the Scripps Aquarium opened in 1903, followed by the Venice (CA) Aquarium/Marine Biological School in 1909, the Philadelphia Aquarium in 1911, the first iteration of the Steinhart Aquarium in 1923, and the John G. Shedd Aquarium in 1929. Of these, only the Shedd continues to operate in its original building. Many of the others continue in newer structures.

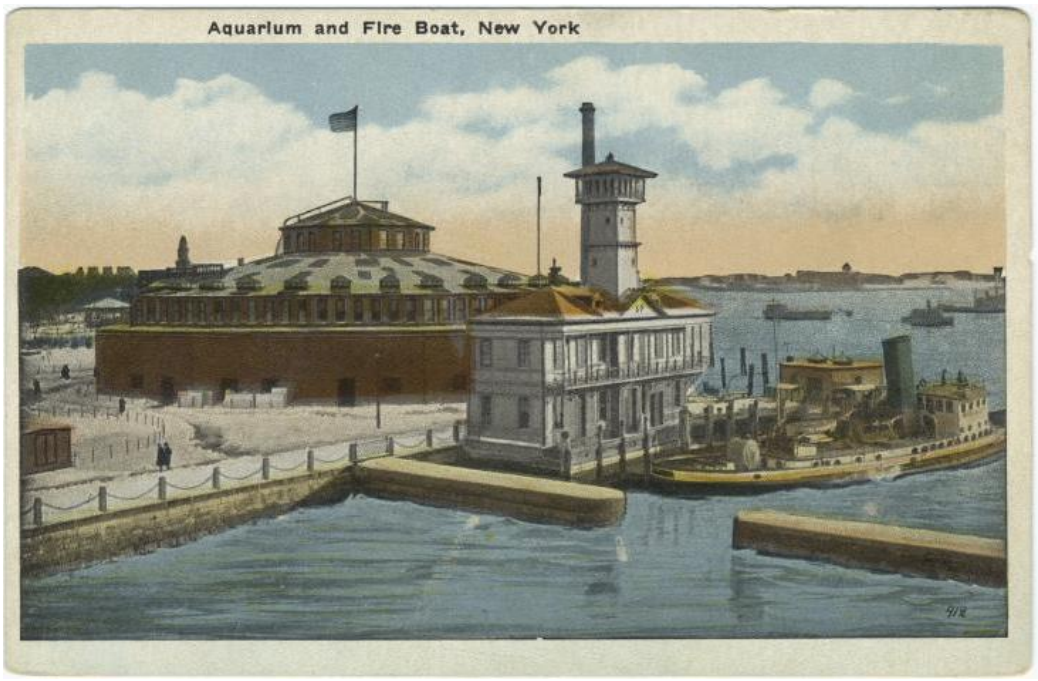


Figure 2. The New York Aquarium at Battery Park "before 1923". Postcard. Wikimedia Commons.

Between 1930 and 1954, most new facilities were stand-alone city or zoo aquariums located in Texas, California and Ohio. While some major aquariums of this type continued to open over the next decade (Vancouver Aquarium and New York's replacement aquarium), for-profit, tourist destinations began to dominate new construction in the mid-1950s. Many of these were oceanariums with increased focus on marine mammal exhibitions. Marineland of the Pacific led the way in 1954, followed by the Miami Seaquarium and Gulfarium in 1955. Additional facilities included Marineland of Canada (1961), Aquarama (1962) the first Sea World in San Diego (1964), Sea Life Park (1964) and Sea-Arama, (1965). Additional oceanariums opened later, including three more Sea World parks (NE Ohio, Orlando, San Antonio), Marineland and Marine World (CA), Sealand of the Pacific, the Mystic Aquarium and a few smaller locations.

Charlie White's Undersea Gardens attractions were a unique series of at least four aquarium barges moored along the west coast starting between 1964 and 1966. Each installation consisted of a barge with underwater viewing, floating within a containment structure that served as the main aquarium. They were located in Victoria, BC (Figure 3), Seattle, WA, Crescent City, CA and Santa Barbara, CA. Some or all were built in Seattle and two remained in operation until recently. The New England Aquarium borrowed the barge idea a decade later to create Discovery, a floating, 1,000-seat marine mammal stadium (decommissioned in 2004).

Figure 3. Pacific Undersea Gardens, Victoria BC, 2011, Richard Eriksson, Flickr.

Oceanariums and oceanarium additions to existing aquariums continued to be built through the late 1980s and early 1990s when the Indianapolis Zoo, National Aquarium in Baltimore and Shedd Aquarium opened their marine mammal expansion projects, largely ending the active phase in a 35-year trend in the construction of cetacean stadiums in North America.

During this "oceanarium era" other types of venues continued to be built. A number of marine science centers began to open in the 1970s and 1980s as well as a trio of North Carolina Aquariums (1976) that were later dramatically expanded.

In 1969 the opening of the New England Aquarium signaled the beginning of a new wave of modern aquarium development. The facility was designed by Cambridge Seven Associates who followed up over the years with major facilities in Baltimore, MD (1981), and Chattanooga, TN (1992). Other large, mostly non-profit facilities have continued to open regularly until the present day, but the peak in construction was during the early to mid-1990s. These are sometimes collectively known as "big box" aquariums. Often created to successfully revitalize tired downtown areas, many initially struggled to meet optimistic budget forecasts. Most adapted over the years, while a couple were rescued and returned to health under new management.

The construction of the first Ripley's Aquarium in Myrtle Beach, SC (1997) demonstrated that new business models for private for-profit aquariums were feasible. Ripley's opened their second, larger facility in Gatlinburg, TN in 2000, and a third in Toronto in 2013. In the meantime, Herschend Family Entertainment had constructed the Newport Aquarium near Cincinnati (1999). They later acquired the struggling New Jersey State Aquarium and relaunched it as the Adventure Aquarium in 2005. Landry's created the Downtown Aquarium in Houston in 2003. They also rescued a financially-exhausted facility, Colorado's Ocean Journey (Denver, CO), and reopened it as their second Downtown Aquarium in 2005. Ripley's, Herschend, and Landry's all have operated larger facilities and have been particular about locating these in markets that would support aquariums of those sizes. In 2008 the first U.S. Sea Life Aquarium opened in Carlsbad, CA. Supported by a large international network of aquariums, they quickly added these somewhat smaller aquariums in Tempe, AZ (2010), Grapevine, TX (2011) and Kansas City, MO (2012). They have continued to open new aquariums every one to five years. All four of the previous companies value their participation in the AZA and work hard to meet those husbandry and operational standards. They have hired staff from the existing public aquarium community and are active in our community.

While new major aquariums, facility expansions, and marine science centers continue to open, the latest wave in new aquarium construction seems to be dominated by new companies specializing in even smaller venues with lots of guest interaction. Many of these aquariums are now opening annually. Some have faced controversy. As of this writing, they are not members of either AZA or ZAA, and their participation in other professional communication resources has so far been limited.

Trends in Openings and Closings

Between 1850 and 1940, a trickle of new North American aquariums opened each decade. Unsurprisingly, no openings occurred during the World Wars, but a couple of facilities closed, one due to decreased rail access caused by WW1. After WW2 there was a brief pulse of construction, then another lull during the Korean War. The first aquarium boom period was 1954 to 1970, followed by a short lull in the early 1970s. The 1980s and 1990s were very busy, with construction at double the levels seen during the first boom. There was another lull in the first decade of this century, roughly coinciding with the economic crash of 2008. While there were only two openings in 2009-2010, the recovery period has been frenetic. New construction exploded in 2011 and has

continued to the present (including announced openings in 2020). Post-recession, most new facilities are smaller and associated with both new and established aquarium chains. A histogram of aquarium opening by half-decade is presented in Figure 4.

Overall, aquarium closing years track with the second wave of aquarium openings, both increasing beginning in the 1970s. This may reflect an impact of the construction of many "big box" stand-alone aquariums on the attendance at older, smaller aquariums lacking large shark or mammal exhibits. The pattern of closings as they relate to opening years has been somewhat random except for an anomaly surrounding facilities that opened between 1954 and 1972, during the first big wave of construction (Figure 5). Eighteen aquarium facilities that opened in this interval closed after 5 to 58 years of operation. Many were early oceanariums or structurally ephemeral attractions such as the floating Undersea Gardens barges. "City" aquariums in Cleveland, Seattle, and Montreal were also shuttered, but were replaced by new facilities under different organizations.

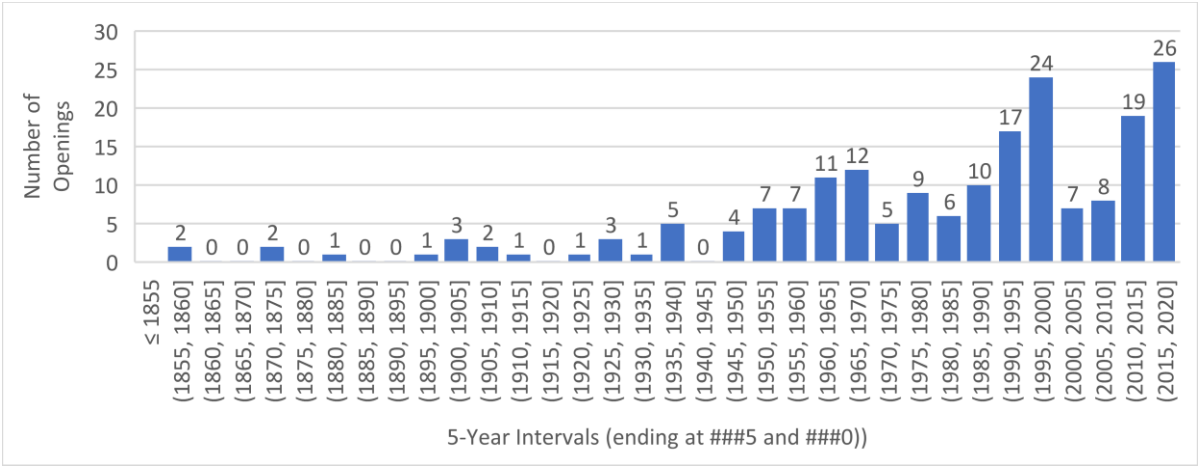


Figure 4. Number of aquarium opening per 5 year interval between 1855 and 2020.

A few aquariums were extremely short-lived. As noted earlier, Boston's Aquarial Gardens closed after 4 years. Two early facilities in Seattle lasted less than a decade. That Undersea Gardens location only survived 5 years and the first Seattle Aquarium closed after 7 years. Aquarama in Philadelphia was also shuttered after 7 years. On the other end of the spectrum, the original National Aquarium (Washington, DC) survived 140 years (as an institution) in a number of locations, although it's first site was far away in Woods Hole, MA, predating the Woods Hole Science Aquarium. One could argue that it still exists, as the unrelated National Aquarium in Baltimore assumed an operational role in 2003. The DC location closed in 2013 and the Baltimore facility has dropped "in Baltimore" from its name. I've chosen to separate the two institutions in my data file as they each deserve an entry.

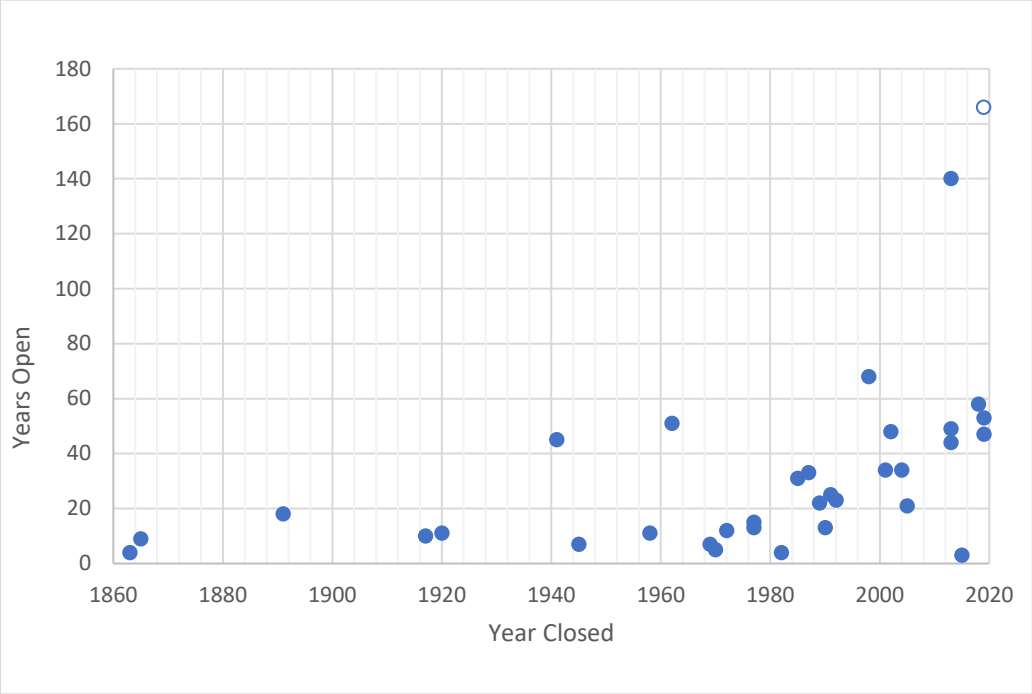


Figure 5. Number of years North American aquarium facilities remained open vs the year they closed permanently. I have included the London Zoo's Aquarium (○) for interest, as it was the first public aquarium to open anywhere and one of the last to close as of 2020.

Future Trends

It is unclear what lies ahead, but you don't need a crystal ball to make some logical assumptions. It seems obvious that we will continue to see the construction of the occasional large aquarium in major cities, and marine science centers in coastal towns. It is a given that existing coastal facilities will face accelerated challenges from hurricanes and rising sea level. Damaged seaside aquariums may need to be reconstructed to new standards, moved to secure inland locations, or closed.

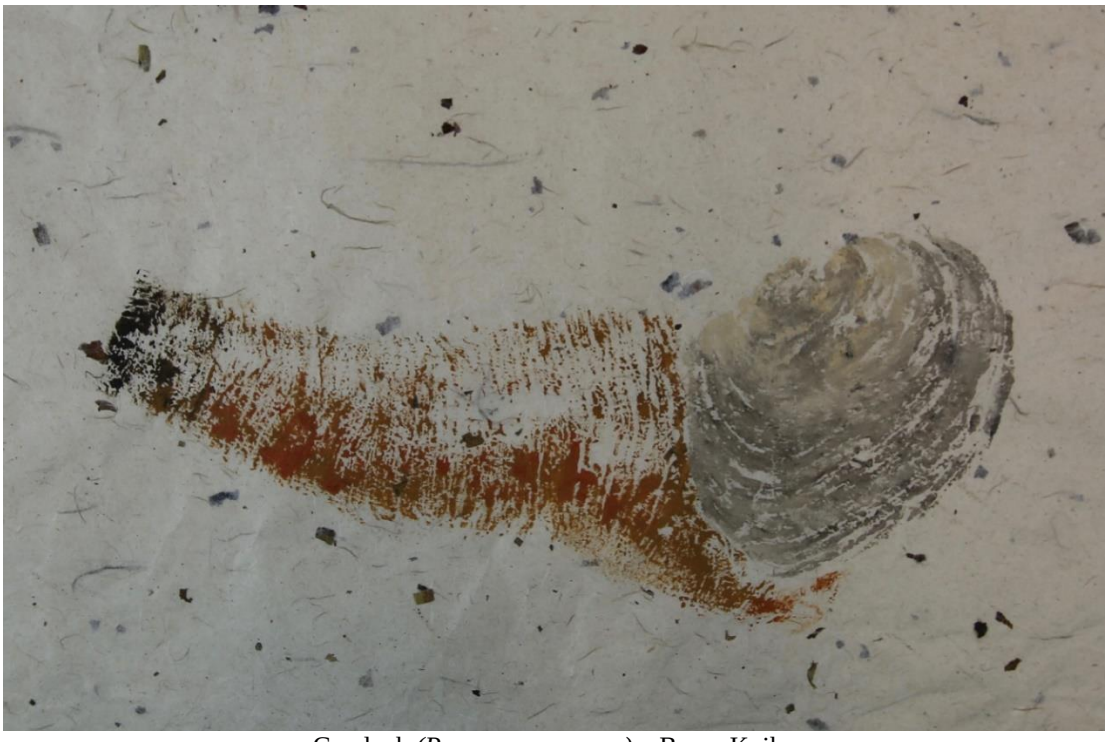
The explosion of small aquariums in suburban shopping areas will probably progress for some time. Some limiting factors on new construction of these and all other types of aquariums could come into play in the next decades. The scope, mission and vision of each aquarium, and how they respond (or fail to respond) to pressures from both the public and zoo/aquarium associations will impact attendance. There may be competition for visitors in metropolitan areas where they can choose among increasing numbers of varied aquarium opportunities with very different missions. Historically there have been a number of cities where two aquariums have co-existed. How many is too many? We will know soon.

The balance point between education and entertainment will continue to move in different directions over time (perhaps at the same time), as it always has. What will be the correct mixes that will have value to future visitors? What will be the rewards or risks associated with embracing or dodging the highest community standards for infrastructure, animal acquisition, and animal welfare?

Major aquariums will continue to be focused on sustainability. Conservation-minded facilities are already choosing short supply chain sources of marine life (aquaculture and known sustainable collectors). Will those continuing to rely heavily on long chain suppliers of marine species face increasing criticism, or will they simply serve a different customer base that is unaware or unconcerned about this issue? Declining wild stocks of charismatic taxa such as certain groups of elasmobranchs may lead to changes in the species composition of large aquarium exhibits. Institutions that support successful research in captive breeding will be more likely to house such species. If restrictions arise for the sustainable collection of marine species, only those institutions that can participate in research into captive breeding may have access to certain species.

I think one thing is certain. Television, virtual reality or other future audio-visual technologies will never fully replace the experience of observing a live marine animal in the flesh. While pressures on wild communities may lead to some reduction in the diversity of fishes in public aquariums, travel opportunities to see marine life in the wild may also become more difficult for the average citizen as pristine areas shrink, disappear, or become less and less accessible. Aquariums will continue to serve as ambassadors for ocean conservation because they create an authentic, in-person connection that is affordable for the average family.

Appendix continued on next page



Geoduck *(Panopea generosa)*. Bruce Koike

Appendix 1. North American Aquarium Openings and Closings from 1956 to 2020.

Year Opened	Institution Name	Year Closed	Years in Operation if Permanently Closed	Years in Operation if Still Open in 2020
1853	London Zoo Fish House, UK. (first public aquarium)	2019	166	
1856	P.T. Barnum's "American Museum" Aquarium, NY.	1865	9	
1859	Boston Aquarial Gardens, MA. (became second Barnum site)	1863	4	
1873	Woodward's Gardens Aquarium, San Francisco, CA.	1891	18	
1873	National Aquarium of Washington, DC.	2013	140	
1885	Woods Hole Science Aquarium, MA.			135
1896	New York Aquarium (Castle Garden in Battery Park), NY.	1941	45	
1903	Scripps Aquarium at La Jolla, CA.			117
1904	Belle Isle Aquarium, MI.			116
1904	Waikiki Aquarium, HI.			116
1907	Silver Lake Amusement Park Aquarium, OH.	1917	10	
1909	Venice Aquarium, CA.	1920	11	
1911	Philadelphia Aquarium, PA.	1962	51	
1923	Steinhart Aquarium, CA.			97
1926	Bermuda Aquarium, Museum, and Zoo			94
1929	Shedd Aquarium, IL.			91
1930	Depoe Bay Aquarium, OR.	1998	68	
1935	Cabrillo Marine Aquarium			85
1936	Tacoma Aquarium, WA.			84
1936	Dallas Aquarium at Fairpark, TX. (Children's Aquarium)			84
1937	Seaside Aquarium, OR.			83
1938	Pier 3 Aquarium / Pier 54 Aquarium (first Seattle Aquarium, WA).	1945	7	
1939	Toledo Zoo's Aquarium, OH.			81
1946	Key West Aquarium, FL.			74
1947	Hermosa Beach Aquarium, CA.	1958	11	
1948	San Antonio Zoo Aquarium, TX.			72
1950	Bo Ginn National Fish Hatchery and Aquarium, GA.			70

Year Opened	Institution Name	Year Closed	Years in Operation if Permanently Closed	Years in Operation if Still Open in 2020
1954	James R. Record Aquarium at the Fort Worth Zoo, TX.	2002	48	
1954	Marineland of the Pacific, Palos Verdes, CA.	1987	33	
1954	Cleveland Aquarium (Gordon Park), OH.	1985	31	
1954	Columbus Zoo Aquariums, OH.			66
1955	Miami Seaquarium, FL.			65
1955	Gulfarium, FL.			65
1955	Westport Aquarium, WA.			65
1956	Marine Life Oceanarium, MS.			64
1957	New York Aquarium (Coney Island), NY.			63
1959	Aquarium du Québec, QC.			61
1959	Memphis Zoo Aquarium, TN.			61
1960	Calgary Brewery Aquarium	1972	12	
1960	Morro Bay Aquarium, CA.	2018	58	
1960	Gavins Point National Fish Hatchery, SD.			60
1961	Marineland of Canada, ON.			59
1962	Seattle Marine Aquarium (Pier 56, second Seattle Aquarium)	1977	15	
1962	Aquarama, PA.	1969	7	58
1964	Undersea Gardens, Victoria, BC. (Pacific Undersea Gardens)	2013	49	
1964	Aquatarium, FL.	1977	13	
1964	Sea World San Diego, CA.			56
1964	Under Sea Gardens, Crescent City, CA.			56
1964	Sea Life Park, HI.			56
1965	Undersea Gardens, Seattle, WA	1970	5	
1965	Sea-Arama Marineworld, TX.	1990	25	
1965	Aquarium of Niagara Falls, NY.			55
1966	Undersea Gardens, Newport, OR.	2019	53	
1966	Montreal Aquarium, QC.	1991	25	
1966	Undersea Gardens, Santa Barbara, CA.	?		54
1967	Oceana Aquarium at Cedar Point, OH.	2001	34	
1967	Seafloor Aquarium, Nassau, Bahamas.	1989	22	

Year Opened	Institution Name	Year Closed	Years in Operation if Permanently Closed	Years in Operation if Still Open in 2020
1967	Pittsburgh AquaZoo, PA.			53
1968	Marine World, CA.			52
1969	Sealand of the Pacific, BC.	1992	23	
1969	Aquarium of Cape Cod, MA.	2013	44	
1969	New England Aquarium, MA.			51
1970	Sea World of Ohio	2004	34	
1970	Gulf World, FL.			50
1971	Ocean Life Park Aquarium, PR	?	?	
1972	Mount Desert Oceanarium, ME.	2019	47	
1972	Huntsman Marine Science Centre, St. Andrews, New Brunswick.			48
1973	Mystic Aquarium, CT.			47
1973	Sea World of Florida			47
1976	North Carolina Aquarium, Roanoke.			44
1976	North Carolina Aquarium, Ft. Fisher.			44
1976	North Carolina Aquarium, Pine Knoll Shores.			44
1977	Seattle Aquarium (Pier 59, third city aquarium), WA.			43
1978	Sea World Shark Institute, FL.	1982	4	
1978	Minnesota Zoo Aquarium (first of multiple projects)			42
1980	Marine Science Center at Mote Marine Lab (Mote Aquarium).			40
1980	Kipp Aquarium at Houston Zoo, TX.			40
1980	Roundhouse Aquarium, CA.			40
1981	National Aquarium in Baltimore, MD.			39
1981	Clearwater Marine Aquarium, FL.			39
1982	New Brunswick Aquarium and Marine Center, Shippagan.			38
1982	Cold Spring Harbor Fish Hatchery & Aquarium, NY.			38
1984	J.L. Scott Marine Education Center and Aquarium, MS.	2005	21	
1984	Monterey Bay Aquarium, CA.			36
1986	The Living Seas, Disney/Epcot, FL.			34
1986	Virginia Marine Sci Ctr (Virginia Aquarium)			34
1987	Sea Center, Santa Barbara Museum of Natural History, CA.			33
1988	Maritime Center opens in Norwalk, CT. (Maritime Aquarium)			32
1988	Pier Aquarium, FL.			32
1988	Indianapolis Zoo's Oceans Aquarium, IN.			32
1988	Indianapolis Zoo Aquarium and Oceanarium, IN.			32
1990	Aquarium of the Americas, LA.			30
1990	Tarpon Springs Aquarium, FL.			30
1990	Gulf Specimen Marine Aquarium, FL.			30
1991	Jenkinson's Aquarium, NJ.			29
1992	Seacoast Science Center, NH.			28
1992	Acuario de Veracruz, Mexico.			28
1992	Tennessee Aquarium			28
1992	Montreal Biodome, QC			28
1992	New Jersey State Aquarium (Adventure Aquarium)			28
1992	Oregon Coast Aquarium			28
1992	Texas State Aquarium			28
1992	Dallas World Aquarium. TX.			28
1992	Calvert Marine Museum's Estuarium, MD.			28
1993	World Aquarium, MO.			27
1994	Nauticus, Norfolk, VA.			26
1995	Florida Aquarium			25
1995	Henry Doorly Zoo, Scott Kingdom of the Seas Aquarium, NE.			25
1995	Maine State Aquarium			25
1995	Living Shores Aquarium, John Ball Zoo, Grand Rapids, MI. Replaced an older aquarium.			25
1996	Underwater World, CA.			24
1996	Sea Center Texas			24
1996	Santa Monica Pier Aquarium, CA.			24
1996	Underwater World, Mall of the Americas, MN.			24
1997	Maria Mitchell Aquarium, MA.			23

Year Opened	Institution Name	Year Closed	Years in Operation if Permanently Closed	Years in Operation if Still Open in 2020
1997	Hatfield Marine Science Center, OR.			23
1997	Ripleys Aquarium, SC.			23
1998	Aquarium of the Pacific, CA.			22
1998	Maui Ocean Center, HI.			22
1998	Estuarium at Dauphin Island Sea Lab, AL.			22
1998	Alaska Sealife Center			22
1998	McWane Science Center, AL.			22
1999	Newport Aquarium, KY.			21
1999	Atlantic City Aquarium, NJ.			21
1999	Mississippi Museum of Natural Science			21
1999	Colorado's Ocean Journey (Downtown Aquarium, Denver)			21
1999	Moody Gardens Aquarium Pyramid, TX.			21
2000	Great Lakes Aquarium, MN			20
2000	Seymour Marine Discovery Center, CA.			20
2000	South Carolina Aquarium			20
2000	Ripley's Aquarium of the Smokies, TN.			20
2000	Shark Reef at Mandalay Bay, NV.			20
2000	Discovery Cove, Orlando, FL.			20
2000	Atlantis Marine World, NY. (Long Island Aquarium)			20
2001	Wonders of Wildlife Museum & Aquarium.			19
2003	National Mississippi River Museum & Aquarium, IA.			17
2003	ECHO, Leahy Center for Lake Champlain, VT.			17
2003	Downtown Aquarium, Houston, TX.			17
2004	Loveland Living Planet Aquarium, UT.			16
2004	Flint River Aquarium, GA.			16
2005	Georgia Aquarium			15
2006	Central Coast Aquarium, CA.			14
2006	Reiman Aquarium at Discovery World, WI.			14
2007	Sitka Sound Science Center, AK.			13
2008	Sea Life Aquarium at Legoland California Resort, CA.			12
2008	Aquarium at Wildlife World Zoo, AZ.			12
2008	MaST Center Aquarium, Des Moines, WA.			12
2009	Shaw Centre for the Salish Sea, BC.			11
2010	Sea Life Arizona, AZ.			10
2011	Sea Life Grapevine, TX.			9
2011	Idaho Aquarium.			9
2011	Aquarium and Shark Lab by TeamECCO, NC.			9
2011	Butterfly House and Aquarium, SD.			9
2012	Portland Aquarium, OR.	2015	3	
2012	Greater Cleveland Aquarium (Flats), OH.			8
2012	Sea Life Kansas City, MO.			8
2012	South Florida Science Center and Aquarium, FL.			8
2012	Peoria Riverfront Museum, IL.			8
2012	Ucluelet Aquarium, BC.			8
2013	Discovery Passage Aquarium, BC.			7
2013	Ripley's Aquarium of Canada, ON.			7
2013	Greensboro Science Center's Wiseman Aquarium, SC.			7
2013	Austin Aquarium, TX.			7
2014	Sea Life Charlotte, NC.			6
2014	San Antonio Aquarium, TX.			6
2014	Acuario Inbursa, Mexico City.			6
2014	Florida Keys Aquarium Encounters, FL.			6
2015	Sea Life Michigan.			5
2016	OdySea Aquarium, AZ.			4
2016	Alberni Aquarium and Stewardship Centre, BC.			4
2016	Via Aquarium, Schenectady, NY.			4
2016	SeaQuest Las Vegas, NV.			4
2016	SeaQuest Layton, UT.			4
2017	Nicholas Sonntag Marine Education Centre, BC.			3

Year Opened	Institution Name	Year Closed	Years in Operation if Permanently Closed	Years in Operation if Still Open in 2020
2017	Shreveport Aquarium, LA.			3
2017	Acuario Michin, Guadalajara, Mexico.			3
2017	Philip and Patricia Frost Museum of Science, FL.			3
2017	SeaQuest Fort Worth, TX.			3
2017	East Idaho Aquarium, ID.			3
2018	SeaQuest Littleton, CO.			2
2018	Electric City Aquarium & Reptile Den, PA.			2
2018	SeaQuest Folsom, CA.			2
2019	St Louis Aquarium at Union Station, MO.			1
2019	SeaQuest Fort Lauderdale, FL.			1
2019	SeaQuest Roseville, MN.			1
2019	SeaQuest Lynchburg, VA.			1
2019	SeaQuest Woodbridge, NJ.			1
2019	Blue Zoo Spokane, WA.			1
2020	Mississippi Aquarium, MS.			0
2020	Sea Life East Rutherford, NJ.			0
2020	Sea Life San Antonio, TX.			0
2020	Blue Zoo Oklahoma City, OK.			0
2020	7 Seas Aquarium, TX.			0
2020	Aquarium at the Boardwalk, MO.			0

Butter clam *(Saxidomus giganteus)*. Bruce Koike

Daphnia CULTURE MADE SIMPLE

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Editor's Note: Doug was the Curator of Fishes at the Belle Isle Aquarium in Detroit, MI. This article is based on experiments done at home in the early 1990s, and later incorporated into standard practices at the aquarium. It has been shared with various hobbyist newsletters, pamphlets, and web pages since the early or mid-2000s.

Instructions for Maintaining Daphnia Cultures

Daphnia are one of the finest and most universally accepted live foods for most fish. Many fish species can be kept in excellent physical condition by feeding live *Daphnia* to them several times per week. *Daphnia* are extremely effective at bringing many fish into spawning condition. This is especially true for cyprinids (carps and minnows) like goldfish, barbs, danios, etc.

Daphnia can be cultured in just about any container that holds water and is non-toxic. Ideally, aquariums should be used, especially for the beginner since you can keep better track visually on the culture's progress, how much to feed, etc. Once you gain experience, other containers may be used such as Rubbermaid tubs or trash cans. The minimum size container recommended is 20 gallons although small quantities may be reared in smaller tanks.

Daphnia prefer cool water. Temperatures between 65 and 70 degrees are about ideal. So, it is best to culture *Daphnia* in your basement or other cool locations around your house. At temperatures above 75 degrees, *Daphnia magna* begins to slow in production. Cultures will survive at warmer temperatures but do not expect much from them during warmest months of summer.

Daphnia Diets

Daphnia cultures can be fed one or several of the following feeds:

1. Spirulina algae (available through aquaculture supply companies and health food stores).
2. Chlorella algae (available at health food stores.)
3. Green water (containing algae like *Ankistrodesmus* and *Scenedesmus* spp.).
4. Microfeast (larval shrimp feed available from aquaculture supply companies).
5. Active baker's yeast (available from wholesale bakery supply stores).
6. Artificial Plankton Rotifer (larval fish feed available from aquaculture supply companies).
7. Powdered split pea soup mix (from a bulk food store).
8. Whole wheat flour (from a bulk food store).
9. Doug's mix.
    a. 1 part soy flour.
    b. 3 parts whole wheat four.
    c. 1 part dry split peas (finely ground to a flour like consistency).
    d. 3 parts baker's active yeast.

e. 1 part paprika.
f. 2 parts dried chlorella algae.

My experiments have indicated that *Chlorella* algae and the active baker's yeast to be the easiest and most effective feeds to work with when culturing.

The split pea soup and whole wheat and soy flour feeds are mixed with powdered paprika (about one part paprika to ten parts soup mix or flour). The paprika is used as a color enhancer for you fish. I have worked out a generalized quantity of feed for *Daphnia* cultures. This quantity is listed in the following chart (Table 1).

Table 1. Approximate Amounts to Feed *Daphnia* Tanks.

Density of *Daphnia* per 20 ml sample	**Culture Tank Size**	
	20-gallon	**40-gallon**
20+	2+ teaspoons (tsp)/day	4+ tsp/day
12 – 20	1 -2 tsp/day	2 – 4 tsp/day
5 – 9	3/4 tsp/day	1 1/2 tsp/day
1 – 4	1/4 tsp/day	1/2 tsp/day
0 – 1	1/8 tsp/day or less	1/4 tsp/day

There are two ways you can feed *Daphnia* cultures. One is a visual judgment of water clarity and is described below. The second is to monitor *Daphnia* densities by using a test tube or cuvette attached to a wooden dowel rod. Randomly sample the culture multiple times at various depths and get an average count of the number of *Daphnia* per milliliter. Use the above table as a guideline for how much to feed. You may need to make your own chart depending on the type of food you are feeding. The above table gives a good starting point. Match the average density of *Daphnia* to the size of tank used and feed an appropriate amount of feed. I believe the above, monitoring density and using the chart method, is better for beginners. This is crucial. Starting *Daphnia* cultures, it is very easy to overfeed because you do not have the bio-mass of animals to utilize the feed added to the tank. The uneaten food rots, produces too much bacteria and fouls the water eventually causing a collapse and loss of the colony. Note: if you get a starter culture of *Daphnia* from a fish auction, they are usually in a fish bag. Adding this bag to a 20-gallon tank you are going to have far fewer than 1 *Daphnia* per 20 ml sample. So, you need to feed a very small quantity probably like a pinch per day. Only when the *Daphnia* have reproduced to some detectable level between 0 and 1 *Daphnia* per 20 ml do you dare to increase the feed to 1/8 teaspoon or so.

The ideal level to feed the *Daphnia* should be enough to cloud the tank up to slightly noticeable opacity. One day later, that same water should be crystal clear. If the water clears up sooner than one day, too little is fed. If the water is still very cloudy the next day too much has been fed. So, adjust quantities accordingly.

Regular water changes need to be performed on *Daphnia* tanks as well as fish tanks. The bare minimum that a *Daphnia* tank, at full production, should be changed is two 25% water changes per week. If water changes are done more frequent than this, it is possible to see an increase in production.

Water changes on *Daphnia* tanks can be performed by siphoning water using a fine screen to cover the intake end of the siphon. Be sure to shake off any *Daphnia* adhering to this screen before the screen is withdrawn from the tank. Alternately, you may combine the water change with harvesting by siphoning off the tank into a very fine mesh screen or net. Feeding the *Daphnia* collected during the water change to your fish.

Aged, dechlorinated tap water is needed for *Daphnia* cultures just like for fish. Chlorine will be rapidly lethal to *Daphnia* so you must dechlorinate or age tap water before it is used on the *Daphnia* culture.

Selective harvesting of *Daphnia* can be done with a course meshed fish net. Using a course mesh will allow the youngest *Daphnia* to escape for further growth while entrapping full grown *Daphnia*.

All *Daphnia* tanks should be at maximum standing crop (10+ *Daphnia* per 20 ml) within several weeks of starting the culture. *Daphnia* tanks not reaching standing crop must be closely inspected for flatworms, hydra, or other pests. If infested, the culture must be discarded, and the container disinfected and cleaned to eliminate the pest.

Daphnia cultures should not be harvested until they reach a minimum average density of 10 *Daphnia* per 20 ml. *Daphnia* should not be harvested at a rate that decreases their numbers below 10 *Daphnia* per 20 ml. In other words, do not harvest more than what would decrease the total average numbers below 10 *Daphnia* per 20 ml. There should always be at least 10 *Daphnia* per 20 ml. Higher numbers should be attained before harvest, only slightly lower after harvest.

Periodically, *Daphnia* culture tanks, especially ones at full production, will need to be cleaned more thoroughly. If the walls of the tank become covered with debris, this can be scraped off. If excessive debris accumulates on the bottom of the tank, this can be siphoned off into a bucket. Allow the debris to settle, then pour the *Daphnia* from above the debris back into the tank and discard the debris. If excess debris is not cleaned out eventually the *Daphnia* will suffer "fouling" of their antennules and thoracic legs (swimming and feeding limbs) and this situation must be corrected. This is easily observed as "junk", debris and spider-web like filaments trailing below the *Daphnia* as it swims. I assume this "junk" prevents the *Daphnia* from properly feeding or perhaps molting its exoskeleton. So, it's very necessary to correct this situation in order for the culture to thrive.

Snails should be present in every *Daphnia* culture to clean up uneaten, settled food particles. If snails become overabundant, they need to be harvested and fed off to snail eating fish. Alternately, some *Daphnia* cultures come with small oligochaete worms (like *Dero digitata*) with them. These perform the same job as the snails, and can be harvested to feed the fish too. It is even possible to culture California blackworms *(Lumbriculus variegatus)* or perhaps tubifex worms in the bottom of *Daphnia* culture tanks. If this is done it may be necessary to feed the cultures at a slightly higher rate to be assured enough food makes it to the bottom for the worms.

Airstones in all *Daphnia* tanks should be running pretty well. Airstones running hard enough to constantly stir and swirl all the *Daphnia* through the water are appropriate. *Daphnia* must not be collecting or grouping up near the water surface or close to lights, etc. If they are doing this there is not enough water aeration. Strong aeration is necessary to keep the *Daphnia* feed in suspension. I have seen some reasonably successful *Daphnia* cultures with very little aeration. However, to achieve the yields listed below adequate aeration is a must. Remember that microscopic food particles need to be kept in suspension for the *Daphnia* to feed. If the food is settling before the *Daphnia* can eat it, you are only feeding your snails or worms.

Following the above instructions, it is possible to produce 4 to 5 ounces (wet weight) of *Daphnia* per 40 gallons of *Daphnia* culture per week. Therefore, if you go through about one pound of frozen brine shrimp per month, you could simply substitute a 40-gallon culture of *Daphnia* to produce the food needed to supplement your fish.

Author's Addendum

I have been a fish hobbyist for about 35 years and a professional fish biologist for 27 years. During many of these years I have had the opportunity to raise *Daphnia* in containers ranging from 2-liter soda pop bottles up to 1,200-gallon vats. The above instructions will not guarantee you will have success with *Daphnia* culturing but should go a long way to getting you started. Here I will share other secrets to success.

In 1992 I conducted an experiment to determine the best and most cost-efficient feed to raise *Daphnia*. This study was inspired by the sudden lack of a very good *Daphnia* feed many hobbyists used in the 1970s through 1980s. Many hobbyists are familiar with Jim Langhammer's successful *Daphnia* culture methods using "split pea and ham soup mix" fed alternately with baker's dried yeast. According to Jim Langhammer, the yeast seems to make the *Daphnia* reproduce quickly, while the split pea and ham soup mix made the *Daphnia* grow big and robust. The ham chunks in the split pea and ham soup mix served as food for tiny *Dero digitata* worms that shared the *Daphnia* cultures. The dehydrated ham chunks would eventually sink to the bottom of the tank and the *Dero digitata* worms clustered and fed on the decomposing ham. These tiny worms, like miniature tubifex worms, are also a great food to feed small fish. This system worked very well for Jim Langhammer and I when first culturing *Daphnia*. The split pea and ham soup mix could be purchased from bulk food stores. Sometime in the late 1980s to the early 1990s the split pea and ham soup mix suddenly became unavailable. Bulk food stores that carried it no longer did. So, I had to find a good substitute.

Doing literature searches on *Daphnia* culture you come up with a bewildering array of ways *Daphnia* can be cultured in the laboratory, primarily for toxicology studies. Everything from manure, yeast, live phytoplankton (micro-algae), finely ground trout chow, alfalfa meal, to snail manure (from apple snails), to soy flour and other finely ground foods have been cited as foods for *Daphnia*. Often, some of these items don't serve directly as food for the *Daphnia*, but the microorganisms like fungi, yeast, bacteria, and protists that grow in the water and are feeding on these items as they decompose is what serves as food for the *Daphnia*. The critical component to using decomposing organic matter as food for *Daphnia* is quantity. If too much is fed, the decomposing matter grows too much bacteria, causing the water to become too cloudy with ensuing water quality problems. IT IS VERY EASY TO OVER-FEED *DAPHNIA* CULTURES

CAUSING CATASTROPHIC COLLAPSE OF THE COLONY. Not sure what kills the *Daphnia* but it could be depleted oxygen, high ammonia, high nitrites, high nitrates or high phosphates. Something kills them off if overfed.

Literature searching on water parameters to which *Daphnia* are sensitive, you find that they are fairly tolerant of ammonia, intolerant of nitrites, somewhat tolerant of nitrates, and have an interesting relationship with phosphorus. It turns out that *Daphnia* use phosphorus as an environmental cue to reproduce or not. In nature, *Daphnia* reproduce most rapidly when algae (phytoplankton) are rapidly growing since micro-algae (phytoplankton) are the usual food for *Daphnia* in lakes and ponds. When algae are rapidly growing and is at a high density, phosphorus in the water is usually low, because the algae are rapidly using this up as a food source. So, *Daphnia* reproduction is linked to phosphorus levels. High phosphorus indicates to the *Daphnia*'s physiology there is no food (i.e. algae) in the water and cease reproduction. Low phosphorus level indicates to the *Daphnia*'s physiology there should be high algae levels so kick reproduction into high gear. This is one of the reasons water changes are very critical to *Daphnia* culture success!

Daphnia sensitivity to nitrites may be the explanation why cultures often fail roughly one to three weeks post initiation. This is just about the right amount of time for the nitrogen cycle to proceed from a high ammonia level to high nitrites. So, *Daphnia* also need biological filtration just as a fish tank does. This is one of the reasons why I advocate using many snails or other aquatic organisms to consume uneaten food. Uneaten food as it decomposes contributes to the ammonia and subsequent nitrite spike. The more quickly this food is assimilated into body mass the less nitrite will end up in the water. Snail shells would also serve as a living bio-media for nitrifying bacteria to convert the ammonia to nitrites and later to nitrates. You can't have a sponge filter or under-gravel filter in a *Daphnia* tank since this media will trap *Daphnia* food before they get a chance to eat it. Therefore, you need some other substrate in the tank to serve as biologically active media. Hence snails, in my opinion, are a quick way to solve this problem on several levels. Snails eat and assimilate some of the uneaten food and their shells serve as bio-media for nitrifying bacteria. I repeat this twice because it's critical.

When you have *Daphnia* in a closed environment it is very easy for phosphorus levels to climb quickly especially when you have a high standing crop, and since you are adding quantities of food. All biological materials contain phosphorus so each time you add *Daphnia* feed to the tank you are increasing phosphorus levels and effectively shutting off their reproduction. Therefore, water changes are necessary to flush away excess dissolved phosphorus. The water changes also diminish nitrites and nitrates which also are detrimental to their welfare. This is extremely crucial when you get a *Daphnia* culture really going in the "maximum standing crop" mode discussed in the first part of this paper. If you do not harvest *Daphnia*, at the same time you do a water change, several times per week, *Daphnia* numbers can quickly plummet and reproduction can be shut down. Think of this mantra…feed, feed, water change, harvest…feed, feed, water change, harvest. If you stick to a schedule like this you will successfully keep *Daphnia*. If you feed, feed, feed, forget the water change, forget to harvest…your *Daphnia* culture will most likely crash. If you forget to feed, forget to water change, you will never have enough *Daphnia* to make it worthwhile.

Close observations of your *Daphnia* are also necessary for success. This is why I advocate using aquariums for the beginner *Daphnia* culturist. If any *Daphnia* predators, such as planaria flatworms like *Dugesia* get into the culture they will eat away the profitability of your operation. Likewise, hydra will wreak havoc too. Close observations of the glass walls of your culture aquarium will tell you if flatworms or hydra are present. If they are it is best you start over again with clean aquaria and new *Daphnia*. If you must use *Daphnia* from the contaminated colony be sure to carefully net out only a small number of "colonists" to start the new colony in order to avoid capturing any hydra or planaria with them. Do not just scoop a bunch of debris and *Daphnia* from the contaminated tank as the debris will likely harbor flatworms, hydra, or resting eggs or cysts of these pests. Be sure to isolate only pure *Daphnia* to go to the new culture.

Daphnia can get parasites. More than one time, some of my *Daphnia* cultures became contaminated with a microsporidian type parasite. These will cause the *Daphnia* to decrease reproduction. Most importantly, this disease will make the *Daphnia* very opaque, or unusually white colored. When you see a large number of *Daphnia* becoming extremely white and opaque, it is best to destroy the colony and start over again.

Close observation of your *Daphnia* will give you more clues to how the population is doing. Remember you are managing a population and therefore you need to know something about demographics in order for your colony to thrive. If you see nothing but small, young *Daphnia*, in your culture, you may be over-harvesting or it's a freshly formed colony just coming into good production. If most of the *Daphnia* are large behemoths then you are probably under-harvesting, or your culture has not been reproducing well so you need to adjust accordingly. *Daphnia* typically only live about 21 to 28 days before they die (at room temperature and in a rapidly growing colony) so plan accordingly. If you see mostly big *Daphnia* a large portion of these should be culled out and fed to your larger fish before they perish, thereby making room for younger animals in the colony. Ideally, a thriving colony should have a healthy mixture of all ages of animals. Plenty of newly born young with a large number of sub-adults and a reasonable number of big old adults all should be represented in the population. If you see any one age class over-represented it may indicate a change in management plans is necessary.

Daphnia will give you other clues to how they are doing. Normally, under ideal conditions, all *Daphnia* are females and reproduce by parthenogenesis. That is, their eggs develop without being fertilized by a male. These eggs all develop directly into tiny female *Daphnia* that are born from the mother. When *Daphnia* colonies become "stressed" by poor water conditions, improper or not enough food, high temperatures, low temperatures, etc. the all-female population will start to produce some tiny males. These males then mate with the females and the resulting eggs that are formed are "resting eggs". These eggs are very different from the normal eggs. The resting eggs form an "ephippium" or saddle on the mother *Daphnia*s' backs. This saddle is a dark brown or blackish "case" carried on the back of the female and is readily visible to the naked eye with close observation. If you see these ephippium forming on *Daphnia* in your colony, it is a clear indication something needs to be changed quickly. Either water changes, more feed, heavy harvesting, etc. Something needs to be corrected before your colony collapses. These resting eggs are designed to survive harsh conditions such as winter freezing, summer hot and dry periods where the pond completely dries up, or periods where there is no food. So, if you see these forming it is a clear indication your *Daphnia* think it's time to aestivate and you must convince them that

conditions are improving enough for them to pull out of this reproductive mode. Remember…feed, feed, water change, harvest…feed, feed, water-change, harvest.

So, back to my experiment mentioned previously. In 1992 I set up a replicated experiment to determine the best *Daphnia* feeds to be substituted for the old split pea ham and soup mix alternately fed with baker's dried yeast. I used twenty-one two-liter soda pop bottles set up on an aeration manifold (each bottle had an air-stem bubbling in it). Each bottle was randomly assigned to one of seven diets. Each diet had three replicate bottles. Each pop bottle received an equal number of *Daphnia* (n=20) and snails (Seminole red rams-horn snails – *Planorbella duryi*) scavengers at the beginning of the experiment. Each container was fed an equal weight (determined volumetrically since all the foods had very similar densities) of food that was assigned to it. (In the end I compensated for slightly different densities by knowing the total weight of food as calculated from the density and volume of food fed. Therefore, the cost of production per *Daphnia* is compensated to the right weight of food.) I monitored *Daphnia* densities in each container and harvested and hand counted all *Daphnia* pulled from the bottles and recorded this over a 44-day period. Data was recorded and graphed as population densities and numbers of *Daphnia* harvested.

Numbers of *Daphnia* produced were noted and then cost of producing 100,000 *Daphnia* was extrapolated by dividing the weight of food used by the number of *Daphnia* produced multiplied by the cost per pound of each feed times 100,000. (Remember these are from 1992 prices)

<u>*Daphnia* Feeds Tested</u>
Diet 1 – baker's active dried yeast.
Diet 2 – dried *Spirulina* algae.
Diet 3 – dried *Chlorella* algae.
Diet 4 – dried, ground, split peas and paprika.
Diet 5 – dried, ground split peas, paprika and baker's active dried yeast.
Diet 6 – baker's active dried yeast, dried spirulina algae, and dried chlorella algae.
Diet 7 – dried, ground split peas, paprika, dried spirulina algae, and dried chlorella algae.

<u>1992 Costs of Select *Daphnia* Feeds Used in Trial</u>
Red Star brand baker's active dry yeast - $1.40/lb.
Ocean Star International brand dried *Spirulina* algae powder - $16.00 /lb.
Now brand dried *Chlorella* algae powder - $35.80/lb.
Ground split peas and paprika mixture (10:1 ratio) - $2.26/lb.

Results

The outcomes of this experiment follow, ranked from best to least effective feeds. Costs are 1992 calculations.

Diet #6 - combination of baker's active dried yeast, dried *Spirulina* algae and dried chlorella algae yielded the best performance. Diet 6 produced more *Daphnia* by 32 days (n=5,240) and by 44 days (n=9,650) than any other diet. It also produced *Daphnia* more consistently with 13 harvests. It produced the first harvest within 14 days. Overall cost was $1.36 to produce 100,000 *Daphnia*.

Diet #7 – dried ground split peas, paprika, dried *Spirulina* and dried *Chlorella* algae was the second best in performance. It produced the second greatest number of *Daphnia* after 44 days (n=6,254) and the third best at 32 days (n=3,236). Had the second most number of harvests (n=11). This diet really performed well with rapidity of harvest – like diet 6 within 14 days. The cost to produce 100,000 *Daphnia* was $2.22.

Diet #3 – just dried *Chlorella* algae was ranked third. Third in total production at 44 days (n=4,857) and second best in production at the 32 days (n=3,396) and third at total number of harvests (n=9). It was also ranked third at producing a harvest quickly-at 19 days rather than 14 days for the above two diets. Overall cost was $4.54 to produce 100,000 *Daphnia*.

Diet #2 – just dried *Spirulina* algae ranked fourth. This diet only produced three harvests and it took 39 days to reach harvest densities. A total of 3,801 *Daphnia* were produced after 44 days. Cost was $1.79 to produce 100,000 *Daphnia*.

Diet #4 – dried, ground split peas and paprika ranked fifth. This diet, like *Spirulina*, only produced three harvests and took 29 days to reach harvest densities. It cost $1.71 to produce 100,000 *Daphnia*.

Diet #5 – dried ground split peas and paprika and baker's active dried yeast. This diet also fared poorly and was similar to yeast alone. Harvests occurred only after 36 days and amounted to only 2,955 individuals. However, cost per *Daphnia* is relatively low at $.30 per 100,000 *Daphnia*.

Diet #1 – strictly baker's active dried yeast cultures did poorly. Although their densities did increase initially up to 10 days post start of the experiment, after 15 days densities remained consistently low and only 2,676 *Daphnia* were harvested at the end of the 44-day period with the first low number harvest (n=846) after 39 days. However, cost to produce 100,000 *Daphnia* was the lowest at $.22 per 100,000 *Daphnia*.

Conclusions

Mixtures of *Daphnia* feed containing whole spray dried algae outperform other types of feeds when it comes to quantities and rapidity which *Daphnia* populations grow. Mixtures of both *Spirulina* and *Chlorella* algae added to either baker's active dried yeast or ground dried split peas and paprika both performed very well. Any time a dried algae product is used it adds considerable cost to *Daphnia* production. However, using a mixture of high cost algae combined with very low-cost yeast or split peas gives rapid yields, with high harvests, at a medium cost to the hobbyist.

Using only a dried algae product (either *Chlorella* or *Spirulina*) gives relatively high to medium yields at a slightly longer period than the above-mentioned mixtures. However, the high cost of these products ends up making the cost per *Daphnia* produced much higher compared to other mixtures and the cheaper single source feeds.

Dried ground split peas and paprika ranked only mediocre. It produced only a modest number of *Daphnia* at a prolonged period and cost was also medium at $1.71 per 100,000 *Daphnia*.

Dried ground split peas and paprika added to baker's active dry yeast and baker's active dried yeast alone did produce *Daphnia*. However, production was much slower (by a factor of 3.7 X) than when using dried algae products. Even though production was slow, and low, the cost per unit was also low at only $.30 to $.22 per 100,000 *Daphnia* respectively.

Further Discussion

Algae (microplankton or phytoplankton) are the natural diet of *Daphnia* in their wild habitat. Therefore, it stands to reason that *Daphnia* should perform (grow and reproduce) well fed on dried algae products. However, other feed items have traditionally proven to be effective at rearing *Daphnia* in captive situations. Feed items like baker's active dry yeast and other plant products like dried and ground split peas will serve as feed as well. These later products, although not natural to *Daphnia* habitats, will support *Daphnia* production at a much-reduced cost. However, production is much slower and lower yielding than when dried algae products are used. Just as with feeding any animal, variety, like mixing several different feed items, offers better nutrition and compensates for deficiencies that any one item may present. In this study, it was demonstrated that a mixture of both natural type feeds (like dried *Chlorella*) combined with other traditional *Daphnia* culture feeds (like ground split peas, paprika, and active baker's dried yeast) gives high production of *Daphnia* at a moderate cost. The hobbyist can therefore select from using very low-cost feeds and be happy with low and slow production, or can add value to the feed with an addition of spray dried algae. Adding spray dried algae to *Daphnia* cultures, one can realize at least a 3.7-fold increase in production.

Dried algae products come with other benefits. These products are far richer in vitamins, anti-oxidants, color enhancing phytopigments, and the proper fatty acids necessary for proper development and health in fishes. Therefore, I would advocate adding dried algae products just for these added values without even considering price.

I also tested, in subsequent trials, the suitability of adding other finely ground plant products to homemade *Daphnia* feed. This grew from the annoying, labor intensive activity, of purchasing dried split peas and grinding these into a flour like consistency using either a blender, food processor, or coffee grinder. Some hobbyists have avoided this step by using canned, human baby food vegetables (peas) purchased in jars from the grocery store. Some substitutes tested and found to be very effective were whole wheat flour and soy flour. These two ingredients can be exchanged for ground split peas or added in addition to the later.

Paprika is still used since it's very high in anti-oxidant pigments and is converted to color enhancing xanthophylls (carotenoids) in the *Daphnia* which are then passed up the food chain to your fish.

Finally, in the last twenty years there has been a huge growth of knowledge in aquaculturing a host of marine and freshwater fish and shellfish. Along with this growth there are now numerous dried, live, and preserved algae products available which all could potentially be used for *Daphnia* feed. Some of these products are very expensive, some available to hobbyists, some not so readily available but with effort could be acquired. Many of the more expensive products have very great potential to increasing the nutritional content of your *Daphnia* fed to your fish. I will only list some of these products here for your potential experimentation. Including but not limited to Artificial Plankton Rotifer (APR), Microfeast (Provesta), *Spirulina* (Ocean Star

International), *Chlorella* (Now Foods), Algamac 2000, Algamac 3000, Algamac 3050, Aqua-grow Advantage, Aqua-grow Advantage Enhance, Beta-Meal, Phyto-feast and Roti- Grow (Reed Mariculture).

Daphnia culturing certainly has the potential to reduce the need of purchasing frozen or live fish feeds like brine shrimp, bloodworms, glassworms, tubifex, or California blackworms. *Daphnia* cultured in your own home also can be considered cleaner and probably more parasite and disease free compared to frozen or live foods collected from various "questionable" and "contaminated" habitats. This comes with a price though. Culturing enough *Daphnia* to feed an entire fish room is possible. I know, I did it with four 40-gallon Rubbermaid trash cans for many years. However, it IS very LABOR intensive. You easily spend as much time caring for the *Daphnia* as you do the fish! I found that "pre-children" I had adequate time to run *Daphnia* cultures and a fish room. Post children and everything changes. I no longer culture *Daphnia* simply because other hobbies, kids' activities, etc. consume too much time. (I now have other very cheap frozen and live food substitutes…but that is a topic for another article!)

I hope this helps for anybody wishing to venture into *Daphnia* production or who have had troubles in the past. *Daphnia* culture can be reduced to science, although for many it may still seem like magic!

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HYPERSALINITY TREATMENT TO ERADICATE *Aiptasia* IN A 40,000-GALLON ELASMOBRANCH SYSTEM AT THE INDIANAPOLIS ZOO

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Indianapolis Zoo, Indianapolis, IN USA 11/27/2019

In late 2018, the Indianapolis Zoo had a severe outbreak of *Aiptasia* occur in our 40,000-gallon exhibit tank (Figure 1) which houses a population of cownose rays (*Rhinoptera bonasus)* and smooth dogfish (*Mustelus canis*). At first, we saw a few *Aiptasia*, however, within months, they had spread to cover all our rockwork (Figure 2). The *Aiptasia* then started spreading along the aragonite sand bottom, up the sides of the tank, and over the plumbing found within the tank. The invasive anemone quickly became a hazard to divers. Several of the cownose rays began showing signs of ulcerations, most likely due to the anemones.

Figure 1. Indianapolis Zoo's 40,000 gallon exhibit tank featuring smooth dogfish and cow nose rays.

When the *Aiptasia* first appeared, we used Aiptasia-X to try and eradicate the anemones, however, there were too many. We also tried injecting kalkwasser into the stalk of the anemones (Figure 3). While the kalkwasser worked on a small scale, it was not practical to inject each individual polyp in this large of a tank. We knew we had to treat the entire life support system to be effective on a long-term basis.

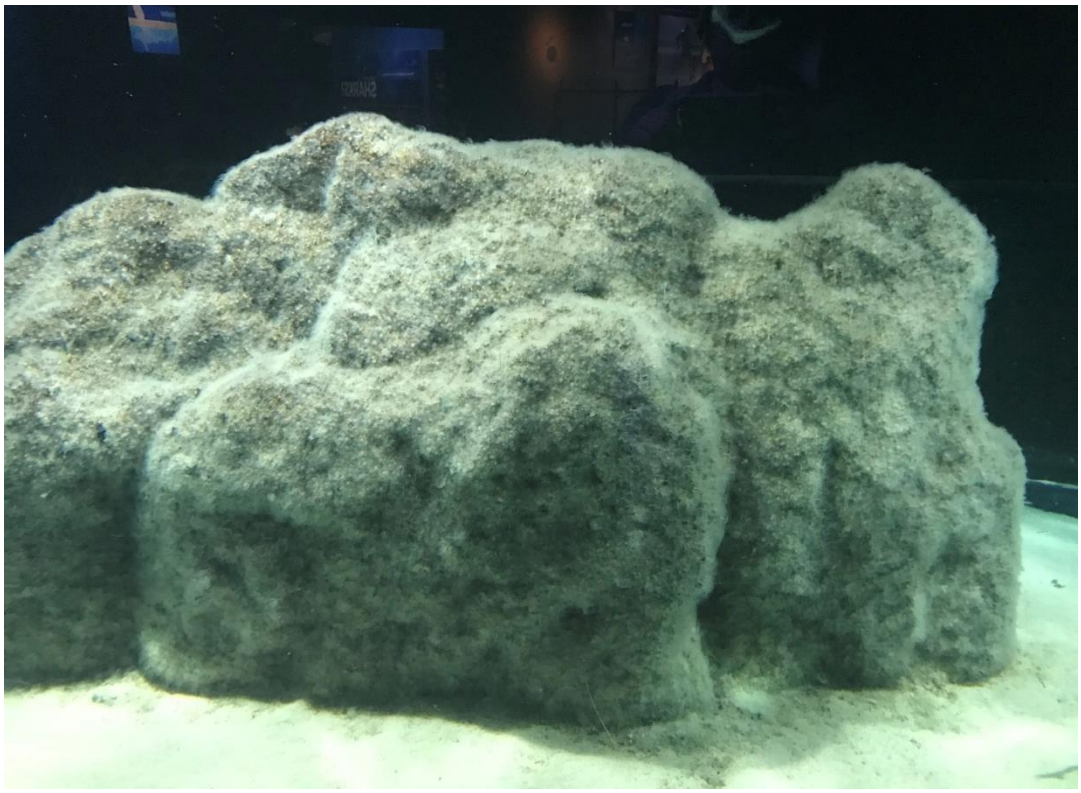

Figure 2. Image taken on 4/23/19 showing very "furry" rocks. All rockwork were entirely covered with *Aiptasia*. The anemone had also started to spread along the back walls, on the plumbing in the tank, and on the substrate.

Figure 3. Image taken on 3/20/19 showing a very small patch of rock where aquarists had been able to eradicate *Aiptasia* using kalkwasser.

After reading posts on Aquaticinfo list server and contacting other facilities, we narrowed our options to either bleaching the entire system or using chloroquine. Our Water Quality Specialist at the time, Sarah Hoback, contacted other facilities to get their recommendations and any insights they may have to offer. She also tracked down dosages, warnings, and products.

Informal in-house trials on the effectiveness of chloroquine, done by Tracy Sipes, Aquarist, didn't prove effective, even after 48 hours 20ppm. Since we don't have a fail proof method of testing for chloroquine and we run ozone to the system, we were concerned about the overall effect to our elasmobranchs' health. These two factors eliminated chloroquine as a potential method, even though it was our cheapest option.

Tracy also ran an informal trial using bleach. The good news was that bleach proved effective. The bad news was we'd have to use extremely high concentrations of bleach, between 500ppm to 800ppm. This would amount to using 250 gallons of 13% sodium hypochlorite bleach. Since our exhibit is indoors, and we have other life support systems immediately adjacent to our tank, we ruled out bleaching our exhibit. We were also very concerned about bleach fumes affecting staff and guests. We also didn't want to risk any bleach water getting into one of the adjacent tanks or have fumes settling on tanks or sumps downstairs.

Small scale trials proved using freshwater or magnesium chloride to be an ineffective means of eradicating *Aiptasia*. On the other hand, the trials showed that leaving *Aiptasia* out of water in the air would kill them. Since we knew we couldn't dry out the insides of our sand filters or pipes, we knew we couldn't use this method for our main eradication plan. With this fact in mind, we did choose to keep the tank dry a few days between the treatment and recommissioning of the system. Both of our original options – using bleach or chloroquine - had now been eliminated. We needed an effective solution that was safe for staff, guests, and animals. We also needed a solution that the entire team at Indianapolis Zoo approved.

While discussing the problem and possible solutions for the umpteenth time after many months, Brady Stoever, one of our aquarists at the time who now works at Florida Aquarium, mentioned a conversation he had with Sean Boyd. Sean, Senior Aquarist at Ripley's Aquarium in Myrtle Beach, mentioned to Brady that we should try raising our salinity to 70ppt. Whether Sean was joking or not, this was a new approach that we hadn't seen in all our research or on Aquaticinfo. In a "we have nothing to lose" effort, we ran an informal trial placing *Aiptasia* in 5 gallons of system water and adding enough salt to raise the salinity to 70ppt. Within hours, all the *Aiptasia* had expired.

We quickly put together a plan to raise the salinity of our 40,000-gallon tank to 70ppt and presented it to our Vet staff, senior Life Sciences team, and Life Support Team. With the support from the Zoo team, we finally had a way forward.

We ran a second test using a salinity of 50ppt in 5 gallons of water to see if *Aiptasia* would die off at a lower concentration. They did. Since we had already ordered salt for 70ppt and we wanted as big of an osmotic change as possible in the shortest amount of time, we stuck with 70ppt as our goal. Just in case.

Below is the course of action we took which proved successful. We have since used this plan for a 300-gallon elasmobranch holding tank. New England Aquarium also recently used this hyper-salinity method on an elasmobranch touch tank with the same success.

Goal

Increase salinity of the system to 70ppt as quickly as possible to eradicate *Aiptasia*.

Warnings

1. You need to remove all your animals from the system.
2. With this method, you'll be completely killing your biofilter.
3. When ordering salt, make sure it's "YPS free" food grade NaCl, which is free of contaminates that could kill fish. Note: salts used for mixing seawater will work, however, they're much more expensive than food grade salt.

Course of Action

1. Move all the animals out of the tank and into different life support systems.
2. Shut off ozone to the system and remove ORP probes.
3. Shut off venturi pumps on protein skimmer.
4. Add 16,600 pounds of food grade salt (NaCl) to 40,000-gallon system (make sure salt is YPS free) to reach salinity of 70ppt.
5. Run the system 24 – 36 hours to thoroughly mix the salt and treat the entire life support system.
6. Drain the system entirely, or as much as possible (almost 100% drained). Flush with freshwater as needed.
7. Tank remains empty for 4 days in order to perform necessary LSS maintenance, dry out any residual *Aiptasia*, power wash, modify rockwork and add artificial corals, etc.
8. Refill the life support system with freshwater and run for 24 hours or more to dilute any residual salt and flush plumbing and filters. Start up filters on backwash.
9. Drain the life support system (further flush out pipes and filters).
10. Refill with seawater & recommission the life support system. Start up filters on backwash.
11. Add Korzyme saltwater bacteria to reestablish biofilter. (We used 12 gallons of bacteria)
12. Add fish within 24 hours of adding the bacteria to start, and then gradually add more over time as water quality allows until fully populated.
13. 48 hours after adding the bacteria, turn on protein skimmer and ozone (at lower percentages to start)

Logistics and Timing

The life support system in question doesn't have a reservoir, which meant that we had to add the salt directly to the tank. Access to the tank is up a set of narrow stairs. The good news is there's a hoist. The bad news is that the hoist isn't rated to haul a supersack of salt. This meant that while the food grade salt we wanted to use was available in supersack sizes, we would have to order the salt in 50-pound bags. Andy Verhey, Area Manager of Life Support, came up with a way to hoist as many bags of salt as possible upstairs. Once upstairs, salt bags still had to be moved manually to the areas surrounding the tank.

It was important to keep elasmobranchs in the exhibit tank for as long as possible. We also had to keep in mind that we had to have fish back in the system for a previously planned evening event in the building. This created a non-flexible end date and a short time frame for our *Aiptasia* eradication project.

We gradually removed animals from the system over a period of 19 days. Cownose rays and dogfish were both moved into two different holding systems. The gradual addition of fish to the two holding systems also helped with maintaining water quality in those systems since neither location had a heavy bio load in several months.

We knew from the start that we had 0.3 pregnant cownose rays. To cut down on stress of being moved into a holding tank, and then moved back into the exhibit, they were all moved into the closest holding tank.

Once salt arrived on site, the pallets were unloaded from the truck and stored in our hay barn until needed. Over a period of two days, many staff from various teams (Aquarists, Life Support, and Marine Mammal trainers) moved salt from outside the building, up the stairs to the top of the tank, and then staged either around the perimeter of the tank, or in the areas adjacent to the tank.

Our hoist isn't rated for a full pallet of salt, so we manually moved bags onto a smaller pallet. Once upstairs, the salt bags were then individually moved and stacked around the perimeter of the tank. The hoist is also slow, so bags were also carried up the stairs to save time.

Bags were placed as close to the edge of the tank as possible. We used plastic lattice work to make sure the bags couldn't fall into the tank prematurely (Figure 4).

Figure 4. Five pallets worth of salt were staged around the tank's perimeter and in the adjacent areas. Plastic lattice work was used to prevent salt bags from prematurely falling into the tank.

It was important to us that all the bags were staged upstairs, either around the perimeter of the tank or as close as possible prior to the salt dump. Having the bags staged beforehand allowed us to dump as much salt into the tank as quickly as possible. We were aiming for the biggest salinity change in the shortest amount of time in order to hopefully cause osmotic changes that the *Aiptasia* couldn't withstand.

Salt Dump

On the morning of the salt dump, aquarists moved the last few remaining animals from the tank and a curtain was placed in front of the acrylic so guests couldn't see what we were up to.

Life Support staff drained enough water from the system to account for the salt being added since we didn't want to overflow the tank. The life support system was run while we added the salt. We turned up airlifts, added additional air stones, and ran a sump pump in the tank to help move water and dissolve salt.

We were able to dump 16,600 pounds of salt into the tank within 20 minutes. This accomplishment was due to a strong team effort which included our Curator, Marine Mammal trainers, Aquarists, and Life Support staff.

While we were dumping the salt, staff wore protective eye wear, gloves, and dust masks. As expected, the water became extremely cloudy during the dumping process. It also turned a lovely shade of ice blue (Figure 5).

Figure 5. The actual salt dump took a team from multiple departments. As we added salt, the water turned electric ice blue, now called "hyper saline blue".

We intentionally held four bags of salt back from the initial dump. Once we drained the tank, we inspected the rocks for *Aiptasia*. Had there been any, we would have used the remaining salt to create a thick paste and apply it to the rocks.

The water began clearing up after the last of the salt was added. After a few hours, we could begin to see the rockwork again (Figure 6) and, what was most important, we could actually see the rocks! Prior to the salt dump, the rocks were covered completely in thick mats of *Aiptasia* and we hadn't seen them for several months. Pieces of *Aiptasia* could also be seen in the water column.

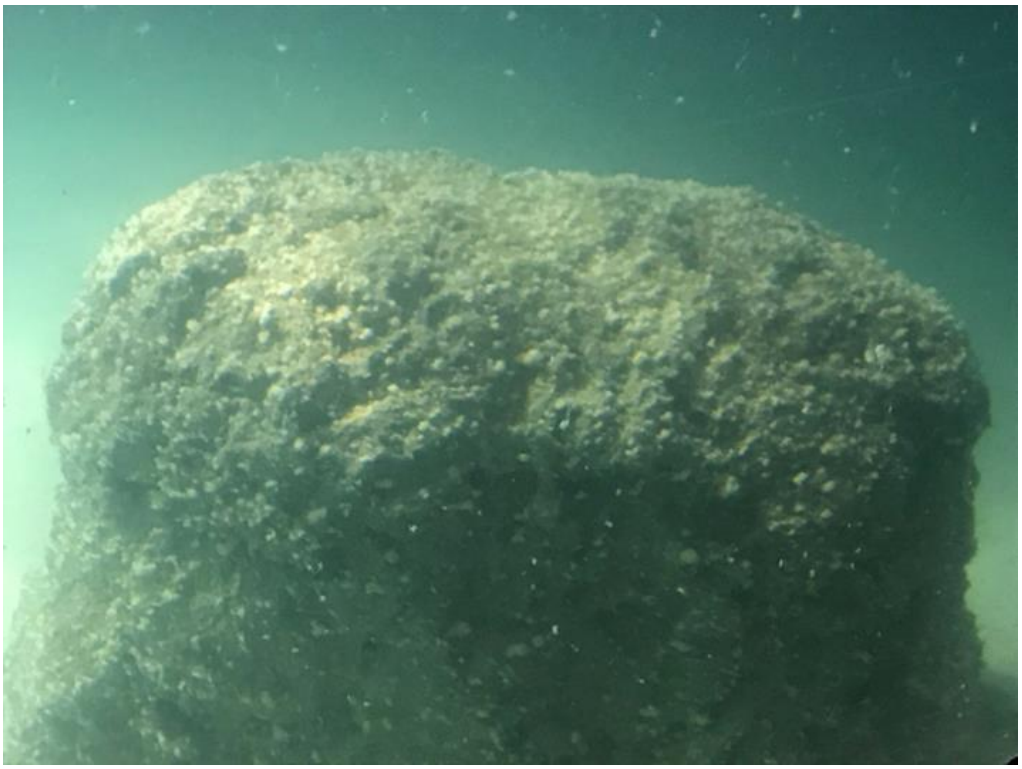

Figure 6. Image taken within hours of the salt addition. You can already begin to see the actual rocks. The white blobs on the rocks are dead *Aiptasia* that had either released from the rock and died, or that had died elsewhere in the tank and had been forced up into the water column and then settled out. These were easily brushed off and into the water column.

While I had focused on the logistics of the actual dump and the treatment process, I hadn't thought too much about the cleanup. My team, however, had. We had one team cutting open and dumping bags, and another removing the bags and taking them downstairs to the dumpsters. Bags were loaded into the upside-down top of a large animal kennel, which was then attached to the hoist and lowered downstairs to be carted away. The actual dump and clean up took under an hour.

Normal salinity of the system is between 30ppt – 32ppt. Our goal was to raise the salinity to 70ppt. Two hours after the salt dump, after the filtration system had turned over twice, salinity was tested between 65ppt – 67ppt. The next morning, salinity had risen closer 69ppt. This was due to salt that had settled on the bottom dissolving over time.

I had expected a stench to develop as the huge amounts of *Aiptasia* died but one never developed. We did get thick brown sludge developing on the water surface. Periodically, this was netted out, bagged, and disposed of properly.

We decided to turn on the venturis on the protein skimmer while we ran the system, which resulted in copious amounts of beautiful protein scum (Figure 7).

Figure 7. The day after adding the salt, we brought our protein skimmer online.

Additional Insurance

We ran the life support system, with the now hyper-saline water for 48 hours. The water was then drained out. While the tank was draining, we sprayed down the walls, acrylic, and rockwork of the tank with freshwater.

When we were working around the tank, during the treatment and after when the tank was dry, we would wear latex gloves in case of any residual nematocysts.

We couldn't drain the tank dry using the life support system pumps. There was over a foot of water in the bottom of the tank when the pumps had to be turned off. We used a sump pump and hoses to further drain the tank down. In the past, when a large tank had been drained entirely and the bottom allowed to dry for days, the pool coat on the bottom delaminated. To prevent this, we kept six inches of water and the substrate in the bottom of the tank during the remainder of our

process. We thoroughly inspected the bottom and substrate to make sure there were no living *Aiptasia*. Had we found any, we would have added enough salt to raise the salinity again.

We planned to let the tank walls and rockwork sit and dry for several days in case any residual aptasia were clinging to the nooks and crannies of the rocks. If we found any, our intention was to also apply a thick paste of salt. We never found any that were alive.

While the tank was dry, we power washed the tank walls and rockwork (Figure 8). We also took advantage of an empty tank. Life support team did some work on the plumbing and electricians worked on lights above the tank.

Originally, we wanted to have the tank dry for between four to five days, however, we started to observe some health issues with the dogfish tails in one of the 9,600 gallon holding tanks. We weren't certain why the tip of the caudal fin on some of our dogfish were bruised and almost butterflied open until Staff saw a cownose ray munching on their tails. A decision was made to move forward with our process so we could get the cownose rays and dogfish moved back into a larger tank. The tank was dry for about three days total instead of the five we had planned.

We filled the system with freshwater and ran it for approximately 36 hours. During this time, thick brown sludge settled out on the bottom and was then siphoned out by divers.

Once the freshwater was drained out, the tank was checked thoroughly for *Aiptasia*. After the final approval was given, we refilled with seawater.

Recommissioning

Due to the size of the tank (40,000 gallons) and the size of our salt water mixing tank (20,000 gallons), it took us a several days to refill the tank. The city water temperature was quite a bit colder than our normal water temperature but, thankfully, the water warmed up quickly in our system

Once the tank was full, the system was running normally (except for ozone and protein skimming) and water temperature reached 72°F, we added 12 gallons of Korzyme saltwater bacteria.

We moved in a small number of animals, four cownose rays and four smooth dogfish, within 24 hours of adding the bacteria. Ozone and protein skimmers were turned back on 48 hours after the bacteria was added.

We tested ammonia, nitrite, and nitrate levels daily until we knew our biofilter was fully established. We continued gradually adding more animals, as water quality allowed, until all animals were back in the tank.

We were able to have the tank up and running with animals before the previously scheduled evening event. All animals were returned to the exhibit within 18 days of adding the Korzyme bacteria (Figure 9).

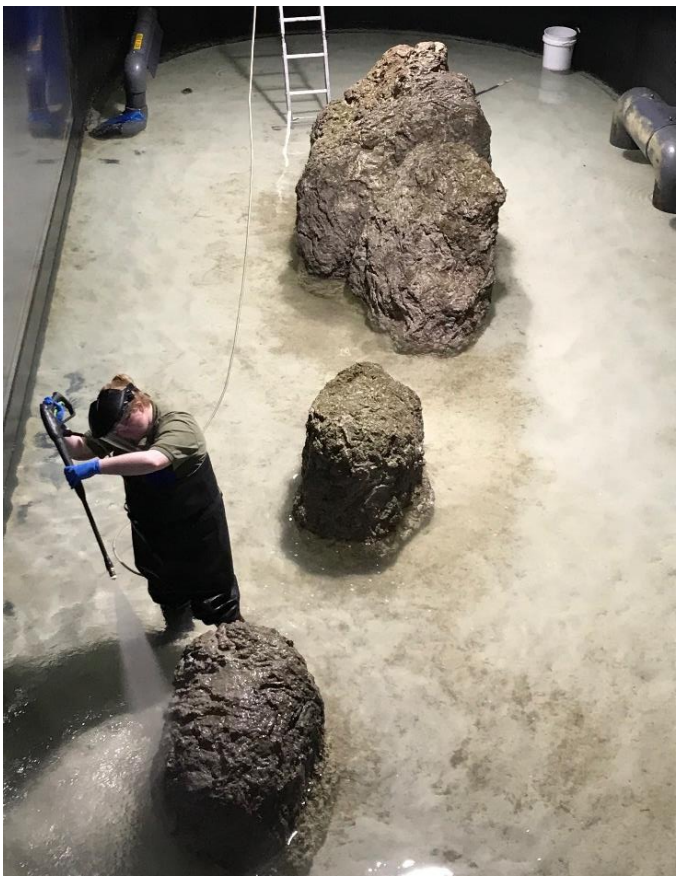

Figure 8. Rocks and tank walls were power washed while the tank was dry.

The Power of "Team"

This treatment wouldn't have occurred without a strong team No one person could have done this treatment. Even the Oceans team, consisting of one manager, four aquarists, and a water quality specialist, couldn't have done this treatment without help from the Zoo team.

Life Support helped problem solve logistics and helped with manual labor. Commissary helped by making room in the hay barn to store extra pallets of salt. Veterinary staff helped by not only providing care for the animals, but also weighing in on treatment options, dosages, and even providing institutional memory on our elasmobranch population. Creative Services provided "pipe and drape" to cover the acrylic and signage for guests, with little notice while working to finish their spring construction projects. Marine mammal trainers showed up to help move endless bags of salt upstairs and then showed up again to help dump the salt and remove the empty bags. This was time they could have spent caring for their own animals. Often, on a team, there is one person who will quietly go about taking care of routine business without calling attention to themselves. On the Oceans team, this person was Cara Van Kleeck, aquarist, who made sure all our other fish and invertebrates and life support systems were well cared for.

Figure 9. Image taken on 5/9/19 after the completion of the hyper salinity treatment and animals were introduced back into the tank.

Ideally

As an added precaution, I would have removed the aragonite sand substrate in the tank and replaced it with crushed coral, however, budget and time didn't allow for it. In the future, we would still like to change out the gravel for aesthetic reasons and to keep the cownose rays from stirring up the sand.

The treatment was done in late April/early May 2019. Both sand filters for the system were due to be repacked later in 2019. Due to budget and time constraints, we couldn't get the repacking project moved up to the spring. In October, we repacked one of our sand filters as originally scheduled. The second filter was repacked the end of November. Had we been able to, I would have repacked both filters while the tank was empty.

The Results

As of writing this article, we have yet to see an *Aiptasia* in the tank (knock on acrylic) and, in the fall, all three of the pregnant cow nose rays had successful births. Moms and pups are doing well.

Thank You

- To the entire Oceans team of Indianapolis Zoo (Tracy Sipes, Cara Van Kleeck, Brady Stoever, Sydney Pitts and Sarah Hoback) who researched options, discussed possibilities, problem solved, developed timelines, gathered supplies, moved lots of animals, and most importantly, cared for our collection.

- To Andy Verhey, Area Manager of Life Support, for his knowledge of our life support systems, his time spent problem-solving logistics, and coming through with the end results we needed.
- To Stacey Green, Curator, and Jodie Baker, General Curator, for support and guidance along the way and for taking the chance on the idea.
- To the Life Support Team of Indianapolis Zoo for all the work involved in not only the treatment, but the recommissioning of the life support system. And a huge thank you for cleaning out the residual gunk in the pump strainer baskets.
- To the Marine Mammal trainers of the Indianapolis Zoo for their work moving numerous 50-pound bags of salt upstairs, dumping it into the tank, and clearing away the bags.
- To Tracy Sipes for helping with editing this article.
- To Sean Boyd, Senior Aquarist at Ripley's Aquarium in Myrtle Beach, for the initial idea, whether he was joking or not.
- To Vet staff of Indianapolis Zoo for their input, research, and work with the animals.
- To Brian Brawner, now of Hayward, for technical advice and moral support.
- To Paula Carlson Branshaw, Dallas World Aquarium, and Barret Christie, Norwalk Maritime Center, who reviewed our treatment plan prior to the actual treatment.
- To all aquarists who have been discussing *Aiptasia* eradication on Aquaticinfo list server.

"Dream big, be a little bit crazy, and never give up."
Steve Winter, National Geographic Photographer

Red Rock Crab *(Cancer productus)*. Bruce Koike

GERMAN OCEANOGRAPHIC MUSEUM, ZOOAQUARIUM DE MADRID AND CORAL DOCTORS CLUSTER TO DEVELOP A PROJECT ON TRAINING OF LOCALS ON REEF REHABILITATION IN THE MALDIVES

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From the 9th to the 24th March 2019, a team of the three institutions developed free workshops on reef rehabilitation on 3 Maldivian islands.

For the third time, members of the German Oceanographic Museum Stralsund and Coral Doctors went to the Maldives with various objectives: impart free workshops on reef restoration to dive center´s staff and local communities and strengthen bonds with local NGOs and local organizations, developing the bases for future collaborations. As the project is growing, this year, ZooAquarium de Madrid joined in a stronger team that hopefully is only the beginning of a long-term effort to raise public awareness and train the locals to work on reef restoration autonomously.

Torsten & Yamila from Coral Doctors, Dr. Nicole Kube from the German Oceanographic Museum and Pablo Montoto from the ZooAquarium in Madrid.

In the local islands of Maafushi, Rasdhoo and Ukulhas workshops took place with theory lessons on general biology and ecology of the coral reef and its restoration, which were complemented with practical lessons at the sea. The participants gained confidence on the different restoration approaches: both biological and physical, with a mean duration of 3 days. These free workshops were open to the main public, since awareness on the threats and the actual state of

their local reefs were emphasized and the urgent need to adopt measures for its conservation. In the last lesson of the workshop, monitoring of the restoration efforts was taught, providing assistance by the European partners – establishing a cooperation that is intended to grow in time with future visits.

Classroom in Maafushi.

Frame practice at Ukuhlas.

The satisfaction of the organizers has been complete, as a mean of 20 attendees participated in each workshop, from dive instructors, scholars, boy scouts, local authorities and general public. In addition, on the island of Villingili, the organizers had the chance to meet with the local NGO "Save the Beach" where they had the opportunity to visit and discuss their efforts on reef restoration, rising awareness, as well as their future plan to build a "Center for Marine Learning", the first center for environmental education focusing on marine life in Maldives.

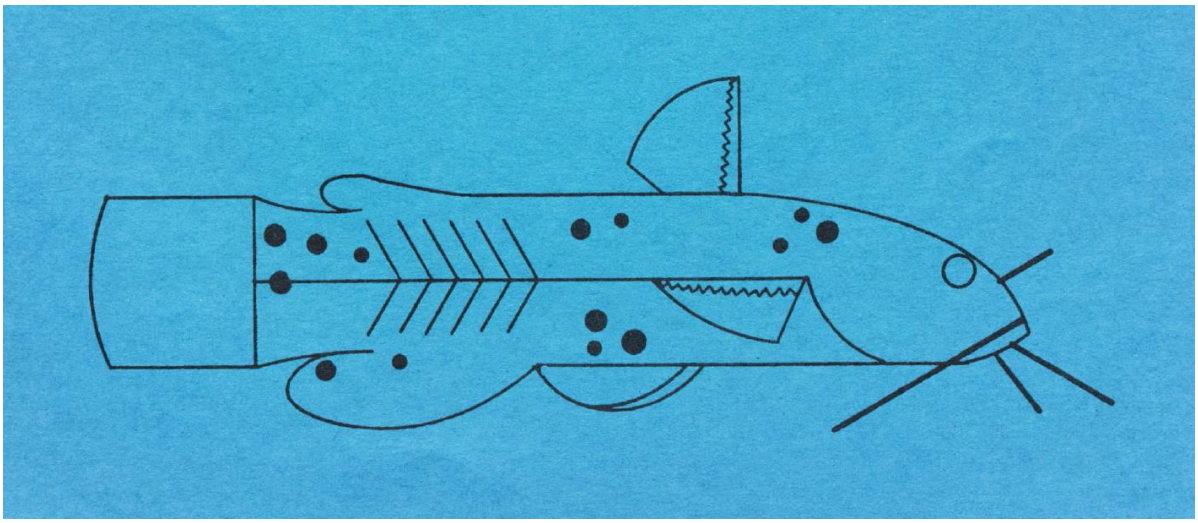
Beybe from Save the Beach checking Restoration trail in Villingili.

This project has been possible thanks to the German Oceanographic Museum Foundation, the European Association of Aquarium Curators (EUAC), the Parques Reunidos Fundation and other private sponsors.

Coral reefs around the world are suffering an unprecedented crisis. In countries like the Maldives, coral reefs play a major role in their citizens live, as the country is formed entirely by reef atolls. The importance of this unique ecosystem reaches its maximum exponent, offering protection to the coastline against erosion, tropical storms and tsunamis, being the main source of protein through fishing and the main attraction to tourists who visit the Maldives to enjoy their fantastic white coral sand beaches and marine life while snorkeling or diving.

In 2016, the Maldives, along with other important regions of the world, suffered a massive bleaching event that killed the vast majority of corals. In this trip, the team had the opportunity to

verify that the visited reefs were recovering from this episode, with important growth of the surviving colonies as well as an important number of recruits. Even though, this can´t be taken as good news, since predictions for the coral reefs are alarming due to climate change, ocean acidification and other threats, being public awareness and restoration vital efforts to try to keep the most divers ecosystem on earth, the coral reefs, alive.



Craig Phillips, Drum and Croaker 1971

EFFICACY OF CERAMIC BIOLOGICAL FILTER BRICKS AS A SUBSTITUTE FOR LIVE ROCK IN LAND-BASED CORAL NURSERIES

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Abstract

Coral aquaculture has been increasing over the years in public aquaria and amongst hobbyists and these practices may help protect threatened reefs by providing a genetic bank of critically endangered species of corals, and by reducing the dependency on harvested corals. One of the most important components of land-based coral nurseries is that they have good biofiltration. Live rock has been widely used, as it is one of the most effective bio filters to help maintain stable water quality for reef-building corals (Yuen et al., 2009). Live rock is typically taken from the wild, which raises concerns over damaging coral reefs that are already stressed (Cato and Brown, 2003). Our study looked at whether biological brick media (bio-bricks) could be used as an artificial alternative to live rock. We compared nitrification rates between five live rocks and five bio-bricks, and found that bio-bricks were equally effective as live rock at nitrification, consuming ammonia at a rate of 0.07 mg l^{-1} h^{-1} kg^{-1}. A t-test revealed that consumption rates were not significantly different.

Introduction

Coral conservation and restoration have been undertaken both in ocean-based (Nedimyer et al., 2011) and in land-based nurseries. Land-based nurseries often do not have direct access to seawater and must employ various forms of filtration to maintain healthy water quality for their marine organisms. Biofiltration, or the living organisms that remove dissolved waste in aquaria, can be cultivated in a number of ways in closed-system life support systems (LSS). Extruded thermoplastic media, sand, gravel, and live rock are all commonly used in aquaria to house bacteria that oxidize ammonia to nitrite and subsequently to nitrate. The use of live rock in particular has been a proven technique in research mesocosms (e.g. Cato and Brown, 2003; Forsman et al., 2015), and in many public aquaria (Sharp, 2008). Corals and coral reefs are exceptionally dependent on pristine water quality (Pawlowsky, 2008; Cooper et al., 2009; De'ath and Fabricius, 2010), and the ability of live rock to act as an effective primary biological filter for coral systems has been quantified (Yuen et al., 2009; Li et al., 2017).

With coral aquaculture being a key tool to increase the sustainability of the coral trade into the future (Rhyne et al., 2012), and conservation projects such as the *ex situ* genetic refugium of Caribbean coral biodiversity (also referred to as the AZA Florida Reef Tract Rescue Project) being dependent on closed-system LSS, there is an obvious need for non-destructive means of establishing natural nitrifying microbiomes. In recent years, a number of aquarium product manufacturers have started to market ceramic or bio-media, claiming that these products have extremely high surface areas. The prospect of using such media, rather than natural live rock, may be an option that increases sustainability, as well as biosecurity, since rocks often are harvested from the wild, and may harbor vectors from disease outbreaks that are threatening natural reefs.

To assess the viability of these manufactured products as compared to natural live rock, a small controlled trial was undertaken.

Methods

To compare the efficiency of an artificial biological brick filter media (hereafter referred to as bio-bricks) to live rock, five bio-bricks (Brightwell Aquatics Xport BIO™) designed for home hobbyists were purchased, sterilized and inoculated in an aquarium with live rock extracted from the Florida Keys for 45d at 26°C. Media was sterilized with peracetic acid and sodium hypochlorite according to the manufacturer's instructions. Once inoculated, five bricks and five pieces of natural live rock were each placed in an HDPE container with 9.47 liters of artificial seawater. No aeration or water movement was added during the trial so oxygen and water flow would not be variables, and lids were placed on each container to reduce contamination from the surrounding environment. Each container was dosed with an equal amount of a 10g/l ammonium chloride stock solution giving a starting ammonia concentration of 1.25mg/l. Ammonia, nitrite, and nitrate were measured via spectrophotographic analysis (HACH™ methods 8155, 8507, and 10206, respectively) at 6h and 26h after the trial began. All biological substrates (bio-bricks and live rock) were weighed so that consumption of ammonia could be expressed as mg/l consumed per kg media, per hour. This normalization of data allows for more accurate comparison of efficacy of artificial vs. natural media. Plots were created using Microsoft Excel™ and data were analyzed using the =TTEST function of Microsoft Excel™, 2-tailed, assuming unequal variances, α=0.05.

Results

Ammonia consumption per hour (mg NH_3 h^{-1}) between the two substrates, live rock and bio-brick, did differ slightly when the mass was taken into account (Table 1). However, the average ammonia consumption between the live rock and bio-bricks were equal, (Table 1, Figure 1). A 2-sample t-test revealed that there was no significant difference between the consumption of ammonia amongst the two substrates (p=0.99).

After being normalized for mass, the two live rocks with the least mass had the highest consumption rate of ammonia per hour (0.11 mg NH_3 h^{-1} kg^{-1}, Table 1). The piece of live rock with the greatest mass (3.6 kg) had the lowest ammonia consumption rate per hour at 0.03 mg NH_3 h^{-1} kg^{-1}. All five bio-bricks had nearly identical ammonia consumption rates over 26 hours, ranging from 0.06 to 0.08 mg NH_3 h^{-1} kg^{-1} (Table 1). To figure out how much bio-brick replaces live rock, a one to one ratio based on average mass was calculated by dividing the average mass of bricks by average mass of rocks (1.9 kg rock/1.36 kg brick = 1.38 kg). Given the nitrification capacities were the same, we can infer based on the mass differences that it takes 1.38 kg of bio-brick to replace 1 kg (or 2.2 lbs) of live rock.

After 26 hours, nitrite and nitrate levels did start to rise, indicating that the nitrification process had begun. Nitrite levels between live rock and bio-bricks were not significantly different (p=0.94). The average nitrite levels for live rock and bio-bricks were equal (Table 2, Figure 2). On the other hand, live rock had an average nitrate level of 7.26 ppm with a standard deviation of 4.08 and bio-bricks had an average nitrate level of 9.27 ppm with a standard deviation of 1.01. Nitrate levels between the two substrates were not significantly different (p=0.34; Table 2, Figure 3).

Table 1. Consumption of ionic ammonia data from n=10 replicates as a function of biological filter mass and time. Ammonia levels after 6h and 26h of time to process ammonia are reported, and these data are further extrapolated to provide the percent consumption of ionic ammonia as well as the ammonia consumption per unit time and normalized by biofilter substrate mass. Averages shown include standard deviation.

Tank	Substrate	Mass (kg)	NH_3 6h (mg/l)	NH_3 26h (mg/l)	ΔNH_3 (mg/l)	ΔNH_3 (%)	mg NH_3 h^{-1}	mg NH_3 h^{-1} kg^{-1}
1	Rock	2.0	0.30	0.10	1.15	0.92	0.10	0.05
2	Rock	1.0	0.23	0.12	1.13	0.90	0.11	0.11
3	Rock	1.0	0.20	0.16	1.09	0.87	0.11	0.11
4	Rock	1.8	0.26	0.19	1.06	0.85	0.10	0.06
5	Rock	3.6	0.19	0.14	1.11	0.89	0.11	0.03
6	Brick	1.4	0.20	0.26	0.99	0.79	0.11	0.08
7	Brick	1.2	0.40	0.09	1.16	0.93	0.09	0.08
8	Brick	1.4	0.52	0.10	1.15	0.92	0.08	0.06
9	Brick	1.4	0.46	0.30	0.95	0.76	0.08	0.06
10	Brick	1.4	0.17	0.13	1.12	0.90	0.11	0.08
Avg.	Rock	-	-	-	-	-	-	0.07 ± 0.04
Avg.	Brick	-	-	-	-	-	-	0.07 ± 0.01

Table 2. Consumption of nitrite and nitrate data from n=10 replicates as a function of biological filter mass and time. Nitrite and nitrate levels after 26h of time. Averages shown include standard deviation.

Tank	Substrate	Mass (kg)	NO_2 (ppm)	NO_3 (ppm)
1	Rock	2.0	0.04	9.48
2	Rock	1.0	0.03	0
3	Rock	1.0	0.04	8.54
4	Rock	1.8	0.04	8.83
5	Rock	3.6	0.06	9.43
6	Brick	1.4	0.04	9.69
7	Brick	1.2	0.05	9.44
8	Brick	1.4	0.05	7.5
9	Brick	1.4	0.03	9.96
10	Brick	1.4	0.05	9.75
Avg.	Rock	-	0.04 ± 0.01	7.26 ± 4.08
Avg.	Brick	-	0.04 ± 0.008	9.27 ± 1.01

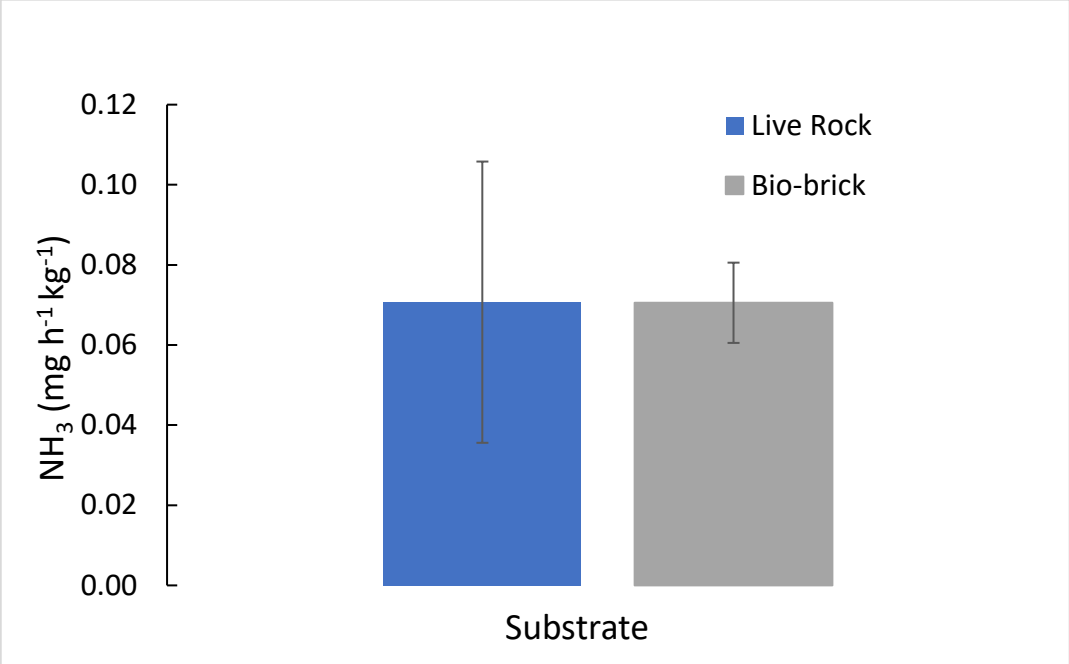


Figure 1. Mean total ammonia (NH_3^-) consumption rates for each substrate normalized for mass. Error bars are standard deviation.

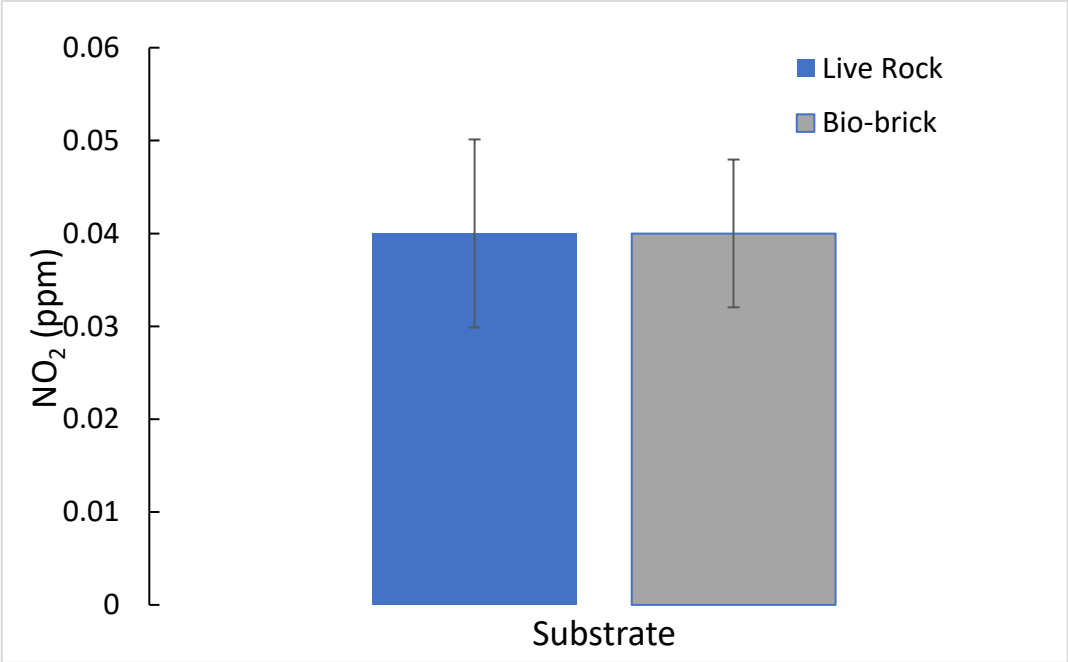


Figure 2. Mean nitrite rates (ppm) for each substrate. Error bars are standard deviation.

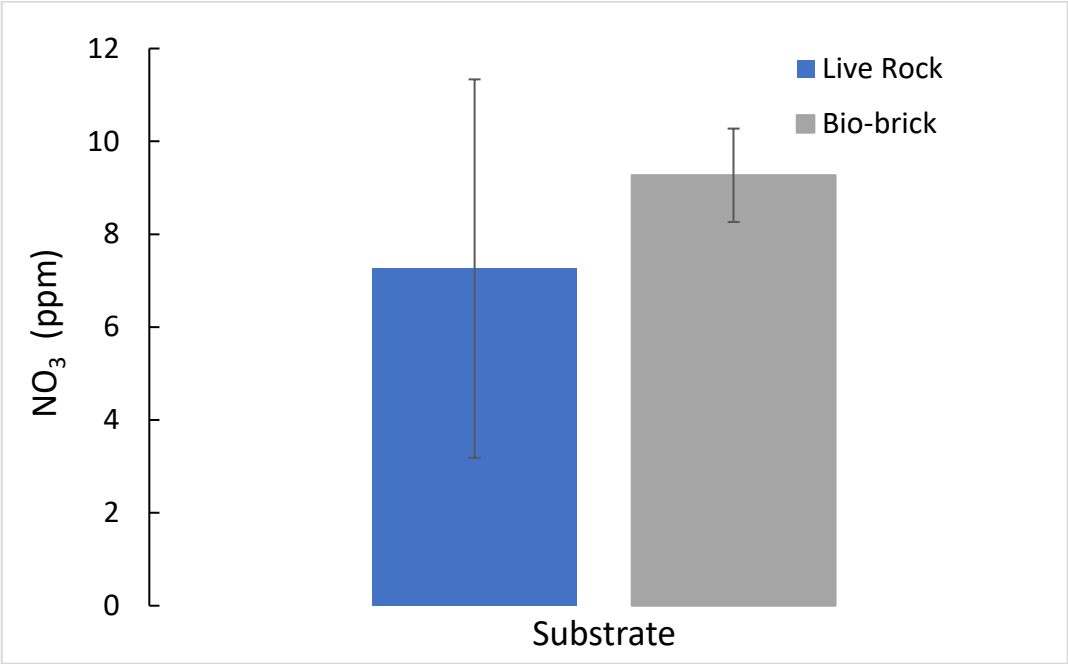


Figure 3. Mean nitrate rates (ppm) for each substrate. Error bars are standard deviation.

Discussion

Live rock has been shown to be one of the most effective ways to remove ammonia and nitrite to maintain optimal water quality for reef building corals (Yuen et al., 2009; Li et al., 2017), and has been the primary source for biological filtration in reef aquariums amongst both hobbyists and public aquariums (Cato and Brown, 2003). Live rock has specifically been largely harvested from Florida for the purpose of keeping corals. If live rock continues to be harvested from the wild, eventually the natural reefs may become negatively affected, which could impact the entire ecosystem. This will not only affect the corals, but also the other species that live on the reef (Cato and Brown, 2003). Artificial bio-media may provide a more sustainable and bio-secure alternative to live rock harvested from the wild. However, there has been little research on whether such artificial media will oxidize ammonia at a rate comparable to live rock.

In this trial, bio-bricks were as effective as live rock in facilitating the nitrification process. Consumption rates of ammonia, did not significantly differ between the two substrates, ($P>0.05$, $\alpha=0.05$). The standard deviation for ammonia consumption rates was higher for live rock, (0.04 mg l^{-1} h^{-1} kg^{-1}), compared to the standard deviation of the bio-bricks (0.01 mg l^{-1} h^{-1} kg^{-1}), indicating that live rock may have oxidized ammonia more efficiently than the bricks. This could be due to more surface area on the live rock compared to the bio-bricks. However, this difference is subtle and in the scope of our study both media are equally suitable substrates for promoting the oxidation of ammonia in reef systems. Repeating this study with similar sized live rock, or extending the study could eliminate this variable.

Though this study did not look specifically at nitrite consumption rates, we did find that nitrite and nitrate levels did not differ significantly between live rock and bio-bricks, with p-values

of 0.94 and 0.34, respectively. There was more variation amongst the NO_2 and NO_3 levels in each trial compared to the variation seen in the NH_3 consumption rates, with the highest variation seen in NO_3 levels. This is most likely because NO_3 is the final by-product of NH_3 oxidation, and is more likely to reflect the subtle variation in each previous step of the process. However, because the levels did not differ significantly between the live rock and bio-bricks, we can assume the oxidation of nitrite was also comparable between the substrates.

Retail price for each brick is about $39.99 (Bulk Reef Supply). Average wholesale and retail prices, for live rock ranges from $7.24 to $19.80 per kg, respectively. Economically, hobbyists in coral aquaculture may find the bio-bricks to be less cost effective than live rock. The amount of live rock needed to maintain stable water quality in a coral reef aquarium varies depending on the size of the tank they are being used in. Typically, 0.5-1.5 kg per gallon of live rock is needed to maintain stable water quality. Given the results of this trial, in order to replace 10 kg of live rock at an approximate cost of $72 - $198, 10 bio-bricks would be needed, which would cost about $400. This is a price difference of up to about 550%, which may be too cost-inhibitive for both hobbyists and public aquaria to completely switch to bio-bricks. The bio-brick is a new product on the market, and not a lot of people are aware of it, or have used it before. As more of this product is purchased, retail prices may eventually drop (Rhyne et al., 2012), and more public and private aquarists will be able to purchase and use both bio-brick and live rock in coral aquaculture. Live rock can vary greatly in size, and depending on size of some rock, may house a larger microbiome due to the variation in surface area. This could have affected our results since the live rock masses were different and the bio-brick masses were very similar.

In the case of the AZA Florida Reef Tract Rescue Project, the primary goal is to protect stony corals from a massive disease outbreak, but it is also imperative that the existing reef is protected to the greatest extent possible, which includes conserving the healthy live rock left on the reef. Products like bio-bricks may allow small and large coral holding facilities to cycle bio-secure tanks without a dependency on locally harvested live rock. This will not only help with shipping costs, but will reduce the amount of live rock being harvested from these stressed and endangered reefs.

Acknowledgements

Thanks to the husbandry advisors and coral holders of the AZA Florida Reef Tract Coral Rescue Project for input and initial discussion on this product as a possible surrogate for live rock. Dr. David Hudson of the Maritime Aquarium provided review of the manuscript. And thank you to Barrett Christie (The Maritime Aquarium) for providing review of the manuscript and supporting the experiment.

Literature Cited

Cato, J.C. and C.L. Brown. 2003. Marine Ornamental Species: Collection, Culture, and Conservation. Blackwell publishing. 208p, 395p

Cooper, T.F., J.P. Gilmour, and K.E. Fabricius. 2009. Bioindicators of changes in water quality on coral reefs: review and recommendations for monitoring programs. Journal of the International Coral Reef Society 28(3): 589-606.

De'ath, G., and K. Fabricius. 2010. Water quality as a regional driver of coral biodiversity and macroalgae on the Great Barrier Reef. Ecological Applications 20(3): 840-850.

Forsman, Z.H., C.A. Page, R.J. Toonen, D. Vaughan. 2015. Growing coral larger and faster: micro-colony-fusion as a strategy for accelerating coral cover. PeerJ e1313.

Li, Y., X. Zheng, X. Yang, D. Ou, R. Lin, X. Liu. 2017. Effects of live rock on removal of dissolved inorganic nitrogen in coral aquaria. Acta Oceanologica Sinica. 36(12): 87-94.

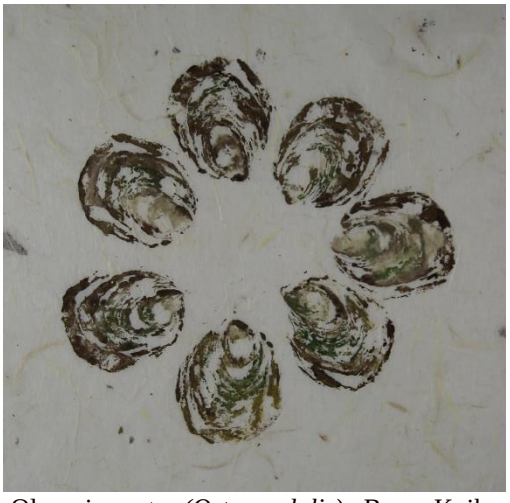
Nedimyer, K., K. Gaines, and S. Roach. 2011. Coral Tree Nursery®: An Innovative Approach to Growing Corals in an Ocean-Based Field Nursery. AACL Bioflux 4(4): 442-446.

Pawlowsky, E. 2008. Macronutrients N P K, levels and management. In Leewis, R.J. and M. Janse (eds.) Advances in Coral Husbandry in Public Aquariums. Public Aquarium Husbandry Series Vol. 2. Burgers' Zoo, Arnhem, Netherlands. pp. 103-114.

Rhyne, A.L., M.F. Tlusty, L. Kaufman. 2012. Long-term trends of coral imports into the United States indicate future opportunities for ecosystem and societal benefits. Conservation Letters. 5(2): 478-485.

Sharp, A. 2008. Ecological purification in the captive reef- natural approaches to water quality management. In Leewis, R.J. and M. Janse (eds.) Advances in Coral Husbandry in Public Aquariums. Public Aquarium Husbandry Series Vol. 2. Burgers' Zoo, Arnhem, Netherlands. pp. 103-114.

Yuen, Y,S., S.Y. Yamazaki, T. Nakamura, G. Tokuda, H. Yamasaki. 2009. Effects of live rock on the reef-building coral *Acropora digitifera* cultured with high levels of nitrogenous compounds. Aquacultural Engineering 41: 35-43.



Olympia oyster *(Ostrea edulis)*. Bruce Koike

Joint Symposium & Workshops

March 28th – April 1st, 2020

Host: Johnny Morris'
Wonders of Wildlife Museum and Aquarium
500 W Sunshine St, Springfield, MO 65807

We, the AALSO and RAW leadership, are thrilled to announce that for the first time, RAW (Regional Aquatics Workshop) and AALSO (Aquatic Animal Life Support Operators) are joining forces for a joint symposium in 2020. This joint conference is an unprecedented opportunity for aquarists, curators, biologists, and researchers to collaborate, share information, and connect with life support operators, water quality technicians, and mechanical systems professionals from across the aquarium and zoological industry.

Registration

You must be a current AALSO Operator Member before registering as an Attendee for the 2020 Joint Symposium & Workshops for taxes and liability purposes. There will be 4 types of registrations this year, Attendee, Exhibitor, Non-Exhibiting Vendor and Guest.

Attendee Registration:

- Become an AALSO member now here;
  - https://aalso.wildapricot.org/page-516287
- Register here:
  - https://www.smartsource-reg.com/2020-aalso/4343/Site/Register
- Early Bird: $395.00 until Sunday March 1st, 2020 at 11:59 PM
- Late Registration: $495.00 after March 1st 2020
- Attendee Day Pass: $125.00
- Attendee Sponsorship Applications will open in the fall with a deadline of February 1st, 2020.

- *This registration is open to all operators, technicians, biologists, aquarists, curators, directors and students*
- Attendee Cancellation Policy: Attendees can cancel with a full refund up to seven (7) days before the start of the Symposium. Substitutions from the same facility can be arranged without penalty. Please send an email to treasurer@aalso.org to initiate a cancellation or substitution.

Exhibiting and Non-Exhibiting Vendor Registration:
All Booths for 2020 Symposium have been sold!
If you would like to be placed on the waiting list, go to the vendor symposium page.
http://new.aalso.org/symposium-vendor-booth-layout

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- University Plaza Hotel, 33 John Q. Hammons Parkway Springfield, Missouri 65806
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The Conference Center
- Springfield Expo Center
- Located across the street from the University Plaza Hotel
- The Lecture Hall and Banquet will be held in the Convention Center, and the Exhibiting Vendor Hall, Registration, and AALSO Store can be found in the Expo Center.

The Airports
The local airport is the Springfield-Branson National Airport (SGF)
- It is served by: United, Delta, American Airlines, and Allegiant.

Other airports in the area:
- 3 hours away in Kansas City: Kansas City International Airport (MCI)
- 3 hours away in St. Louis: Lambert-St. Louis International Airport (STL)

Further Details
All of the above information and more can be found at:
http://new.aalso.org/2020-joint-aalso-raw-symposium-and-workshop-springfield

AALSO / RAW Joint Symposium FAQs:

Why is the registration fee more expensive than usual for a RAW conference?

The early symposium registration rate will be $395, significantly higher than previous RAW conference registration fees. But in addition to all standard RAW conference program opportunities, the registration includes:
- Free public admission to Wonders of Wildlife on Sunday, March 29th.
- 2020 Conference T-shirt
- Daily hot breakfast AND lunch during the main conference.

- Icebreaker event admission to WoW, including transportation, food and (2) beverages
- Includes all hands on, dry and/or classroom style workshops.
- Includes all hands on, wet workshops on the B.A.W.L (Big Automated Water Loop)
- Includes all admittance to all lectures
- Includes ability to take LSS or Water Quality Certification exams
- Admission to final night Certification Banquet, with buffet dinner
- Access to ~75 industry vendors at the expo.

We've negotiated a very low, $121 / night rate at the main conference hotel which will be more affordable than most recent RAW conference hotels. We expect that the total cost for attending 2020's conference will be in line with past RAWs or even cheaper, depending upon your travel logistics.

Why do I have to become an AALSO member to register for the conference?

As a legal functioning body, AALSO must provide insurance to cover the conference activities of a formal membership body. The nominal $20 AALSO membership fee provides you AALSO membership for one year. You MUST be a member of AALSO before you can finalize your registration. If you are not already an AALSO member, you'll be prompted to join prior to your online symposium registration.

What will be different this year from past RAW conferences?

In addition to our traditional robust schedule of talks, networking opportunities, and TAG activities, the AALSO / RAW joint symposium will offer exam opportunities for both LSS and WQ certification programs, access to dozens of hands-on workshops, a massive vendor hall, and the world-famous BAWL (Big Automated Water Loop). It'll be everything you've come to expect from RAW and more!

Will there be a registration cap this year? How about presentations?

There will be no registration cap for the 2020 joint conference. We expect at least 600 attendees and potentially many, many more. There will, as always, be limited space in the program for formal presentations and a call for abstracts will go live in the fall of 2019.

What about next year and the future of RAW?

For now, the RAW / AALSO conference partnership is a **one-time** event. We'll return to a standalone RAW conference in 2021 in Orlando, FL.

Rough Agenda *(next page)*

2020 Symposium Agenda

Saturday, March 28	
8:00 am to 4:00 pm	**OSHA Part 1**
8:00 am to 4:00 pm	**CPO Course**
9:00 am to 12:00 pm	Marine Fish Taxon Advisory Group Steering Committee- Closed
9:00 am to 5:00 pm	**BAWL Setup**
1:00 pm to 4:00 pm	Freshwater Fish Taxon Advisory Group Steering Committee- Closed
4:00 pm to 7:00 pm	Aquatic Invert Taxon Advisory Group Steering Committee- Closed
Sunday, March 29	
8:00 am to 4:00 pm	**OSHA Part 2**
8:00 am to 6:00 pm	**Attendee and Guest Registration**
9:00 am to 5:00 pm	**BAWL Setup**
10:00 am to 12:00 pm	**Marine Fish Taxon Advisory Group- Public**
10:00 am to 12:00 pm	**LSS 3 and WQ 1&2 Exam Review Sessions**
1:00 pm to 3:00 pm	**Freshwater Fish Taxon Advisory Group- Public**
1:00 pm to 3:00 pm	**WQ 3 and LSS 1&2 Exam Review Sessions**
12:00 pm to 6:00 pm	**Vendor Registration**
3:00 pm to 5:00 pm	**Aquatic Invert Taxon Advisory Group- Public**
5:00 pm to 8:00 pm (6:30 pm to 7:30 pm)	**Ice Breaker/Vendor Interaction** Poster Session
Monday, March 30	
7:00 am to 8:00 am	**Registration and Breakfast**
7:30 am to 8:00 am	**Vendor Meeting- Vendor Hall**
8:00 am to 8:30 am	**Opening Ceremonies**
8:30 am to 8:50 am	**Host Facility Spotlight**
8:50 am to 9:25 am	**Exhibiting Vendor Introductions**
9:25 am to 09:40 am	**First Time Attendee Presentation**
9:25 am to 10:10 am	**Break / Vendor Interaction**
10:10 am to 12:00 pm	**Lecture Series & Workshops**
12:00 pm to 1:00 pm	**Lunch / Vendor Interaction. Felts Vendor Hall**
1:00 pm to 2:30 pm (1:00-2:30) (1:30-2:30)	**Certification Test WQ 3 & LSS Levels 1, 2 & Workshops** WQ Level 3 LSS Levels 1 & 2
2:30 pm to 3:15 pm	**Break / Vendor Interaction. Felts Vendor Hall**
3:15 pm to 4:45 pm (3:15-4:45) (3:45-4:45)	**Certification Test LSS 3 & WQ Levels 1, 2 & Workshops** LSS Level 3 WQ Levels 1 & 2
6:30 pm to 10:00 pm	**Evening Event at Wonders of Wildlife** Busses depart hotel at 6:15
Tuesday, March 31	
7:00 am to 8:00 am	**Breakfast**
8:00 am to 9:20 am	**Lecture Series & Workshops**
9:20 am to 10:00 am	**Break / Vendor Interaction. Felts Vendor Hall**
10:00 am to 10:45 am	**Lecture Series & Workshops**
11:00 am to 12:00 pm	**The Future of AALSO, Business Meeting, Elections (OK/IL)** **RAW Business Meeting (Lecture Hall)**
12:00 pm to 1:00 pm	**Lunch / Vendor Interaction. Felts Vendor Hall**
1:00 pm to 2:30 pm	**Lecture Series & Workshops**
2:30 pm to 3:00 pm	**Break / Vendor Interaction. Felts Vendor Hall**
3:00 pm to 5:30 pm	**Lecture Series & Workshops**
Wednesday, April 1	
7:00 am to 8:00 am	**Breakfast**
7:30 am to 8:00 am	**Vendor Meeting Elections- Vendor Hall**
8:00 am to 9:40 am	**Lecture Series Block 6 & Workshops**
9:40 am to 10:30 am	**Break / Vendor Interaction. Felts Vendor Hall**
10:30 am to 12:00 pm	**Lecture Series & Workshops**
12:00 pm to 1:00 pm	**Lunch / Vendor Interaction. Felts Vendor Hall**
1:00 pm to 3:40 pm	**Vendor Breakdown**
1:00 pm to 2:30 pm	**Lecture Series & Workshops**
2:30 pm to 2:45 pm	**Break**
2:45 pm to 4:00 pm	**Lecture Series & Workshops**
6:30 pm to 9:30 pm	**Certification Banquet**

RAW 2019 ABSTRACTS

"ReRAW," The Regional Aquatics Workshop, May 13-17. The Columbus Zoo and Aquarium. Columbus, OH, USA.

AZA Aquatic TAG Steering Committee Meetings – May 12th.

Monday, May 13th

Session 1

Welcome;
Doug Warmolts, Vice President of Animal Care, Columbus Zoo and Aquarium

Keynote Address:
A Brief History of RAW
Pete Mohan
Akron Zoo / *Drum and Croaker*
p.mohan@akronzoo.org

Chambering the Chambered Nautilus:
Raising *Nautilus pompilius* Hatchlings in Pressure Chambers
Ellen Umeda
Monterey Bay Aquarium
eumeda@mbayaq.org

Chambered nautilus eggs have never been found in the wild and rarely hatch out in public aquariums. As a result, determining the appropriate parameters for a sustainable culture has proved challenging. In the wild, nautiluses live at depth under high pressure. This pressure at depth likely influences the rate and development of new chambers in the shell of a nautilus. The Monterey Bay Aquarium built pressure chambers to examine the life span of nautilus hatchlings when kept under pressure. In addition, we hatched eggs and raised hatchlings at various temperatures to examine the effects on hatch rate and life span. In total, the Monterey Bay Aquarium hatched out 16 hatchlings with the longest living 167 days. Although our results remain inconclusive, we expanded our nautilus culturing knowledge and created a foundation for new ideas and methods moving forward.

Behavioral Complexity in Cephalopods (and I'm Not Talking Octopus...)

Gregory Jeff Barord, PhD
Central Campus and Save the Nautilus
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The behavioral plasticity of nautiluses (Family Nautilidae) is so simply, complex that it has been overshadowed by its more conspicuous coleoid cousins. Leaving aside the mind-boggling complex ability to adjust its internal atmospheric pressure as it jets through the water column, recent behavioral observations have started to show nautiluses as something more than a dumb octopus in a shell. A population of nautiluses surveyed in Palau displayed behaviors not observed previously which suggests a flexible repertoire of behaviors available, depending on the conditions. And that these behaviors may be shifted over the course of just a couple of generations. Currently, husbandry and management are relatively similar for all nautilus populations. I propose that we change this way of thinking to improve husbandry practices and development management strategies that result in sustained sustainability.

Sponsor Presentation:
Aqua Logic Inc

Utilization of Advanced Diagnostic Imaging in a Zebra Shark (*Stegostoma fasciatum*)

Katie Seeley
The Columbus Zoo and Aquarium
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Elasmobranchs are popular exhibit animals in both aquaria and zoological institutions and serve a vital role as ambassadors for their wild counterparts. Many of the standard diagnostic tools used in veterinary medicine can be difficult to apply to aquatic species and require modifications for the aquatic environment. The use of advanced diagnostics imaging, specifically computed tomography (CT), has become more commonplace and serves as an important tool when ultrasounds and radiographs are insufficient. A 15-year-old female zebra shark (*Stegostoma fasciatum*) presented with a distended abdomen. Ultrasound showed evidence of eggs, but there were areas of concern within the liver tissue. With careful planning and logistical input from the husbandry and veterinary team a CT was performed which provided essential information and allowed for appropriate clinical management of the shark. This case illustrates that with creativity and forethought CT can be safely utilized in aquatic species.

Session 2

Sponsor Presentation:
The Aquarium Vet

AZA Aquatic Invertebrate Taxon Advisory Group (AITAG)
Reporting Meeting

Sponsor Presentation:
Animal Professional

Session 3

The Use of "Omics" in Freshwater Mussel Conservation

Ieva Roznere
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Freshwater mussels are the most endangered group of animals in North America. A common conservation technique involves translocating mussels to different habitats or bringing them into captivity. However, this often results in increased mortality and slower growth and, despite the necessity, we know very little about freshwater mussel health. We use "omics" techniques, such as transcriptomics and metabolomics, to better understand the physiology of these animals and how they respond to stress. Transcriptomics is the study of transcripts, the subset of genes that are being expressed at a certain time period. Metabolomics is the study of metabolites, the intermediates and products of metabolism. Because gene expression and metabolite production are closely associated with environmental conditions, studying changes in these biological molecules is especially helpful in understanding how animals react to environmental stressors.

Freshwater Mussel In Vitro Research

Jacqualyn Halmacher
The Ohio State University, The Columbus Zoo and Aquarium, and Ohio Division of Wildlife
Halmbacher.2@osu.edu

Since 2002, the Columbus Zoo & Aquarium's Freshwater Mussel Conservation and Research Facility has been dedicated to reintroducing mussel populations back into Ohio's rivers. The success experienced by this facility has resulted in the release of tens of thousands of mussels in Ohio via propagation and reintroduction; receiving accolades such as "The North American Conservation Award" in 2011. Recently, the facility has experienced a significantly higher degree of success in propagating freshwater mussels by implementing a cell culture technique known as in vitro. The Columbus Zoo & Aquarium's Freshwater Mussel Conservation and Research Facility is among a handful of institutions across the United States successfully transforming juvenile mussels with this innovative technique. In vitro offers an alternative: eliminating host fish from the equation. The protocol allows thousands of juveniles to be cultured in one petri dish. This presentation will give insight into the in vitro research conducted at the Columbus Zoo & Aquarium's facility partnered with The Ohio State University and Ohio Division of Wildlife.

Sponsor Presentation:
Tenji Aquarium Design and Build

Flexing Our Mussels, Part Deux

Andy Allison and Mikaela
National Mississippi River Museum & Aquarium
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The National Mississippi River Museum & Aquarium has made a commitment to help its partners to restore and interpret freshwater mussels, considered by many to be among the most threatened taxa in North America. Our live freshwater mussel display has been a great introduction for much of the public who do not otherwise get to see live mussel beds. We intend to describe the husbandry methods that have been successful or us. In addition, our collaborative partnerships have been instrumental in jumpstarting additional conservation work, opening the door to resources that would not have otherwise been available to us. Learn how our simple early collaboration efforts have led to big opportunities to do conservation and research. Our hope is that this presentation will inspire other facilities, both big and small, to start or expand their own mussel projects.

Citizen Science, Ex-Situ, and In-Situ Unionid Mussel Conservation: Possibilities for Zoos and Aquariums

Barrett L. Christie
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Unionid molluscs are the most endangered fauna in North America, at present we are losing species faster than their populations can even be assessed. Avenues for conservation action include a range of possibilities such as citizen science, rescue, and small-scale propagation efforts. Aquarists, researchers, and even educators can utilize their skillsets in animal care, transport, aquaculture, field collection, or science communication to contribute to conservation activities or promote awareness of this unique and fascinating taxon. In one case study, a small aquarium in the western U.S. was able to survey over 10,000 animals, rescue another 3500 from drought, and evolve to propagating threatened species never before bred in captivity. An overview of the scope of freshwater mussel conservation programs from aquaria, zoos, museums, and their partners will show how a facility with any size budget and resources can work towards ensuring the survival the most imperiled animals on our continent.

Session 4

A Study of Culturing and Maintaining Jellyfish in Captivity: An Evolution of the Techniques Demonstrated with the Culture of *Chrysaora fuscescens*

Marie-Lyne Deshaies
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Jellyfish aquarists know how developing expertise in their field goes mainly by using a trial and error approach. The Aquarium du Québec has eleven tanks exclusively reserved for the display of jellies since the opening of a new building in 2012. This presentation is an overview of the lessons learned over the past six years. The team is now reproducing and maintaining seven different species all year round. The focus here is on the culture of C. Fuscescens because it allows observing the responses to changes over a single generation. In order to be successful, the Aquarium developed specific equipment and used different iodide solutions. Moreover, a proper feeding schedule with enrichment including SELCO and "Gel diet'' was really a game-changer. The evolution of the techniques enabled to develop a team with expertise, creativity and methodological skills. With good established practices, it is possible to display great cultured jellyfish exhibits.

Sponsor Presentation:
Flying Sharks

The Use of Visible Implant Elastomer (VIE) Tags in Jellyfish

Rachel Stein, Bianca Milano, Lindsey Levine
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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 in the field, there are no known methods for identifying and tracking smaller individuals in aquariums. We tested visible implant elastomer (VIE) tags on Aurelia aurita medusae ranging from 2.5 to 16 cm bell diameter. Tags were retained for 5 month and counting, did not cause significant deformities in the animals, and inspired additional research projects by staff. To our knowledge this presentation documents the first time VIE tags have been used in jellies at a zoo or aquarium, and reveals a new tool for record keeping, research, and monitoring the success of jelly culture and care.

Aiptasia Anemones: An Overlooked and Cost-effective Invertebrate Enrichment

Sara Stevens
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Dermasterias imbricata (Leather star) is a species housed by numerous zoos and aquariums around the world. Aiptasia spp. are pests that any aquarist can grow in almost any system. Put them together and you get unparalleled leather star enrichment. After initiating several feeding trials it was found that leather stars consistently preferred the pest anemones when presented with a choice of aiptasia spp. to standard diet items. Quantitative analysis for speed of movement and duration of foraging behavior allowed for measurement of sea star engagement with the new enrichment item. In conclusion, this dietary experimentation found a cost-effective and sustainably-sourced enrichment and nutritional supplement for leather star that most facilities can easily reproduce without breaking the bank.

Session 5

AZA Elasmobranch Species Survival Plan (SSP)

Open Stakeholder Meeting

Session 6

Florida Reef Tract Rescue Plan (FRTRP)

Stakeholder Meeting

Tuesday, May 14th

Session 7

History and Evolution of Collection Gear and Species Caught Over the Last Fifty Years

Forrest A. Young
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During the course of the last 50 years the author has been an active participant in the marine life fishery for the express purpose of supplying display animals to public aquariums, zoos, research facilities and the hobby. During that time gear has evolved substantially. Diving technology for the author's work has gone from simple open circuit SCUBA beginning in 1969 to technical diving with mixed gas rebreathers (2000-present) and finally with submersibles (2006-present). Collection gear has also seen tremendous evolution. Starting off with tiny plastic hand nets and slurp guns and developing into deployment of sophisticated barrier nets and submersible deployed gear. The species list has also seen a tremendous evolution as new species have been added to evolving public display husbandry abilities. Gear technology and the evolution of the species targeted will be summarized on a chronological basis in addition to a full comparison to

gear use in the undeveloped world that supplies the bulk of marine tropical species to the pet trade and public display.

Sponsor Presentation:
ReefBrite

Duped by a Fish:
Livebearing Adventures in Larval Rearing of the Black Brotula, *Stygnobrotula latebricola*

Allison Waltz-Hill and Jeremy Brodt
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The black brotula, *Stygnobrotula latebricola*, is an Atlantic Ophidiiform of the family Bythitidae (viviparous brotulas). Black brotulas are infrequently displayed in public aquaria due to their reputation as a cryptic species with complex husbandry requirements. As a result, their life history and reproductive habits are poorly understood. The New England Aquarium has successfully displayed this species in a mixed taxa exhibit for six years. In 2017, an individual that was placed in holding with a conspecific became pregnant and began a cycle of recurring pregnancies and births (five thus far), culminating in three captive-raised black brotulas to date. These events have allowed us to determine that the approximate gestation is 3-4 months and larval yield ranges from ~1,700-3,600. The female's lack of access to a male prior to two pregnancies suggests sperm storage or parthenogenesis as a reproductive strategy in this species. Further inquiry is ongoing to identify which reproductive strategy is being utilized as well as to refine larval husbandry.

Operation Chill Out:
New Husbandry Techniques for Split-Fin Flashlight Fish *(Anomalops katoptron)*

Nikki Eisenmenger & Tasha Esaki
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The Tennessee Aquarium opened a new gallery that included a 3000 gallon split fin flashlight fish *(Anomalops katoptron)* exhibit that holds approximately 300 flashlight fish. Flashlight fish are commonly kept in the 22-25.5°C (72-78° F) range, however wild caught specimens have been found at depths of up to 365m (1200 ft) where temperatures have been reported below 15.5°C (60° F). There would be several advantages to keeping the fish at this lower temperature some of which would be decreased metabolism, increased oxygen for the light organ, and disease management. A small system was built that would allow us to test lower temperatures on the flashlight fish. A group with varying levels of light were moved into this testing system to see what impacts there would be to metabolism, light brightness, light recovery, and overall health.

Sponsor Presentation:
Aquatic Exhibits International

Session 8

Sponsor Presentation:
McRoberts Sales Co. Inc.

AZA Marine Fishes Taxon Advisory Group (MFTAG)
Reporting Meeting

Sponsor Presentation:
Dynasty Marine Associates, Inc.

Session 9

Sponsor Presentation:
Piscine Energetics

Advances in SAFE Sharks and Rays

Beth Firchau
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SAFE: Saving Animals From Extinction (SAFE) is an Association of Zoos and Aquariums (AZA) initiative that focuses the collective expertise within AZA-accredited zoos and aquariums and leverages their massive audiences to save species. Established in 2015, the projects of the SAFE: Shark and Ray Program's Conservation Action Plan (CAP) fall within four specific thematic areas including public action, policy and legislation, research, and Species Survival Plans®. Each project, designed to incorporate collaborator expertise to maximize efficiency and effectiveness, is comprised of detailed goals, actions, timelines, budgets. Project coordinators leading the efforts within the Shark and Ray Program will provide updates on progress towards CAP goals including new and innovative products created to enhance collective approaches to communicating shark and ray messages, efforts in conservation and advances in animal care. Avenues for participation and collaboration within existing efforts and future efforts with the new Conservation Action Plan development in 2019 will be shared.

AZA Shark SAFE, Animal Care Manuals

Kelli Cadenas
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Pulling together leadership from the Marine Fish Taxon Advisory Group, The AZA Shark and Ray Saving Animals from Extinction (SAFE) project has created a template for elasmobranch care manuals that will be used to make species specific care manuals for species with SAFE SSPs (Species Survival Programs.) Next steps for the project include working with SSP teams and making calls for participation. This presentation will go over how people can help with this project and why it's important. By creating well written and easy to use species specific manuals, we can increase the level of care for our elasmobranchs and better identify what husbandry research is needed in the future.

Sponsor Presentation:
Abyzz by Venotec GmbH/CoralVue

#ChondroCensus 2019: A Roll Call for Chondrichthyans

Jennie Janssen[1], Alan Henningsen[1], and Tony Niemann[2]
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The International Census of Chondrichthyans in Human Care (ICCHC), part of the AZA SAFE: Sharks and Rays sustainability project, has established a user-friendly web-based platform to house and maintain a global census of chondrichthyans in public aquaria, research facilities, and beyond. The goals of the ICCHC include facilitating communication and supporting cooperative research, conservation of at-risk species, and collaborative breeding programs. Over 20 Regional Coordinators have been recruited worldwide to rally and assist ICCHC participants. Each facility participating in the ICCHC is considered a team with a coordinator that approves or denies user permissions to view or edit their team's data. In this way, each facility maintains control of their ICCHC data. Incorporating data from the former AES International Elasmobranch Census, the ICCHC already includes over 200 species from more than 130 facilities representing over 30 countries.

Supplementation of Elasmobranchs: Are We Hitting the Mark?

Jennifer Wyffels
South East Zoo Alliance for Reproduction & Conservation
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Elasmobranchs in aquaria often receive a supplement which provides vitamins and trace minerals designed to mimic the essential nutrients found in the diets of wild sharks. This is necessary due to vitamin degradation during food storage, differences in water quality, and/or not meeting the nutrient requirements in the wild-type diet. Because elasmobranchs in aquaria are susceptible to goiter caused by chronic iodide deficiency, iodine usually is included in

supplements. Thyroid hormones are important regulators of growth and metabolism and influence reproduction. For mature male sand tiger sharks in aquaria, blood plasma iodine was higher and thyroid hormone lower than wild mature male sand tiger sharks for samples collected during spring and summer. No difference in iodine or thyroid hormone concentration was observed between seasons. For male sand tiger sharks in aquaria, high iodine and low thyroid hormone may contribute to the lack of reproductive success historically observed for this species.

Retro Brevi-RAW-stris: Caring for Geriatric Lemon Sharks

Kassie Harold and Alyssa Daily
OdySea Aquarium
kharrold@odyseaaquarium.com, adaily@odyseaaquarium.com

Uncommon to exhibit in human care, *Negaprion brevirostris* pose certain challenges in their everyday care. These challenges are exacerbated when the sharks exceed 25 years of age. At OdySea Aquarium, 2.1 geriatric lemon sharks are handled with much different care than their elasmobranch counterparts. From their arrival at OdySea, the simple task of handling these sensitive individuals proved to be the first of many learning experiences. These animals required more attention to detail when establishing a feeding method and showed many signs of medical anomalies from the beginning. The challenges continued with tumultuous interactions with other species housed in their 400,000 gallon exhibit, resulting in medical care rarely practiced on sharks of this size and age. With persistence, these animals have improved not only in their natural behaviors, but also in their interactions with staff. The care for these elderly sharks has transformed OdySea's views on traditional elasmobranch husbandry.

Session 10

Parthenogenesis in the Epaulette Shark *Hemiscyllium ocellatum*

Sarah Tempesta
New England Aquarium
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Parthenogenesis, or the development of an embryo without fertilization, has been recorded in at least six species of shark and two species of ray. However, it has never been recorded in the epaulette shark *Hemiscyllium ocellatum*. At the New England Aquarium, we have confirmed via genetic testing one birth as the product of parthenogenesis. Test results showed the offspring to be homozygous across all microsatellite loci tested. There have been three other births here under similar conditions and are presumed to be parthenogenesis as well, though the animals are not yet large enough to be tested. This has occurred in two different exhibits, meaning at least two of our ten female epaulettes are producing offspring without the presence of a male. We have had an epaulette breeding program here for a decade and will compare differences in birth size, growth rate, and behavior between our normal and parthenogenetic offspring.

Sponsor Presentation:
Tracks Software

Power of Ultrasound:
A New Technology Provides Insight into The Reproductive Biology of Elasmobranches

Taketeru Tomita
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In the Okinawa Churaumi Aquarium, approximately 40 species of elasmobranchs have given birth in captivity, and many of them are world-first records. Since 2008, we have used an "underwater ultrasound" technique to observe previously unknown embryonic behavior in the maternal body. Accordingly, we revealed two reproductive mechanisms of viviparous elasmobranchs. First, many viviparous shark embryos use buccal pumping, which is the first direct evidence that these embryos obtain oxygen from the uterine fluid through their gills. Second, embryos of some shark species have a strong swimming ability, which may be an adaptation to search for nutritive eggs in utero. These findings emphasize that public aquaria have high potential for extending knowledge about the reproductive mechanisms of large aquatic animals that are difficult to study in the wild.

Advances in the Care and Keeping of *Mobula hypostoma*

Frank Young
Dynasty Marine
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Mobula hypostoma are a dynamic display animal that can be added to most medium to large displays with the proper husbandry techniques and preparation. The proper care for this species as well as other mobulids is more labor intensive and exact than any other rays. Dynasty Marine was worked with this species since 2014 and have successfully collected and supplied them to numerous facilities around the world. Over the course of this time, the husbandry protocol has been significantly advanced but further exploration is still needed. Like most advances in science, the best lessons are learned from failure. The majority of these failures have been overcome and new protocols are in place to avoid repeating past mistakes.

Sygnathid Stakeholder Meeting

Wednesday, May 15th
Session 11

Sponsor Presentation:
US Mysids, Inc.

Moving the Needle for Aquatic Collections Sustainability

Hap Fatzinger
NC Aquarium at Fort Fisher
Hap.fatzinger@ncaquariums.com

Aquatic collection sustainability has been a priority across our facilities and AZA for decades. Great work has been accomplished and AZA animal programs continue to grow and build upon the foundation of work developed by leaders in our field. Over the past year, a surge of efforts has captured the attention and support of AZA and institutional leadership. This presentation will discuss the development of the AZA Board-approved Aquatic Collections Sustainability Special Committee, outline the five priorities identified for action and share future opportunities for supporting the work.

Considerations for a Sustainable Animal Collection

Sandy Trautwein, Ph.D
Aquarium of the Pacific
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Increasing public pressure and new regulations affecting the marine ornamental trade have made it more difficult for public aquariums to procure wild specimens. The development of sustainable animal collections that include ethical species acquisition choices, the establishment of propagation programs, and the creation of robust collection plans can provide a solid platform for mitigating public concerns. In addition, a focus on maintaining excellent care and welfare standards can extend the life of aquarium animals and reduce the demand for wild-caught specimens. This presentation will focus on the importance of developing a robust institutional collection plan, and tips for extending the life of our animal collections, an approach to assessing where our animals come from, and how their welfare and sustainability can be improved.

Approach to Assessing Where Animals Come From

Anna Hildebrandt and Chris Andrews
SEA LIFE
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Unlike zoos, and aquariums specializing in freshwater organisms, a large proportion of the marine fish, elasmobranchs and invertebrates in public aquariums are obtained from the wild, exposing them to potential criticism regarding how the animals are collected, and their care and welfare during the acquisition, transport and acclimation process. This presentation with discuss

one approach to better understand the supply chains used to provide these wild-caught animals for public aquariums, and how the results may be used to improve animal welfare and – ultimately – sustainability practices.

Sponsor Presentation:
TJP, Inc.

Transports Then and Now: A New Paradigm in Marine Animals Collections

Joao Correia, Rui Guedes, David Silva, Luis Silva, Pedro Marques and Telmo Morato
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The opening of the Oceanário de Lisboa, where Flying Sharks' founding staff originated from, involved the collection and transport of animals from literally every corner of the planet to Lisbon, which was a monumental exercise in the development of long-term transport techniques. These techniques were then refined over two decades, allowing for the collection and transport of species once considered 'impossible', such as *Scomber sp., Sarda sarda, Mola mola, Naucrates ductor*, and an assortment of jellyfish, among multiple others. Such advancements include the replacement of 12 V systems for 220 V, while ammonia and pH are no longer a concern, thanks to recent developments in quenching and buffering agents. Additionally, a new paradigm in marine animal transport is presented, whereas buffering agents are used preventively and not correctively, while L.S.S. is designed for long-term maintenance and not just transport conditions. This turned our 'transport unit' into a 'mobile holding station'.

For the Love of Fish, We're in It Together;
The Importance of the Aquarium Hobby to Public Aquaria

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The aquarium industry is under attack. Both public and private aquaria are being demonized by activist groups around the world. This criticism is causing a divide in an industry that should be united. As public aquarium aquarists we need to understand the link between our businesses and the hobby; how private and public aquaria are inextricably linked. Most innovation in aquarium keeping has come from the private sector or by companies supplying it. Public aquaria are making important contributions like education and conservation but the reality is that the world of aquarium keeping is driven by hobbyists, whether it be lighting technology, advances in life support systems, water chemistry/quality analysis or provision of livestock, suppliers could never survive on public institutions alone. The entire industry is under scrutiny, we need to be allies. Supporting and working together is the only way to survive and continue the work we love.

Are Your Fish Legal? An Analysis of the Regulatory and Permitting for Wild Sourced Display Animals out of Florida and the Eastern U.S.

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As an active participant in the marine life fishery to public aquariums for more than 3 decades the complexity of fisheries management, reporting, permitting and compliance has increased substantially. Many species are newly being evaluated for additional protections at state, national, and international levels using SAL, HMS, ESA, and CITES. Taking an active part in the fishery management process and being a proactive participant in helping to craft intelligent conservation regulations to insure long term sustainability is essential. Industries hand in developing best practices for legally and properly collecting and shipping these specimens is discussed. The objective within is to help public aquariums to understand what is necessary to ensure that your collection is legal and that aquariums can continue to source wild caught animals in a legal, sustainable, and ethical way into the future.

Session 12

Accomplishments and Challenges for Rising Tide Conservation

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Rising Tide Conservation is a stakeholder-based initiative designed to promote aquaculture of marine ornamental species. Since the inception, the program has involved stakeholders from both the hobby industry and display aquaria. Major accomplishments of this program include the successful rearing and promoting commercial propagation of yellow tangs. Success in propagation of Pacific blue tangs was also achieved but no commercial propagation has succeeded as yet (now over 2 years from the first successes with this species). Training of students has resulted in identifiable capacity building. Two former program biologists, Matt Wittenrich and Kevin Barden are both now involved in commercial propagation companies. The greatest current challenge is a need for more display aquaria as stakeholders. Now that Rising Tide has something to share, facilities can become stakeholders by holding and conditioning broodstock, collecting eggs, developing aquacultured display tanks, and including aquaculture programs in conservation efforts and messaging.

Update: Unified Vendor Reference Process for Animal Transactions with Non-AZA Facilities

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AZA member institutions are required to evaluate any facilities that they acquire animals from for animal care, sustainability, as well as legal and ethical considerations. Because many aquatic AZA facilities utilize the same non-AZA facilities to acquire animals, it would be helpful if the results of the evaluation process could be shared among AZA institutions. Over the past few years the aquatic TAGs have been working on such a unified vendor reference process for aquatic animal transactions, which has included the formation of the Consolidated Supplier Reference Taskforce (CSR). Representatives of the CSR Taskforce will highlight the progress made so far, and discuss the need for site inspectors and volunteers to help move this effort forward.

Sponsor Presentation:
Asahi/America, Inc.

Can I Get a Garibaldi Without Going to Jail?

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Many institutions either want or have California animals on display. Unfortunately, there is much confusion on what, where, when, and what size of marine life we are permitted to take or possess, as well as who can collect them. I will guide you through the California Department of Fish and Wildlife's new permit process which includes chain of custody, marine aquaria, entity or standard permits, as well as what fish and invertebrates can be collected, gifted, or purchased with or without a scientific or commercial permit.

Marine Conservation & Seafood Security: How OGL's DNA bank Advances Research

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DNA contains a wealth of information about an organism's adaptations, interactions, and life history. At Ocean Genome Legacy (OGL), a non-profit marine genome bank that preserves marine DNA samples, we collaborate with academic researchers, museums, governmental agencies and aquariums to collect marine samples from around the world. OGL's collection now contains more than 27,000 genomic (DNA) samples of marine animals, plants, fungi and bacteria. We make these samples available to researchers and scientists in diverse disciplines. Our samples have been used for the development of seafood reference materials that are critical for seafood species identification and the maintenance of sustainable fisheries, detection of genetically

modified salmon, increased understanding of unusual animals like narwhals, and the conservation of protected species, such as black corals.

The Future of Odor Control and Disinfection

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Chlorine dioxide technology has really taken off over the past couple of years in the farming/ hydroponic agriculture and medical research industries. Chlorine dioxide has been around since 1941, and has been for years used by for black mold remediation, and for large scale water sanitation. The EPA lists Chlorine dioxide as the #1 chemical for disinfection, due to its effectiveness at low concentrations and the absence of harmless byproducts – a stark contrast to bleach and other chemical disinfectants. These issues are exactly why in 1983 the EPA recommended Chlorine dioxide as the ideal disinfectant of potable water.

Until recently, Chlorine dioxide was expensive and difficult to produce, and only cost effective in large commercial applications. Now that it is commercially available in small and stable quantities, Chlorine dioxide has shown to be a great alternative to Chlorine/Bleach and other chemicals. It is safer to handle, less corrosive and environmentally safer than bleach. There are a many more reasons why Chlorine Dioxide is the new choice for water sanitation and disinfection.

Zoo Day

Thursday, May 16th
Session 13

Thinking Outside the Aquarium:
Taking an Interdisciplinary Approach to Animal Husbandry

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By using some creative thinking, The Aquarium of the Pacific reached beyond the traditional and found alternative methods to improve the husbandry of corals. Our process of discovery and eventual success involved looking outside of our aquarium box and instead to horticulture, art, dentistry, medicine and orthopedics. From these disciplines, we adapted more efficient techniques for cleaning, fragmenting and treating coral, and doing so has led to better coral growth and recovery from disease. This use of unconventional methods to solve difficult problems could help others in our industry succeed in displaying species we never thought we could.

Florida's Ongoing Coral Disease Outbreak:
Status, Key Research Findings, and Conservation Opportunities

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Since 2014, the Florida Reef Tract has been experiencing a devastating outbreak of the novel Stony Coral Tissue Loss Disease (SCTLD). SCTLD is known to affect roughly 20 coral species in Florida, including major framework-builders and species listed pursuant to the Endangered Species Act. SCTLD is a contagious disease with high species-specific prevalence and mortality rates, leading to significant impacts to nearshore coral communities and the near extirpation of highly susceptible species from some impacted reefs. A key component of the management response to SCTLD is the Florida Reef Tract Rescue Program. This program seeks to collect colonies of susceptible species from unimpacted areas in order to preserve some of the remaining genetic diversity. Rescued colonies will be distributed among land- based care and propagation facilities, including affiliates of the Association of Zoos and Aquariums, to serve as the basis for future coral restoration efforts in Florida.

The Coral Crisis:
Assessing the Conservation Potential of Captive Coral Populations in Aquariums and Zoos

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Marine ecosystems are facing unprecedented threats that lead to continuous biodiversity loss. Among the affected taxa, corals are disappearing at a fast pace leaving behind empty environments for species that depend on them. In 2011, 75% of the world coral reefs were considered threatened by human activities and many of them might vanish by 2050. At the current rate of loss, protecting the remaining corals might not be sufficient and aquariums are seen as crucial for the conservation of the ecosystems. This project assessed the number of corals in the Species360 ZIMS database network to understand the potential of captive individuals for conservation of corals reefs by comparing the population against IUCN Red List status, vulnerability to climate change, and species evolutionary distinctness. Aquariums can provide genetic information and expertise on how to successfully handle, reproduce and propagate corals. The wealth of data can help them recover from years of damage.

Sponsor Presentation:
Aquatic Equipment and Design

Mysis shrimp: An Invasive Species the Lakes of British Columbia, Canada and the Pioneering of a Sustainable Freshwater Fishery

Nuri Fisher
Piscine Energetics
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Mysis diluviana was introduced into Okanagan Lake in 1966 to provide an additional food source for rainbow trout and kokanee salmon, but this theory has proven to be largely incorrect and the introductions caused competition between mysids and kokanee for the same food source. In the deep lake, the Kokanee eat few of the shrimp, and instead, the shrimp compete with juvenile Kokanee for selective macrozooplanktons such as Daphnia sp. Invasive, Mysis shrimp have decreased the quantity of food available to Kokanee. The experimental harvest of mysids began on Okanagan Lake with the long-term objective of removing enough mysids to provide kokanee with a competitive advantage. Under the auspices of the British Columbia Ministry of Environment, Forests, Lands and Natural Resource Operations, Piscine Energetics pioneered and invented fishing technology for environmentally sustainable harvesting and removal of the invasive Mysis shrimp from Lake Okanagan. The technology enables the simultaneous live harvesting of Mysis Shrimp and facilitates the live reintroduction of non-target species (i.e. Kokanee fry) unharmed and in pristine condition back to the lake.

Captive Propagation of a Federally Endangered Species, the Laurel Dace (*Chrosomus saylori*)

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Chrosomus saylori (Laurel Dace) is a federally endangered species known from eight streams on Walden Ridge of the Cumberland Plateau. Populations have suffered from heavy siltation and declining water quality and recent sampling indicates this species persists in only two streams. Thus, this species is in critical need of conservation including the development of captive propagation protocols. Here, we report the findings from 2018 and preliminary results from 2019 from propagation at the Tennessee Aquarium Conservation Institute in Chattanooga, TN. Broodstock were collected from Bumbee Creek in Rhea County, TN in 2016 and 2018. Spawning occurred from April to May, when the water temperature was 20 - 24.6°C. A total of 809 eggs were collected and survivorship was 54%. Previously unknown reproductive and developmental strategies were documented. These findings represent the first account of captive spawning of the Laurel Dace, and provide invaluable insight into an understudied and imperiled species.

Session 14

AZA Freshwater Fishes Taxon Advisory Group (FFTAG)
Reporting Meeting

Aquatic Animal Welfare – Discussion of New AZA Standards

Sponsor Presentation:
Cairns Marine

RAW Business Meeting

Sponsor Presentation:
Gulf Specimen Laboratories, Inc.

Session 15

The Joy of Cooking, Water Chemistry Edition: How to Homebrew Your Own Nitrate Test

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A previous project from our facility created a method to read the nitrate level in a saltwater sample using the API Nitrate test kit with a Hach spectrophotometer. This not only removed the hazardous waste produced by the cadmium reduction method designed for the Hach machine but was also more accurate and cheaper to run per test. The purpose of this project was as a follow-up to create a 'recipe' for a reliable and potentially cost-effective colorimetric nitrate test for the Hach machine to read nitrates in a saltwater sample at standard aquarium levels using commercially available reagents. Due to the time constraints, this project was not able to test the shelf life of the formulated solutions and their reliability after extended storage; however, this method did prove to be successful in its original goals.

Killing Three Birds with One "Green" Stone: Making a Hill-William Heat Exchanger

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We all have a surplus of grand ideas but usually we find ourselves with a deficit of money to implement them. But when animal welfare is your priority, we need find a way to make things work. In this instance, we were able to solve three issues with one simplistic design. By using off the shelf materials, we were able to create a heat exchanger which solved our cooling needs in one 4200 gallon saltwater system, our heating needs in another 6000 gallon freshwater system, and of course implementing the solutions without spending a lot of money. Additionally, we were able to eliminate the need to add any mechanical equipment or any additional energy demands to solve the problems.

Design and testing of a Self-Contained, Wirelessly Monitored and Controlled, Automated LSS System for the Transport of Large Teleosts and Elasmobranchs

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Historically, the long-distance transport of large teleost and elasmobranch specimens, weather newly collected or moved between collaborating facilities has been occasionally problematic. Even when purged and/or sedated, the high metabolic demands of a large mass animal often result in deterioration of water quality over long hours of transport. The window for successful transport is narrow and fear of mortality/morbidity is an ever-present during these operations. The Walt Disney EPCOT Water Science Department, in collaboration with Aquatic Equipment and Design Inc. (AED), and McDaniel Consulting, LLC (MDC) have devised a fully portable, remotely monitored, animal transport life support system to greatly increase the margin of safety during transport. The system actively monitors and controls flow in the transport container, controls temperature, captures suspended solids, and maintains dissolved gas in water by using innovative technology re-purposed from the pharmaceutical and semi-conductor industry without the need for atmospherically vented or column de-gassing systems.

Session 16

Sponsor Presentation:
Aquatic Solutions

Vancouver Aquarium and Vancouver International Airport;
A Unique Partnership and Opportunity for Ocean Conservation

Patti Beer
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Google “the coolest things to see in an airport” and on many of those lists you will find the 30,000-gallon kelp forest aquarium at the Vancouver International Airport (YVR). The Vancouver Aquarium has managed that facility for over 11 years with all the challenges that security in an international airport brings. This talk will tour this facility and explain the challenges that heightened security, burgeoning passenger traffic, and airport expansion create. I will also share stories of the partnership between these two non-profit organizations (YVR is a non-profit!) and the innovative relationships they nurture with an eclectic mix of partners. There are vastly different initiatives to improve current circumstances in the oceans within this creative network of associates. An aquarium and an airport have come together to protect our oceans. I want to encourage imaginative partnerships towards more of the same.

Renovating a Two-Decade Old Artificial Reef:
A Review of Our Tropical Reef Restoration Project

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After twenty years, the coral in the 350,000-gallon Tropical Reef exhibit at Aquarium of the Pacific has lost a lot of color due to sun bleaching, broken coral, and algae and invertebrate fouling. To help mitigate costs of purchasing new artificial coral, we decided to repair and restore broken pieces. By doing so, we are not only able to recycle hundreds of corals, but also have control over the quality of color and appearance of repainted pieces. This presentation will cover the products, methods, success stories, and challenges that helped return color and coral distribution throughout this exhibit.

Flip or Flop – Mote's Journey through DIY Exhibit Upgrades

Amanda Hodo and Kerry Lee
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Mote's largest exhibit, our 135,000-gallon shark habitat, was in dire need of an aesthetic improvement. Upgrades had to be completed with limited resources, and without removing animals or otherwise closing to the public. The complete in-house overhaul of the exhibit included the installation of a large panel kydex backdrop, reef balls, and skylight alterations. With a bit of MacGyver ingenuity and teamwork, the outcome was better than anticipated. We observed an increase in small fish survivorship, improved animal health, and an enhanced overall aesthetic appeal.

Sponsor Presentation:
Kessil/DiCon Lighting

Movement and Transport of 200+ Kilogram Grey Nurse Sharks…
What Could Go Wrong?

Dr Rob Jones and Aaron Sprowl
The Aquarium Vet and St. Louis Aquarium at Union Station zoOceanarium Group, LLC
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At the end of 2017 Merlin Entertainments, decided to close the 53-year-old Manly SEA LIFE Sanctuary in Sydney Australia. Engineering reports indicated a rebuild was needed due to structures beyond repair. Home to over 1000 different animals careful planning was required for their relocation. Six of the fish were 25 to 40-year-old Grey Nurse Shark aka Sand Tiger Shark (GNS, Carcharias taurus). These sharks were all in excess of 3.5 metres (11.5 feet) and 200+ kg. The species is no longer able to be obtained from the wild in accordance with the Australian Department of the Environment Recovery Plan for GNS. The movement of the GNS required the development of systems and equipment that would allow the sharks to remain submerged and

supported by water through all transfers and avoid traditional capture stress issues. Through careful planning, many long days and nights, and 17 interstate transports each over 1200 kilometres, Merlin Entertainments successfully relocated over 1000 animals including the six aging GNS with zero transport-related mortalities.

Using Novel Behavior Training to Increase Visitor Impact

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The human-animal bond is a powerful conservation tool which zoos and aquariums foster daily. Touch tray habitats are the petting zoos of the aquarium world and one of the few ways to physically interact with aquatic life. Many locations have hands-on encounters; however, it is rare to find teleosts in these exhibits. We will demonstrate novel and husbandry behaviors with teleosts, trained through positive reinforcement, which astound guests and add a dimension to their relationship with fish beyond the dinner plate. Our first-hand experiences show that a deeper appreciation for these complex and wonderful animals is as impactful as our marine mammal interactions and further spreads the message of humane care for all. By leveraging connections with teleost ambassadors through guest interaction, training and social media you can expand the reach of your institution, generate interest and gain advocates for the valuable role of aquariums and marine parks.

Sponsor Presentation;
Aqua-Tech Co. / NextBite

Reproduction of *Gymnura altavela* in the Main Tank of Marine Aquarium of Rio de Janeiro (AquaRio)

Tiê Ferreira, Matheus Félix, Danela Lutfi, Rodrigo Marraschi, and Marcelo Szpilman
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Like other aquariums for public visitation, AquaRio comes with the objective of reproducing marine ecosystems so that visitors can know, respect and want to preserve. In the second year of operation the reproduction of the *Gymnura altavela* was carried out inside the main tank. During the first weeks of March of 2018 several persecutions of males behind females were observed, characteristic act in the reproduction of the rays. In August of 2018 the female was taken to the main tank procedures area and with the help of the veterinary team 5 babies were delivered, 2 males and 3 females. These were taken to the quarantine for biometrics to be made. Also successful in breeding the species *Dasyatis hipostyigma* and *Rhinoptera bonasus*. Works such as this make public aquariums, as well as a tourism equipment, an important center of sustainability for society.

Social Interactions and Feeding Competition in Rio Sao Francisco Piranhas at Cleveland Metroparks Zoo

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To explore whether aggressive interactions might have caused reduced welfare and death in some Rio Sao Francisco Piranhas *(Pygocentrus piraya)* at Cleveland Metroparks Zoo, we analyzed 24 video recordings. DLTdv7 software showed that individuals maintained consistent territories. Individuals were organized in a linear dominance hierarchy, and higher-ranked individuals performed more aggressive bouts and fewer escapes than lower-ranked individuals. Higher-ranked individuals also spent more time behaving aggressively and less time escaping than lower-ranked individuals. We also analyzed 23 videos recorded during feeding and found dominance rank was associated with amount of food consumed. Finally, casual observations indicated increased swimming after one individual was moved to a much larger, multispecies aquarium. Our data suggest that territoriality and aggression may have been elicited by limited available space, as predicted by resource defense theory. Welfare of Rio Sao Francisco piranhas, and other species, might improve in larger enclosures.

Visualizing Sand Tiger Shark *(Carcharias taurus)* Space Use in Aquariums using ZooMonitor and ArcGIS

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Determining how animals move within their environment is fundamental knowledge that contributes to effective management and conservation. In the case of the sand tiger shark (STS), telemetry studies have been conducted on coastal waters, but STS habitat use in human care is poorly understood. This collaborative study across four aquariums used a software program called ZooMonitorTM to record spatial use patterns of 15 STS's during one year to date.

Shark location in the habitat was analyzed by ArcGIS. Heat maps revealed patterns of strong avoidance between two males at one aquarium, which contrasted with heavy spatial overlap

by a third male. Heavy use of the habitat perimeter was observed at one facility while many sharks at other facilities preferred interior habitat space. Interior habitat use by several sharks occurred in preferred core areas. This data can shed light on STS social dynamics and habitat preferences.

Sponsor Presentation:
Fritz Industries

What's YOUR Welfare Score? Using Aquarium-Specific Welfare Assessments To Generate Scores on a Tank-By-Tank Basis.

Sarah Sprague
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AZA welfare standard 1.5 requires that facilities put into place a welfare assessment process that includes both proactive and reactive components. In response, SEA LIFE Michigan Aquarium developed a welfare assessment tool specific to the aquarium environment called Aquatic Welfare Audits (AWA). As a result of each quarterly audit, numerical welfare scores are generated for each tank, or welfare group within a tank. This score determines whether or not action is required to improve any identified deficiencies in welfare conditions. The assessment process uses robust welfare criteria and observation guidelines, while not demanding a significant time commitment or generating unnecessary paperwork. Results are presented in an easy-to-access format, which acts as a database that can be directly applied to husbandry decisions. By creating a tool that is both comprehensive and user-friendly, our facility has found a practical way to apply AZA welfare monitoring standards to the aquarium environment.

Elasmo-Ethology: Data-driven Behavior Management

Zac Reynolds
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As behavioral husbandry continues to be a growing focus to those caring for aquatic animals, so does the interest to make evidence-based decisions on training and enrichment. Through a novel program, Sea Life Michigan Aquarium has partnered with local Oakland University to allow student researchers the opportunity to assist husbandry staff by collecting behavioral data on Sea Life Michigan's elasmobranch population. Using ZooMonitor, behavior budgets and heat maps are generated, which allow aquarists to have a more detailed understanding of the behaviors displayed and locations frequented by sharks and rays in this mixed species exhibit. This information is applied when identifying and planning behavior modification needs. Goals of this project include using an animal's use of exhibit space as one metric of welfare, introducing enrichment to encourage species-specific behavior, and providing a platform for students in the community to participate in research.

Session 18

Sponsor Presentation:
Aquarium at the Boardwalk / Kuvera Partners

Not a Fluke!
Eliminating *Neobenedinia sp*. from a Group of Wild Caught Pacific Fish in Quarantine

Rachel Moote
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Neobenedenia sp. is a marine monogenean flatworm parasite, which can be found in wild caught and aquacultured fish. During a recent quarantine at the New England Aquarium, we acquired a group of wild caught fish presenting with this parasite. After six and a half months of treatments, we were able to fully eradicate the parasite from this population. Our methods included formalin dips, formalin immersion baths, treating with Praziquantel, hyposalinity, and transferring the fish to a new system. Our success was largely due to vigilant surveillance. Samples were taken regularly from both the system and animals, and were carefully analyzed to determine the current parasite load. This presentation will highlight the success of our treatments, and their impacts on water quality and fish mortality. I will also touch on changes we have made to our entrance exam procedure and system design, which have helped us to mitigate further ectoparasite infections.

Eel Surgery, From Trauma to Recovery

Steve Burns, DVM
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Unfortunately, traumatic events can occur in the wild and in aquariums. In November 2018, a tesselata moray eel at SEA LIFE Michigan received a traumatic injury from another animal. While the eel was severely injured, it's behavior and response to stimulus made the veterinary and animal care team choose treatment as the best option, and moved forward with intensive treatment and care. Although the injuries were severe, quick action and dedication to care allowed the animal to recover. This presentation will include an overview of anesthetic procedures, surgery, treatments, and after trauma care including antibiotics, assisted feeding, and husbandry techniques.

Emerging Zoonotic Issues within Public Aquariums and their Health and Safety Implications

Dr Rob Jones
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In public aquariums, there are a variety of potential zoonosis sources that include fish (both teleosts and elasmobranchs), reptiles, birds and marine mammals. Many of these have been known for some time, however there are some new possible pathogens emerging. Potential routes of infection and the issues they can cause will be examined. A toxin, that can cause health and safety issues (whilst not strictly a zoonosis) will also be examined.

A BRIEF GUIDE TO AUTHORS

Updated 2019

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!

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

The approximate deadline for submissions is December 15th. As has always been the case, materials in *Drum and Croaker* may be reproduced unless otherwise specified. Please credit *Drum and Croaker* and the contributor. I expect and assume that all submissions to D&C (papers, photographs, etc.) have been authorized by all original authors or co-authors, do not infringe on any copyright or prior publication agreements, and have successfully completed any internal review process required by your institution.

Submit articles via email as a Microsoft Word document (or a file that can be opened in Word). **My E-mail address is petemohan55@gmail.com.**

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 are compressed 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".
- Double-space after your "institution name" to begin the body of your text. When correct, the title and headings formatting should look like this:

(sample title is continued on next page)

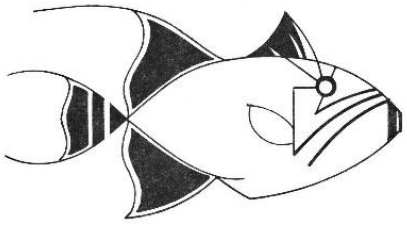
USE OF DUCT TAPE IN THE HUSBANDRY OF *Genus species* AT FISHLAND

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

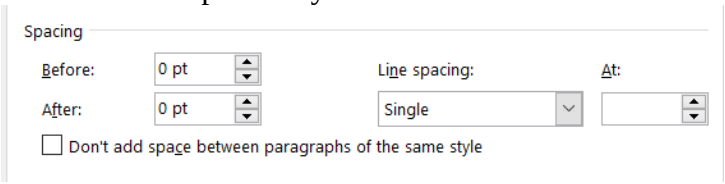
Figure 1. Legends should appear under the photo or graph in this format in 10-point font, aligned with the sides of the image or figure (center or justify). Very short legends can be centered. Photographs should be pasted into the document in the proper location by the author. All photos MUST be formatted 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.