DRUM and CROAKER

A Highly Irregular Journal for the Public Aquarist

Volume 55

January 2024

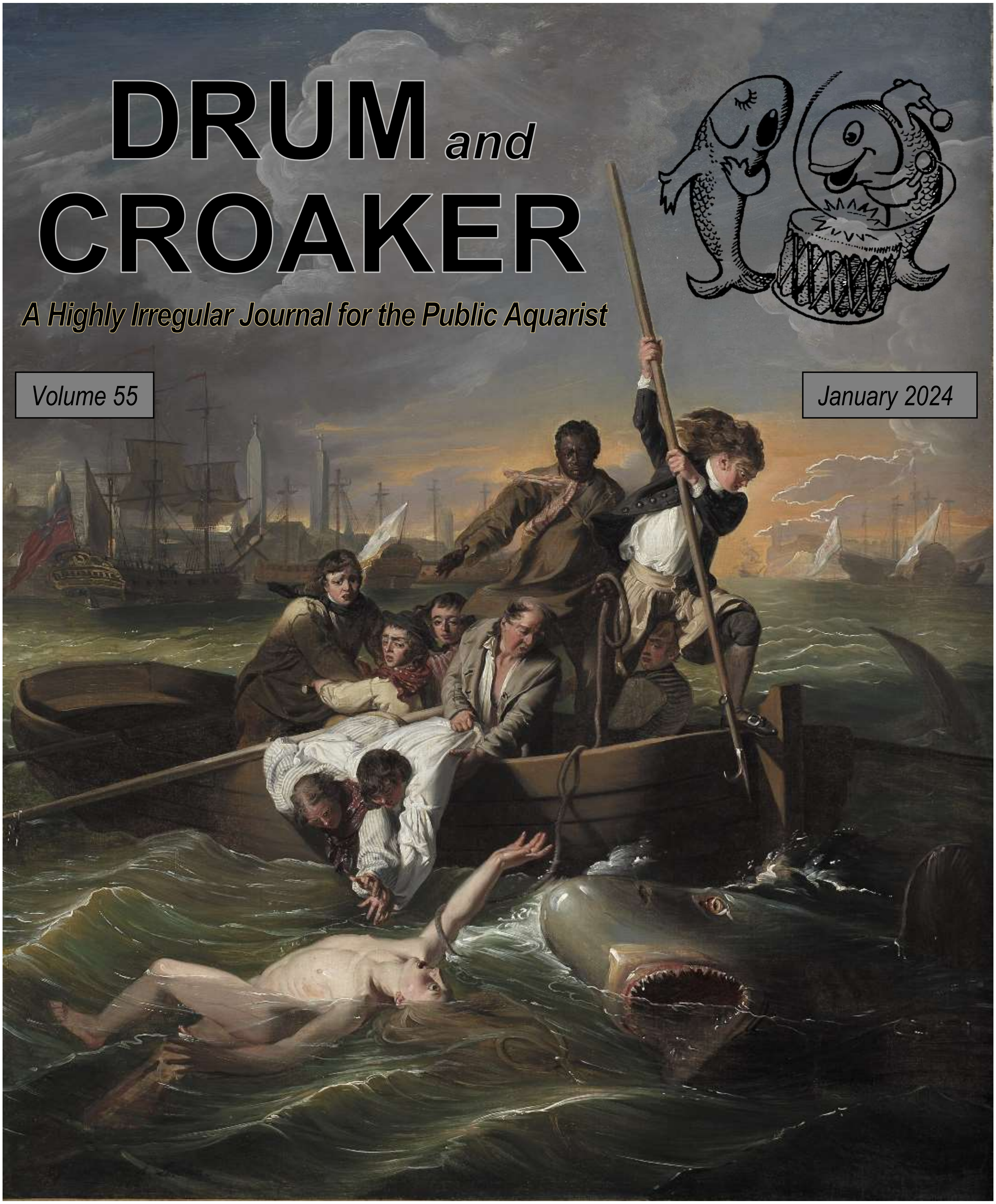


TABLE OF CONTENTS
Volume 55, 2024

Cover Credit: John Singleton Copley, Watson and the Shark, 1782, oil on canvas.
Detroit Institute of Arts, Founders Society Purchase, Dexter M. Ferry, Jr. Fund, 46.310.
Used by permission from DIA.
Interior Gyotaku: Bruce Koike, https://www.brucekoike.com/
Interior Line Art: Craig Phillips, D&C Archives

THE FELLOWSHIP OF FISHES ENDURES
Letter from the Editor, January 2024

Pete Mohan

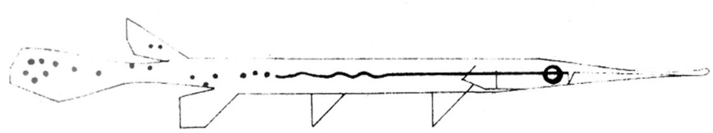
This is a somewhat unusual issue of Drum and Croaker. Change is afoot. Not only have regular paper submissions resumed, but there has been an outpouring of contributions celebrating recent professional gatherings and losses of significant members of our community.

Early 2022 saw continued Covid outbreaks. We gathered anyway at Moody Gardens for our first Regional Aquatics Workshop (RAW) since 2019. This was a smaller meeting than had been typical but reinforced the importance of our "Fellowship of Fishes." Late 2022 found the zoo and aquarium community still struggling to catch up after the impact of Covid. While many of our institutions reopened at least partially in late 2020, illness, weird staffing patterns, and budget struggles continued in 2021 and were amplified locally with other natural events such as mass-strandings of cold-stunned sea turtles and outbreaks of avian influenza. Although our community was often still in post-crisis mode (non-writing mode) in 2022, I was able to create a 2023 issue of Drum and Croaker with the help of a few intrepid outside authors and some scrambling by the newly minted contributing editorial and ankle-biter crew (Barrett Christie and Steve Bailey). Many of our personal shelved projects came out of storage. RAW regenerated a bit more in 2023 thanks to our hosts at the National Mississippi River Museum & Aquarium in Dubuque, Iowa. The huge number of first-timers surprised us. In late 2023, Covid is still around but part of the background noise. I managed to avoid it for years but "helped" delay the family Christmas gathering this year. And…writing is again a thing.

It's been wonderful to see papers coming in at normal or even above-normal levels again. As noted above, we've added several sets of abstracts to the 2024 issue. These include records of presentations and posters from AALSO, and the National Aquarium's "Jelly camp," in addition to the usual RAW contributions.

We are also celebrating the lives of a number of our colleagues; friends who made important contributions to our community, but have "crossed the bar" as Tennyson's 1889 seafarer's elegy goes. All of those memorialized in this issue are deserving of additions to your personal lexicons. They have created foundations for the important work we do today. Searching old issues of Drum and Croaker will reveal some of their contributions from the '60s and beyond.

I look forward to seeing many of you at RAW 2024 in Tacoma, WA!



DRUM AND CROAKER 50 YEARS AGO
June 1974 (Volume 15)

Steven L Bailey and Pete Mohan

Reproduction in the Giant Octopus of the North Pacific
Susan Gabe, Vancouver Public Aquarium.

An impressive account of very deliberate efforts to initiate copulation, egg-laying and tending, the rearing and then care of *Enteroctopus doeflini*. Excellent observational notes, measurements, and photos document this pioneering effort of over fifty years ago. Some of the young octopodes survived for 6 weeks and were likely cannibalizing their siblings. Even in 2024, success eludes most public aquarium professionals with the Order Octopoda, although other cephalopod groups' reproduction e.g. sepiids is now considered commonplace.

The San Juan Aquarium
Robert A. Martin and Ernest Bodner.

A thorough description of Ocean Life Park Aquarium and the 27 indoor aquatic exhibits comprising the exhibit path. Complementing those were an outdoor dolphin tank, a Japanese deer park, an alligator pool, spider monkeys, an aviary, and a variety of species that "children enjoy." The aquatics collection was focused on Puerto Rican fauna except for "two colorful South Pacific reef tanks." The Aquarium was a retrofit of the WWII Lancaster Coastal Battery at Punta Cangrejos. It had direct access to ocean water via intakes just below low tide and relied upon a flow-through life support strategy. No photographs are readily retrievable via the usual internet searches of this well-situated public aquarium in close proximity to the City. Current satellite images reveal no trace of the Aquarium building; the tip of Punta Cangrejos is currently occupied by numerous restaurants and their parking lots.

Sea World of Ohio Announces "World of the Sea" Triquarium
Anonymous. Sea World, Inc.

"The newest of Sea World of Ohio's fifteen exhibits is the $1 million "World of the Sea" Triquarium. The new three-roof complex which sets out over Geauga Lake features many of nature's highly unusual marine species. Supported by 111 sixty-foot-long pilings, the 10,000 square foot building is serviced by multiple entrances and exits to accommodate free traffic flow and provide good ventilation."

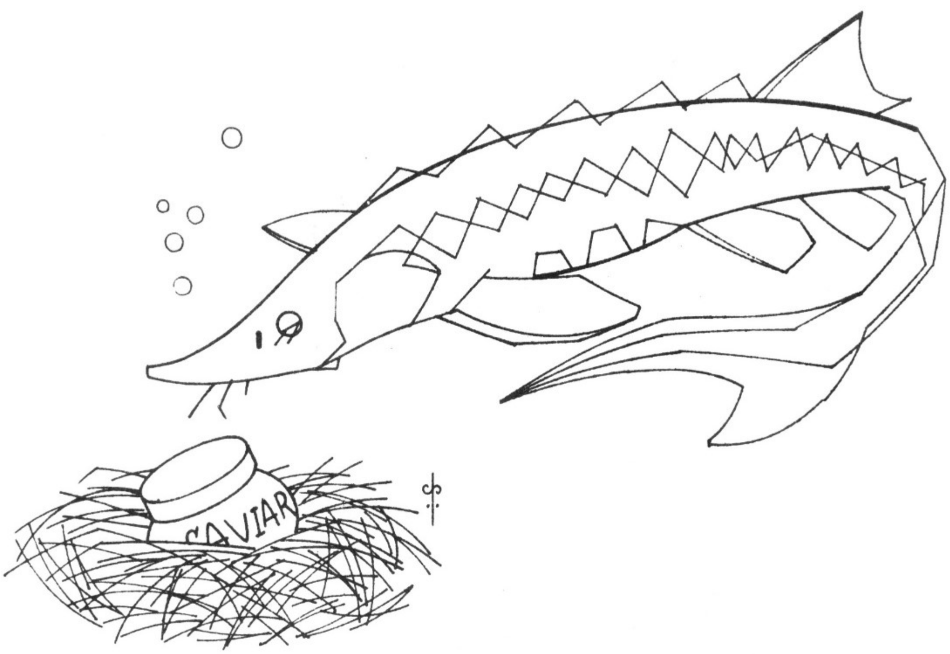
The building was originally constructed as a doorless, open pavilion that could be fitted with winter panels for winter use as an education classroom. The three triangular peaked roofs each covered a 10,000-gallon aquarium, hence the "Triquarium" moniker. A smaller area, known as "center display" featured 5 small aquariums at the time of construction. The nine original displays, which included an outdoor touch tank (covered in winter), were continually remodeled. Fourteen additional displays of various sizes were added over the years, including the first successful coral and jelly aquariums in Ohio, and the nation's only exhibit of Arctic fishes and invertebrates. The touch tank became usable year-round. If you hadn't guessed, this is Pete's alma mater. The building ended its life as an aquarium in 2004, when the property was sold to Cedar Fair and was converted into a water park. Recently the local government has arranged to buy the property and use it as a lakeside city park. The aquarium may be repurposed.


Left: The building under construction.   Right: As it looked in later years – about 1998.

"White Water. Blue Death"

Kym Murphy, Sea World

This is an interesting and unusual case study of exhibit mass mortality, and an issue of which every modern aquarist and LSS operator should take note. Breakpoint chlorination can be used used to remove nitrogenous compounds from system water treated in a reservoir tank. High levels of chlorine are then neutralized by sodium thiosulfate. An excess of thio can later trigger a sudden cascade reaction that causes pH and dissolved oxygen concentration to plummet. This is accompanied by a white-out effect due to the formation of a sulfur colloid. This is a problem that rarely occurs these days, as breakpoint chlorination is a less practical solution to high nitrogen levels in giant aquariums. Anyone using this water treatment process should review this article.



NOVUS AQUAS (NEW WATERS)

Barrett L. Christie, enteroctopusdofleini@yahoo.com

Imagine, if you will, an aquarium with only a couple dozen species of the most robust fishes. The hardiest species, pellet-pigs from hatcheries, maybe a few game fishes…hues of dull green and brown, maybe silver, but little else. Displaying the fauna of a far-flung corner of the oceans is impossible here. Likewise, keeping invertebrates, sharks, syngnathids, jellies, cephalopods, and other rarities are far beyond the capabilities of this hypothetical facility. How would that compare to the modern public aquarium of today? Would the reach be the same? How many more visitors would leave thoroughly underwhelmed or uninspired?

We can only assume that the myriad of species maintained in aquaria today would be a marvel to the earliest aquarium curators. These successes, like all fruitful endeavors, are built upon innumerable failures. To put it bluntly, the techniques we have now came at the expense of animal mortalities along the way. This reality is uncomfortable to some, but should not be feared so reflexively. Objectively speaking, loss of life is inevitable in refining husbandry, mortalities are only empty or futile when nothing is learned, and so much has been learned over the years.

Forty years ago, keeping so many of the coral species readily available today was all but impossible, most corals imported and kept in aquaria lived a brief period and quickly became bleached, lifeless skeletons (which were popular as tank décor at the time). Sixty years ago, the same was true of so many sharks, as our industry was just beginning to learn the intricacies of care for so many of these species. Where would we be today if earlier curators had decided not to pursue the husbandry of these animals? Would our aquaria resemble the hypothetical facility described above?

Both progress and stagnation can arise from a foundation of the same good intentions. As our industry responds to outside criticisms, we are putting a greater focus on the welfare of the animals in our charge. From this we are seeing positive trends, though one could argue that we can equally envision some trajectories that merit closer consideration. Since the opening of the first public aquarium 170 years ago (and 2,060 years since the first Roman *piscinae*), our collections and our exhibits have grown in size and diversity through trial and error. This process of refinement leads to our ability to keep the myriad of species we display today, through the very art of experimental husbandry.

Today hundreds of elasmobranch fishes and thousands of corals, jellyfishes, and other delicate invertebrates are routinely maintained. Public aquaria today continue to lead the way in pioneering techniques for creatures that are truly novel. One thinks of whale sharks, sailfish, tunas, blue sharks, and deep-sea invertebrates and fishes, just to name a few. Our collective achievements go far beyond just keeping these animals, we are also *breeding* many of these species, gaining valuable insights into their biology and natural history. Ctenophores and cubozoans can now be propagated, stony corals can be coerced into broadcast spawning on cue, hundreds of larval marine fishes may now be reared, and studbook keepers are overrun with pups from carcharhinid sharks, stingrays, and other creatures that were an impossibility just a few decades prior.

The ability to push the envelope and display these marvels is imperative for aquaria to broaden our collections, our collective expertise, and ultimately our reach to the public as we seek to fulfill our missions. However, there is a growing school of thought, that while well-intentioned, can stifle the ability to experiment, learn, and grow as biologists. I think none would argue that the drives toward sustainability and improving welfare are valid and worthy goals, however, like so many things in animal care, fixation on any one metric to the exclusion of others rarely yields optimal results.

While using life span as a metric, as one example, can illuminate areas for improvement in overall sustainability of collections, or welfare of individual animals or a particular species. However, if this is done in a short-sighted fashion, one unintended consequence is gradual reduction in diversity. Selecting against species because they fail to live an expected lifespan in captivity is certainly one way to superficially improve your averages. It is tempting to claim such shallow victories in the name of welfare and sustainability, but in a sense, this is antithetical to the spirit of curiosity and progress that has defined aquarists as long as there have been aquariums. A better approach to recognizing that a species is not living an expected lifespan is to ask what can we do differently next time? What data can we extrapolate from our experiences, or what natural history information may be gleaned from the literature that may improve our odds?

Where the above questions are unanswerable or the potential solutions ambiguous, the solution is to collect more data. Trial and error from an informed approach can be quite revealing, and from the very beginning of public aquariums the ability to conduct research on these topics has been a hallmark of what we do well. Especially now as aquaria and zoos seek to re-establish ourselves as centers of biological inquiry, and not solely entertainment or menageries. Such research initiatives may be colossal projects with countless collaborators contributing to the base of knowledge on megafauna such as whale sharks or deep-sea fishes, or they may be as simple as aquarists experimenting with desert spring snails or imperiled killifishes in a back room. As Kurt Vonnegut once wrote, “New knowledge is the most valuable commodity on earth, the more truth we have to work with, the richer we become.” Look no further than the pages of D&C for countless examples of aquarists bringing new knowledge to bear, including this very issue with an excellent piece on sea cucumbers.

I have heard from colleagues who feel their institutional culture has shifted in such a way that they dare not propose keeping species where care is not completely established or for which success is uncertain. Some have also noted some opinions voiced by leadership that have stifled or discouraged experimental husbandry. Such uninformed declarations are more often than not made by individuals blithely oblivious to whose shoulders they’re standing upon.

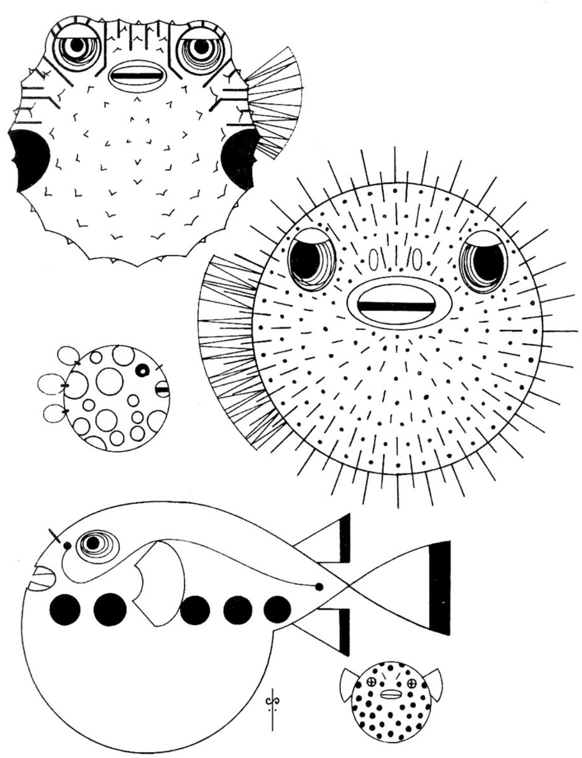
While well-intentioned, what will happen to the diversity of our collections if this trend continues, and greater lists of species become ‘ineligible’ for display in a public aquarium? I would hope that more and more institutions strike a balance between staple species and those that require chances to be taken. This shows faith and trust in your people, above all else, to solve problems and refine best practices. I can only hope the aquaria of 2073 would be as alien and fantastical to us as today’s aquaria would be to a curator from 1973, and the only way this dream will be realized is if aquaria commit to creating a culture of curiosity and experimentation with the freedom to fail.

Catering to the common denominator will create stable displays, and boost sustainability and welfare in the short-term, but at great cost to the array of possibilities, and ultimately progress.

The ability to synthesize all of the known, and through extrapolation visualize a potential path (or paths) into the unknown is where the science ends and the art begins in animal husbandry. Where and when breakthroughs are made, we all benefit from a broadened arsenal of technique and fundamental knowledge, and ultimately those we seek to inspire benefit the most, as we can present them with species that otherwise might have been unimaginable to them.

Should we fail to continue challenging what is possible by daring to push the limits of species that inspire the public, we do ourselves, our progenitors, and our visitors a disservice. And while this cautionary rant may seem pessimistic, I have optimism because I know there will always be both institutions and talented aquarists who push these very boundaries and expand what is known. Aquarists will always be among us who dare to try and to fail, and try again, so that we might be able to stand atop these failures armed with new knowledge and see a new path further into the unknown. We must embrace this process and the failures that come with it, and more importantly, we must share this hard-won knowledge, so that others may carry it even further.

"*If I have seen further, it is because I have stood upon the shoulders of giants*"
-Issac Newton



AQUACULTURE OF THE WARTY SEA CUCUMBER, *Apostichopus parvimensis*, AT THE MONTEREY BAY AQUARIUM

Ellen Umeda, Senior Aquarist eumeda@mbayaq.org

Monterey Bay Aquarium, 886 Cannery Row, Monterey, CA 93940

Abstract

The Monterey Bay Aquarium displays warty sea cucumbers, *Apostichopus parvimensis*, as an accessory species in many exhibits highlighting the marine habitats of Monterey Bay. Although warty sea cucumbers can be collected locally via SCUBA diving, culturing reduces collection pressures on wild populations and provides an opportunity to study the larval development of this species. This paper describes the methods used for successfully culturing warty sea cucumbers in a public aquarium setting, provides images documenting larval development, and discusses improvements and recommendations for future culture attempts.

Introduction

Warty sea cucumbers range from Monterey Bay, CA, USA to Baja California, Mexico (Hamel and Mercier, 2008). They prefer low energy environments and depths ranging from the intertidal to 30 m. Their long, reddish-brown cylindrical bodies can grow up to a length of 30-40 cm. Along the dorsal surface, they have black-tipped papillae, hence the name “warty” sea cucumber (Bruckner, 2006). In the wild, warty sea cucumbers use their tentacles to feed on the top few millimeters of sediment and play an important role in bioturbation (Yingst, 1982; Rogers-Bennett and Ono, 2001).

Due to the increased demand for sea cucumbers as food, particularly in Asia, sea cucumbers face overexploitation. This increased demand combined with limited mobility, slow rates of sexual maturation, and low recruitment rates, make sea cucumbers particularly vulnerable (Rogers-Bennett and Ono, 2001). Warty sea cucumbers are primarily collected by the dive fishery in California, USA, and Baja California, Mexico (Chavez *et al*., 2011). In California between 1997-1999, sea urchin and abalone divers shifted toward sea cucumbers due to the abalone fishery moratorium and a reduced sea urchin fishery caused by El Nino conditions (Rogers-Bennett and Ono, 2001). During this time, warty sea cucumber densities in the Channel Islands declined 33-83% (Schroeter *et al*., 2001).

Although there is no known warty sea cucumber aquaculture in North America, sea cucumber aquaculture has expanded in recent years for other species. In Asia, the two main species found in aquaculture include the Japanese sea cucumber, *Apostichopus japonicus*, and the sandfish, *Holothuria scabra* (Bruckner, 2006). In Ecuador and Mexico, the Galapagos sea cucumber, *Isostichopus fuscus*, has been successfully cultured in land-based systems (Mercier *et al*., 2005; Mercier *et al*., 2012). Many of the techniques used in sea cucumber aquaculture were applied to culturing warty sea cucumbers at the Monterey Bay Aquarium.

This paper explains the methods used for culturing warty sea cucumbers at the Monterey Bay Aquarium. Culturing warty sea cucumbers provides the opportunity to study the larval development of sea cucumbers and reduce collection pressure on wild populations. In addition, warty sea cucumber larvae can also be displayed under the microscope in the Aquarium's plankton exhibit (*Tiny Drifters Under a Lens*) and the juveniles can be displayed in a jewel tank exhibit highlighting juvenile echinoderms.

Spawning

Seven mature warty sea cucumbers were collected from the *Kelp Forest Exhibit* at the Monterey Bay Aquarium during a SCUBA dive. The *Kelp Forest Exhibit* is a 335,000-gallon, 28 ft deep, open-system exhibit featuring live giant kelp, *Macrocystis pyrifera,* along with fish and invertebrate species found in the kelp forest ecosystem. The sea cucumbers were removed from the exhibit using plastic collection bags, transferred to a 20-gallon blue tub upon surfacing for transport, and then placed in 10-15 gallon holding tanks behind the scenes. The holding tanks were on a semi-open system, primarily used for the main jellyfish gallery. Life support for this system included sand filters, UV filtration, and a heat exchanger for maintaining a system temperature of 12-13 °C.

In the wild, warty sea cucumbers are broadcast spawners with a natural spawning season from late spring to summer (Rogers-Bennett and Ono, 2001). To coincide with natural spawning seasonality, spawning was induced a few days after collection on March 28, 2021. A previous spawning attempt in August of 2020 resulted in only one male spawning. At this point in the season, all the cucumbers had likely exhausted their reproductive resources. In addition, sea cucumbers experience an annual visceral atrophy cycle during the fall so timing is important when spawning (Frankboner, 2002).

Warty sea cucumbers were induced to spawn using heat shock. This method is commonly used for other species of sea cucumber in an aquaculture setting (Mazlan and Hashim, 2015). The day before the spawning attempt, two 20-gallon blue tubs were filled with 0.35 micron filtered seawater. A stick heater was added to each tub and set to 5°C warmer (approx. 18 °C) than the current system water temperature (12-13 °C). A compressed air supply line was added to each tub for circulation. These tubs were setup to provide heated water for the temperature shock and to separate males and females once spawning had occurred (Figure 1).

Figure 1. Pre-spawning setup. Consists of 20-gallon blue buckets with stick heaters and compressed air supply line. This water was used for the heat shock treatment and for separating males and females once spawning had occurred.

To induce spawning the next day, the makeup water to the adult warty sea cucumber holding tank was turned off. Approximately 80% of the tank water was quickly removed using beakers. Then heated water from the 20-gallon blue tubs was quickly added to the holding tank to replace the removed water. Once the tank was refilled, a stick heater set to 18 °C, and a compressed air supply line were added to the tank to maintain temperature. In addition, the makeup water was turned on to add a slow trickle of water while still maintaining 18 °C. The temperature was regularly checked every 10-15 minutes.

The male started spawning 30 minutes after the onset of the heat shock treatment, releasing sperm identifiable by its milky, white coloration. The male was quickly separated into one of the heated 20-gallon blue tubs to prevent polyspermy. About 40 minutes after the start of the heat shock treatment, two females started spawning and released pinkish-orange oocytes. Three more females began spawning within the next 10 minutes. All females were moved to the second 20-gallon blue tub. Most of the individuals continued to spawn for at least an hour. The seventh individual never spawned.

Eggs from multiple females were placed in Pyrex crystallizing dishes (190 mm diameter, 100 mm height) with one to two ml of sperm from the male using a pipet. Dishes were placed on water tables set to 13 °C, 16 °C, and 19 °C. The 13 °C water table was a flow-through system using ambient system water. The 16 °C and 19 °C water tables were separate semi-open, recirculating systems with a heater in the reservoir. When the spawning process finished, the adult warty sea cucumbers were returned to their holding tank and the heater was turned off to allow water temperature to acclimate back down to ambient system temperature.

Larval Setup

The water in the dishes was changed daily or every other day. For less densely populated dishes, water changes were done by individually pipetting all larvae into a new dish and topping off with new seawater. For more densely populated dishes, a 25-micron sieve was placed inside the dish. A 3/16" tubing was placed inside the sieve and used to siphon water out of the dish. The larvae remained outside the sieve, preventing any loss during the siphoning process. Approximately 75% of the water was removed and the dish was topped off with new seawater. All dishes were fed 50 ml of microalgae following a water change.

After a week, the larvae in the dishes were transferred to diffusion tubes (Figure 2), a tube within a tube tank design initially developed at the Monterey Bay Aquarium for culturing ctenophores (Patry *et al*., 2020). Diffusion tubes consist of an acrylic inner tube with a 55 µm mesh siliconed to the bottom opening. The inner tube sits within a wider, outer acrylic tube with a square acrylic baseplate attached to the bottom to contain the seawater. The inner tube also sits on a two cm riser made of Vexar mesh to allow water exchange via passive flow between both tubes. A supply line consisting of ¼" tubing is placed between the two tubes approximately five cm from the bottom. This setup provides a passive environnment for the larvae and removes the need to do water changes.

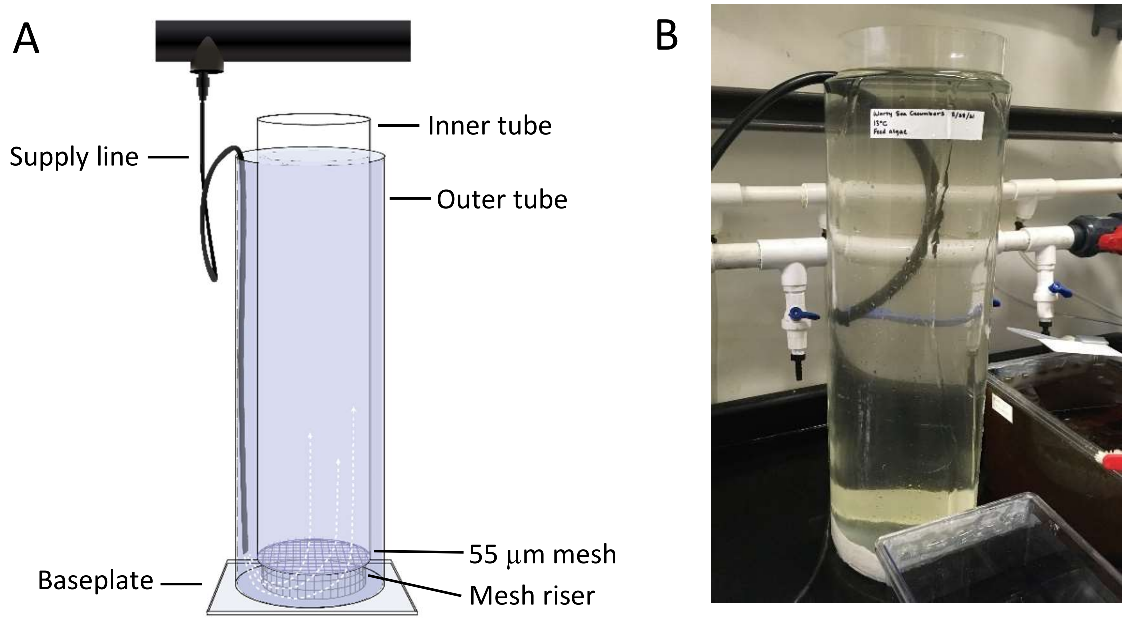


Figure 2. A) Diffusion tube setup B) Larval setup using a diffusion tube. Drawing credit: Audrey Sauble

Larvae were fed micro algae, primarily *Isochrysis galbana* and *Rhodomonas lens*, but also received *Chaetoceros gracilis* or *Dunaliella tertiolecta* when available. Tubes were fed 50-100 ml of micro algae twice per day. Algae densities were not measured since they varied daily. In addition, the different supply line flow rates and variable larval densities in each tube made standardizing feeding amounts challenging. Therefore, the amount fed was based on the presence of microalgae in the tube prior to each feeding and the presence of microalgae in the stomachs of the larvae.

Prior to settlement, the larvae were removed from the diffusion tube and placed in a glass dish. The larvae were not necessarily in the doliolaria stage but appeared to be negatively buoyant. In one case, the larvae were not moved out of the diffusion tube early enough and settled inside the tube. To remove these, the inner tube was gently removed and inverted over a glass dish. The inside of the tube was gently rinsed and sprayed with filtered seawater in a Nalgene bottle. A flashlight was used to check for any remaining individuals stuck to the tube. It was unclear whether the exposure to air during the rinsing process affected the survival of settled individuals, so ideally the larvae would be transferred out of the tube prior to settlement.

Larval Development

As a meroplankton, the larval warty sea cucumbers developed through several planktonic stages before settling on the benthos. The early gastrula stage began to develop two days post fertilization (dpf) and reached the gastrula stage three dpf. By seven dpf, the larvae developed into auricularia. The Auricularia continued to grow until reaching the doliolaria stage. During the doliolaria stage, the larvae shrank in size and began searching for a place to settle (Li *et al*., 2010). This stage was missed during our observations for many of the settled warty sea cucumbers, so metamorphosis can occur quickly once reaching this stage. The length of time for development varied depending on the water temperature with larvae raised in warmer water temperatures developing faster. Photos were taken under microscope (Zeiss Stereo Zoom V16, Canon EOS Rebel T5i) every few days to document the development of the larvae (Figure 3).

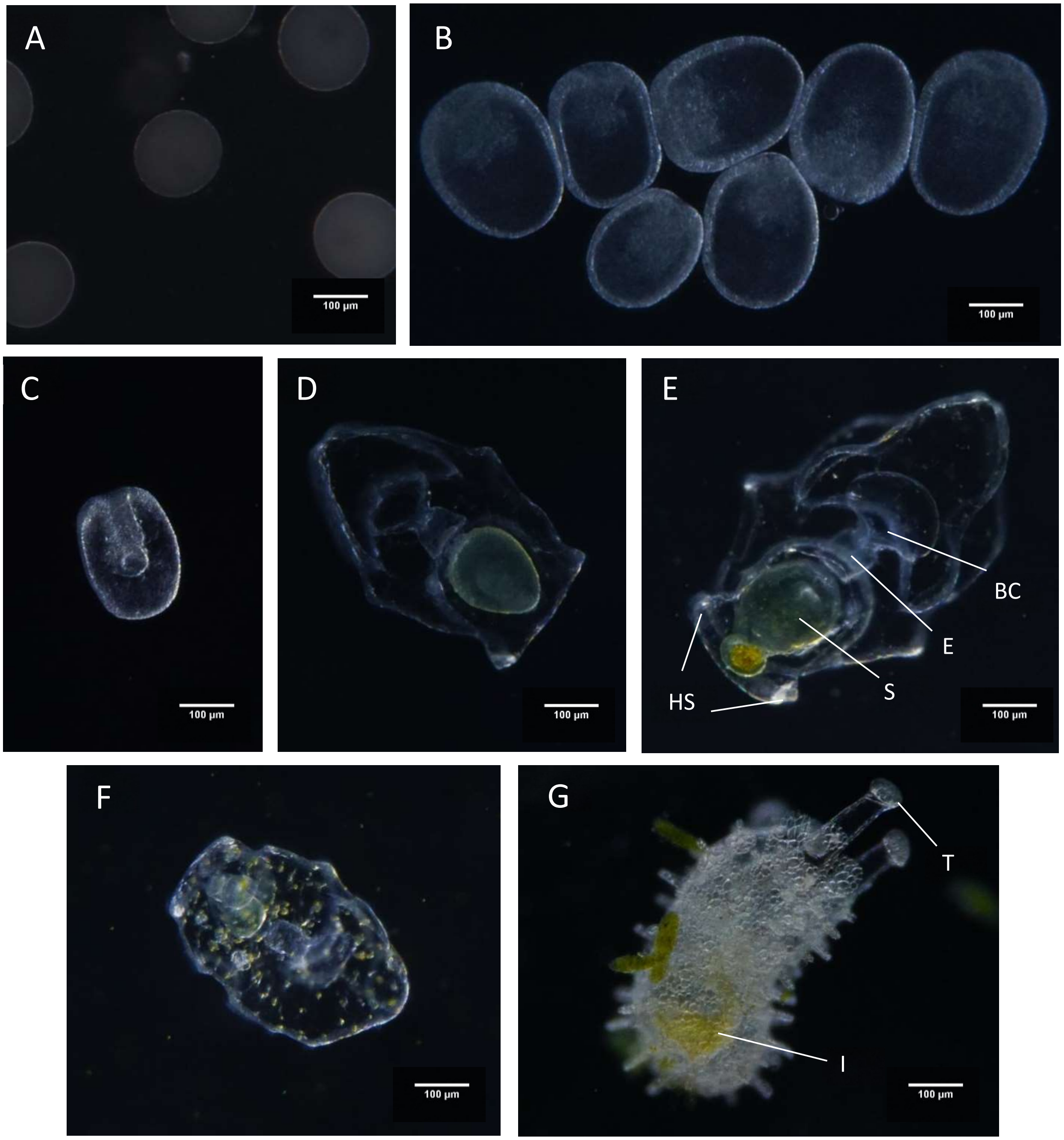


Figure 3. Warty sea cucumber, *Apostichopus parvimensis,* development. A) Unfertilized eggs. B) Developing gastrula. C) Gastrula. D) Early auricularia. E) Auricularia. (HS) Hyaline spheres. (S) Stomach. (E) Esophagus. (BC) Buccal cavity. F) Doliolaria. G) Recently settled larvae. (T) Tentacles. (I) Intestine.

Settlement

Settlement began at 28 dpf for the larvae in the 16 °C diffusion tube. The larvae in the 19 °C diffusion tube disappeared within two weeks. The inner tube was checked for settled warty sea cucumbers, but none were observed so it was assumed this population died during development. The larvae at 13 °C developed and remained in either the auricularia or doliolaria stage for over two months. They never settled and eventually disappeared. The doliolaria stage is a non-feeding stage and the inability to settle may have caused the larvae to slowly die off.

Once settled, the warty sea cucumbers were placed in acrylic containers (Lee's Kritter Keepers ®) with five mm holes drilled 1" from the top on the short ends. The holes were covered with 300 µm mesh to prevent escapees (Figure 4). For feeding, beakers of microalgae were set aside to allow the cells to die off and settle out. The settled microalgae combined with pieces of macroalgae, including giant kelp (*Macrocystis pyrifera*) and sea grapes (*Botryocladia pseudodichotoma*), were added to the 0.75-gallon Kritter Keepers. Occasionally the mesh screens were brushed to prevent the tanks from overflowing. Otherwise, no maintenance was done on the holding tanks to allow diatoms to grow within the tank. After a year, the largest warty sea cucumbers had reached two cm in length and were moved to a 10-gallon holding tank with fine sand on the bottom, rocks, and larger pieces of macroalgae.

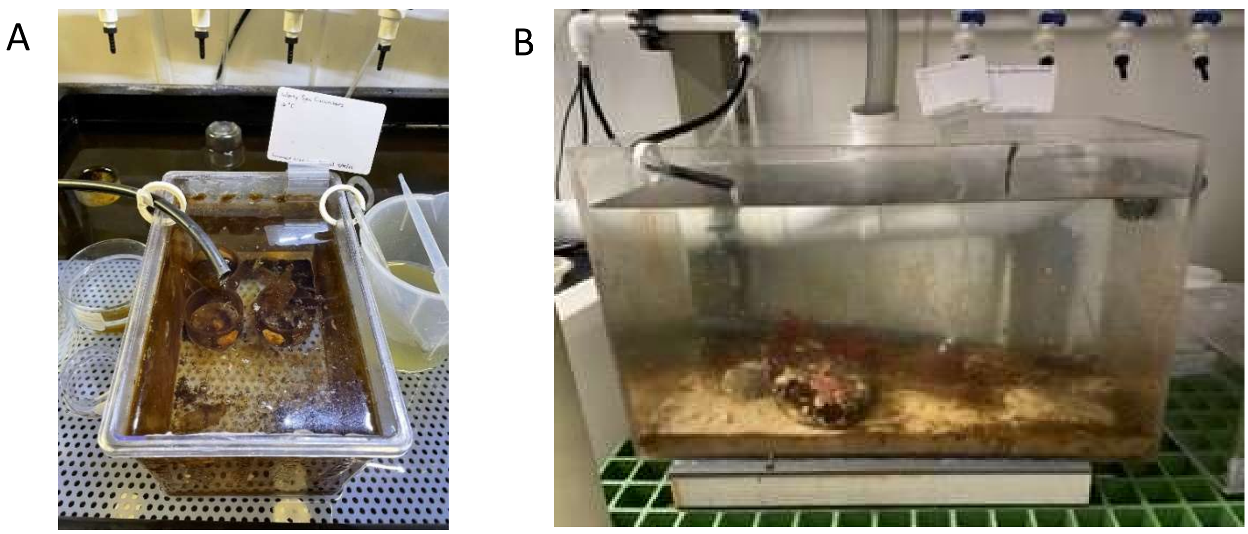


Figure 4. A) 0.75-gallong Kritter Keeper setup. B) 10-gallon holding tank setup.

Results

Only the larval cohort of warty sea cucumbers kept at 16 °C survived until settlement. Although an exact number of settled warty sea cucumbers was not counted, dozens settled based on observations. However, as of two years later, only 15-20 individuals remain. Some have grown to a length of eight cm when stretched out. Initially, most juveniles had a semi-transparent, white coloration (Figure 5). Upon reaching around four cm in length, many began to turn a reddish-brown color like their adult counterparts while the smaller ones maintained their lighter coloration. It appears the change in coloration depends on the size or maturation of the sea cucumber rather than age.

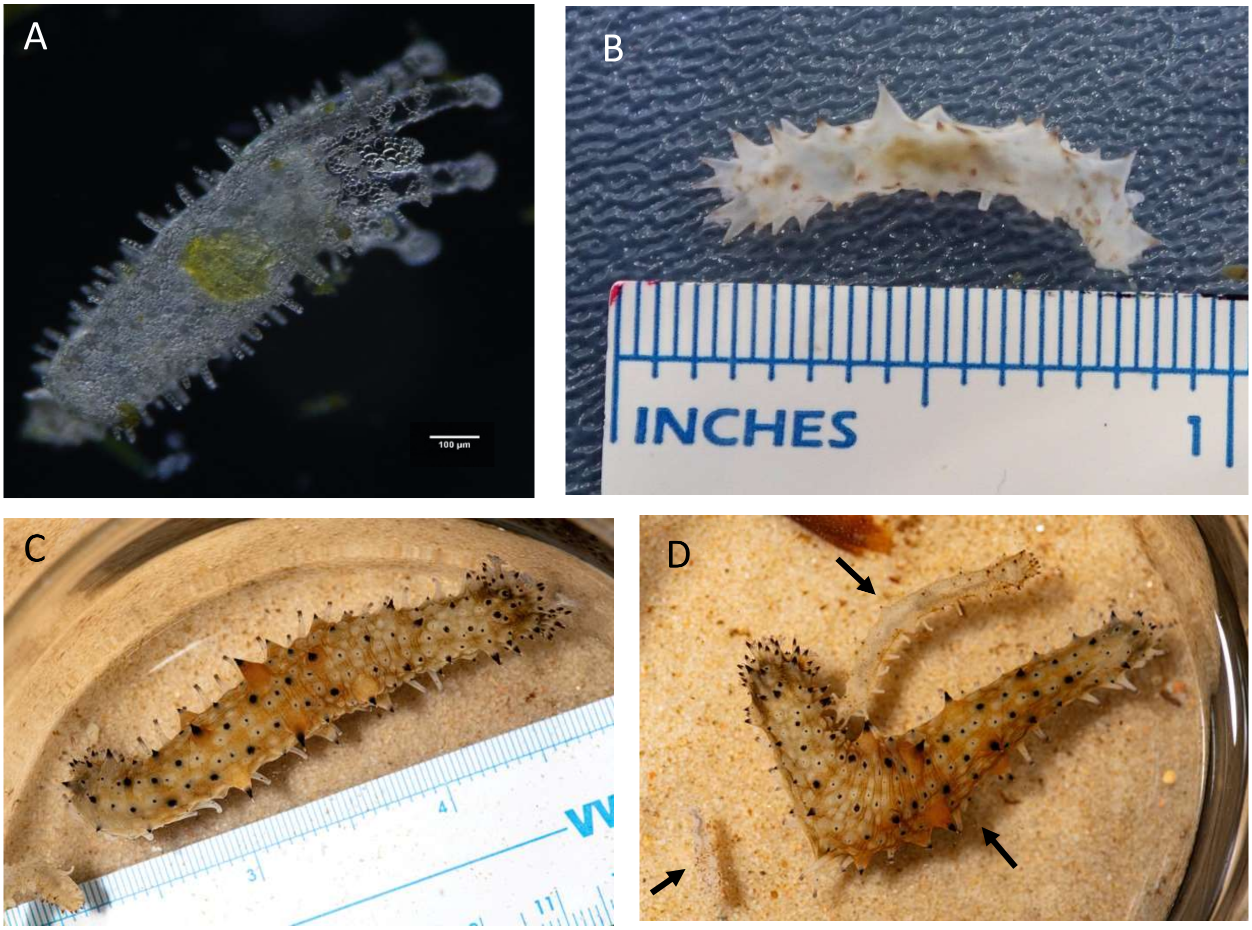


Figure 5. A) Settled pentactula larvae 1.5 months post fertilization. B) Juvenile nine months post fertilization. C) Juvenile 30 months post fertilization. D) Size disparity between three juveniles 30 months post fertilization. Note the difference in coloration. Photos: E. Umeda and T. Rininger, Monterey Bay Aquarium

Discussion

This was our first successful attempt at spawning and settling warty sea cucumbers. However, many questions remain, and more culturing attempts are needed. From our initial attempt, a water temperature of 16 °C appears to be ideal for raising this species. The larvae at 19 °C disappeared within two weeks and the larvae at 13 °C never settled. The increased energy requirements for warmer water temperatures may affect the larvae's ability to go through metamorphosis. Therefore, increased temperature may allow for faster growth but may decrease survival in the long term (Li *et al*., 2010). The density of larvae in each diffusion tube was not measured. In aquaculture, the recommended larval density for *A. japonicus* is 0.1-0.2 larvae per ml (Han *et al*., 2006). Analysis of larval densities for warty sea cucumber culture would allow efficient use of the space available and provide the larvae with an appropriate amount of space to grow and feed.

It is unknown if there was a specific settlement cue for warty sea cucumbers. There was light diatom growth and settled particles of microalgae on the bottom of the tubes and dishes, which may have induced settlement. One study found that *A. japonicus* preferred to settle on plastic sheets covered with diatom films or effective microorganisms (Li *et al*., 2010). The warty sea cucumber larvae at 13 °C never settled, which may have been due to the lack of the appropriate settlement cue. In addition, different water temperatures may cause different bacteria or species of diatom to grow. Future attempts should look at a variety of settlement surfaces to maximize settlement.

Once the warty sea cucumbers settled, many disappeared over time and improvements to increase post-settlement survival would help the long-term success of the culture. The lack of food likely contributed to this die-off, as most of the settled larvae were placed in clean Kritter Keepers with no substrate. Settled microalgae and small pieces of macroalgae were added to the tanks, but this was likely not enough food or the appropriate food. In the wild, warty sea cucumbers feed on the top few millimeters of sediment and prefer areas with the highest organic matter (Yingst, 1982). Pre-seeding tanks with sand, rocks, and macroalgae prior to settlement would provide the settled warty sea cucumbers with sufficient food and the appropriate nutrition for growth. Also, finding appropriate food sources that don't rely on local macroalgae or access to an open system would help as well.

In addition, tracking the growth rates of warty sea cucumbers post settlement would provide additional information about their development overtime. Current observations of growth from this spawning attempt indicate that growth rates are slow for this species. It is estimated that juveniles take four to eight years to reach maturity (Rogers-Bennett and Ono, 2001). In addition, there was a huge size disparity within the population with the longest measuring eight cm and the shortest measuring less than two cm despite being the same age. It is unclear whether this is related to nutrition, genetics, or lack of predators to weed out the smaller individuals. Similar patterns in size disparity have been observed in bat stars, *Patiria miniata* (personal observation; personal communication with Ben Morrow).

In summary, this was the first successful attempt at culturing warty sea cucumbers at the Monterey Bay Aquarium and future culture attempts will help to refine the process. Future efforts should focus on finding the ideal larval density, determining potential settlement cues, improving post-settlement nutrition, and tracking long-term growth. This will provide insight to improve the efficiency and success of warty sea cucumber aquaculture.

Acknowledgements

I would like to thank the Monterey Bay Aquarium for allowing me to pursue this project. A special shoutout to the Pelagic Magic Team for their support and for allowing me to work with a different animal besides jellies. I would also like to thank Manny Ezcurra for reviewing and providing feedback for this paper.

Literature Cited

Bruckner, A. 2006. Proceedings of the CITES workshop on the conservation of sea cucumbers in the families Holothuriidae and Stichopodidae. National Oceanic and Atmospheric Administration, Silver Spring, MD.

Chavez, E.A., De Lourdes Salgado-Rogel, M.A., and J. Palleiro. 2011. Stock assessment of the warty sea cucumber fishery (*Parastichopus parvimensis*) of NW Baja California. *California Cooperative Oceanic Fisheries Investigations Report* 52:136-147.

Frankboner, P.V. 2002. Seasonal visceral atrophy and response to salinity by *Parastichopus californicus* (Stimpson): Osmoregulation? *SPC Beche-de-mer Information Bulletin* 17:22-26.

Hamel, J.F. and A. Mercier. 2008. Population status, fisheries and trade of sea cucumbers in temperate areas of the Northern Hemisphere. *FAO Fisheries and Aquaculture Technical Paper 516*. Food and Agriculture Organization of the United Nations, Rome, Italy. 34 pp.

Han, Q., Keesing, J.K., and D. Liu. 2016. A review of sea cucumber aquaculture, ranching, and stock enhancement in China. *Reviews in Fisheries Science & Aquaculture* 24:326-341.

Li, L., Li, Q., and Q. Kong. 2010. The effect of different substrates on larvae settlement in sea cucumber, *Apostichopus japonicus* Selenka. *Journal of the World Aquaculture Society* 41:123-130.

Mazlan, N., and R. Hashim. 2015. Spawning induction and larval rearing of the sea cucumber *Holothuria scabra* in Malaysia. *SPC Beche-de-mer Information Bulletin* 35:32-36.

Mercier, A., Hidalgo, R.Y., and Hamel, J.F. 2005. Aquaculture of the Galapagos sea cucumber, *Isostichopus fuscus*. *FAO Fisheries Technical Paper*:347-358.

Mercier, A., Ycaza, R.H., Espinoza, R., Arriaga-Haro, V.M., and Hamel, J.F. 2012. Hatchery experience and useful lessons from *Isostichopus fuscus* in Ecuador and Mexico. *Asia-Pacific Tropical Sea Cucumber Aquaculture*. ACIAR Proceedings 136:79-90.

Patry, W.L., Bubel, M., Hansen, C., and Knowles, T. 2020. Diffusion tubes: a method for the mass culture of ctenophores and other pelagic marine invertebrates. *PeerJ* 8:e8938.

Rogers-Bennett, L. and Ono, D.S. 2001. Sea Cucumbers. *California living marine resources: a status report*. California Department of Fish and Game, CA.

Schroeter, S.C., Reed, D.C., Kushner, D.J., Estes, J.A., and Ono, D.S. 2001. The use of marine reserves in evaluating the dive fishery for the warty sea cucumber (*Parastichopus parvimensis*) in California, USA. *Canadian Journal of Fisheries and Aquatic Sciences* 58:1773-1781.

Yingst, J.Y. 1982. Factors influencing rates of sediment ingestion by *Parastichopus parvimensis* (Clark), an epibenthic deposit-feeding holothurian. *Estuarine, Coastal and Shelf Science* 14:119-134.

SHARK BODY CONDITION SCORING TOOL DESCRIBED

**Megan A. Perry, Aquarist II (mearney@georgiaaquarium.org),
and Jillian M. Sawyna, Senior Aquarist (jsawyna@georgiaaquarium.org)**

Georgia Aquarium, 225 Baker St NW, Atlanta, GA, USA

The physical fitness of an individual serves as a cornerstone for analysis of proper animal welfare. While routine handlings provide the most comprehensive means for examining animal health, situations present where these measures are not feasible. A new habitat, *Sharks: Predators of the Deep*, at Georgia Aquarium, comprised solely of five elasmobranch species, represents such a case. Due to their size and/or sensitivity to stress during handlings, certain species are not candidates for routine workups. Therefore, a non-invasive and unbiased method to determine physical health is needed.

Body condition scoring (BCS) on elasmobranchs was previously established by Kamerman et al. 2017 with spotted eagle rays (*Aetobatus narinari*). It was also described in teleosts using zebrafish (*Danio rerio*) as a model (Clark et al. 2018). To account for differences in shark and ray body forms, an image of a bronze whaler (*Carcharhinus brachyuru*) was used to expand criteria to a shark model. Requiem sharks represent a common family in aquarium collections and their morphologies can be readily standardized for benthic and pelagic shark species. Based on the images derived from Kamerman et al. 2017 and Clark et al. 2018, similar key anatomical features were identified, along with those that pertained specifically to sharks. The features examined were coelomic surface, gill arches, pectoral girdle, head, and caudal peduncle (Figures 1a, b). Lateral and dorsal views were depicted for each body condition score. The scores were ranked on a scale of “1-5”. “1” illustrated an emaciated individual, “2” represented an under-conditioned individual, “3” was optimal body condition, “4” illustrated an over-conditioned individual, and “5” constituted an obese individual. The ideal body condition score of “3”, is commonly found in wild specimens; however, accredited aquariums often target a score of “4” in the event the individual undergoes a fasting period.

Body condition scoring assessments were conducted for every individual in the collection at the same time each month, along with health assessments. These health assessments entailed descriptions and photographs of existing or new diseases / injuries (i.e., abrasions, lesions, ulcers, erosion, etc.) for each animal, then compiled into a shared file to monitor progress.

This method of body condition scoring helped mitigate bias among husbandry and veterinary staff when analyzing animal fitness. It also served as a tool for teaching new staff about shark anatomy and how to properly describe physical fitness. Scores following each evaluation aided in management decisions regarding diet allotment and training criteria. Furthermore, they provided a more standardized means for assessing progress when an animal was compromised and undergoing veterinary treatment.

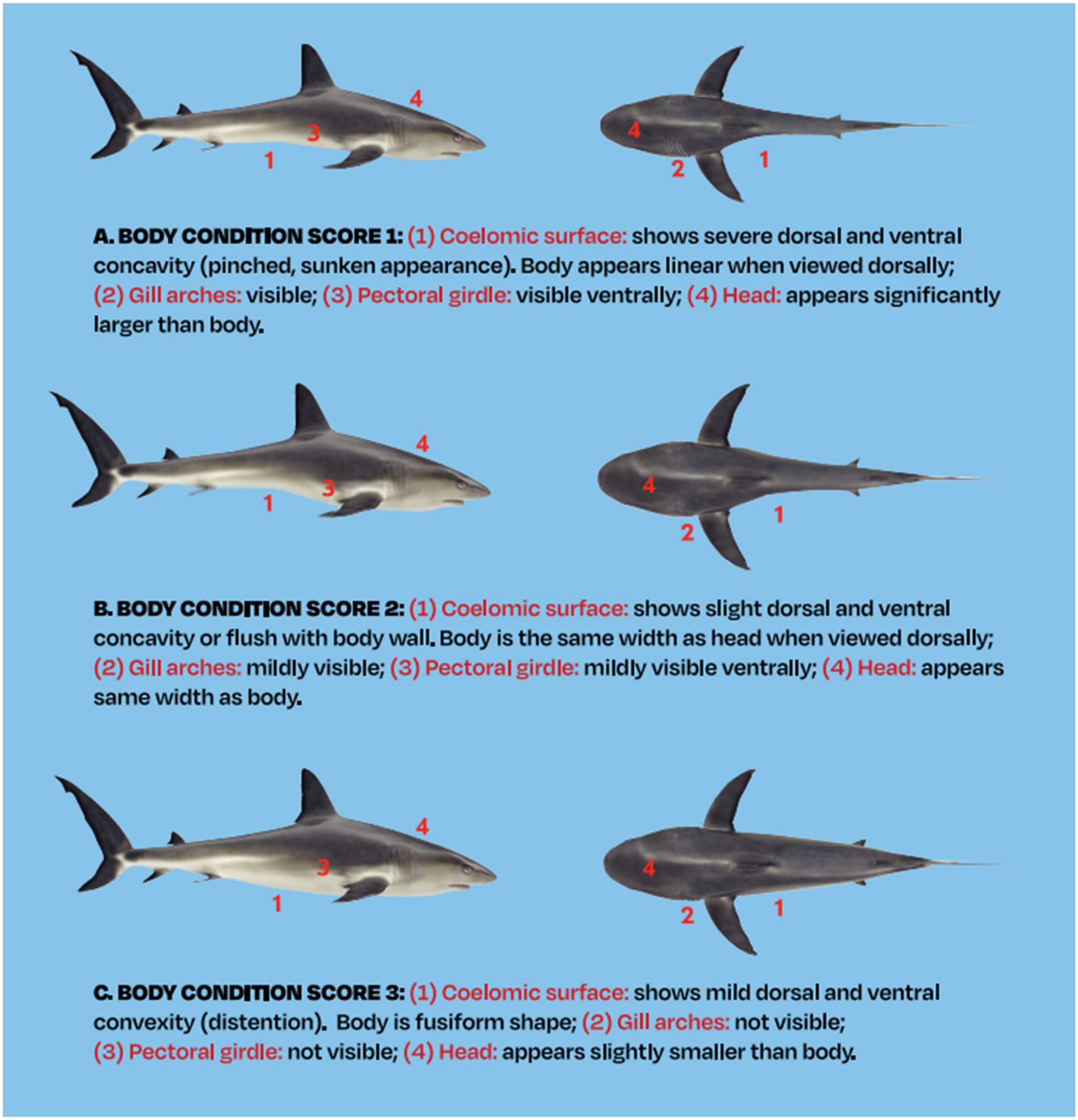


Figure 1a. Shark Body Condition Scoring utilized a carcharhinid model to illustrate body proportions and key anatomical features viewed laterally and dorsally. Scores 1 through 3 shown. See Figure 1b for scores 4 and 5.

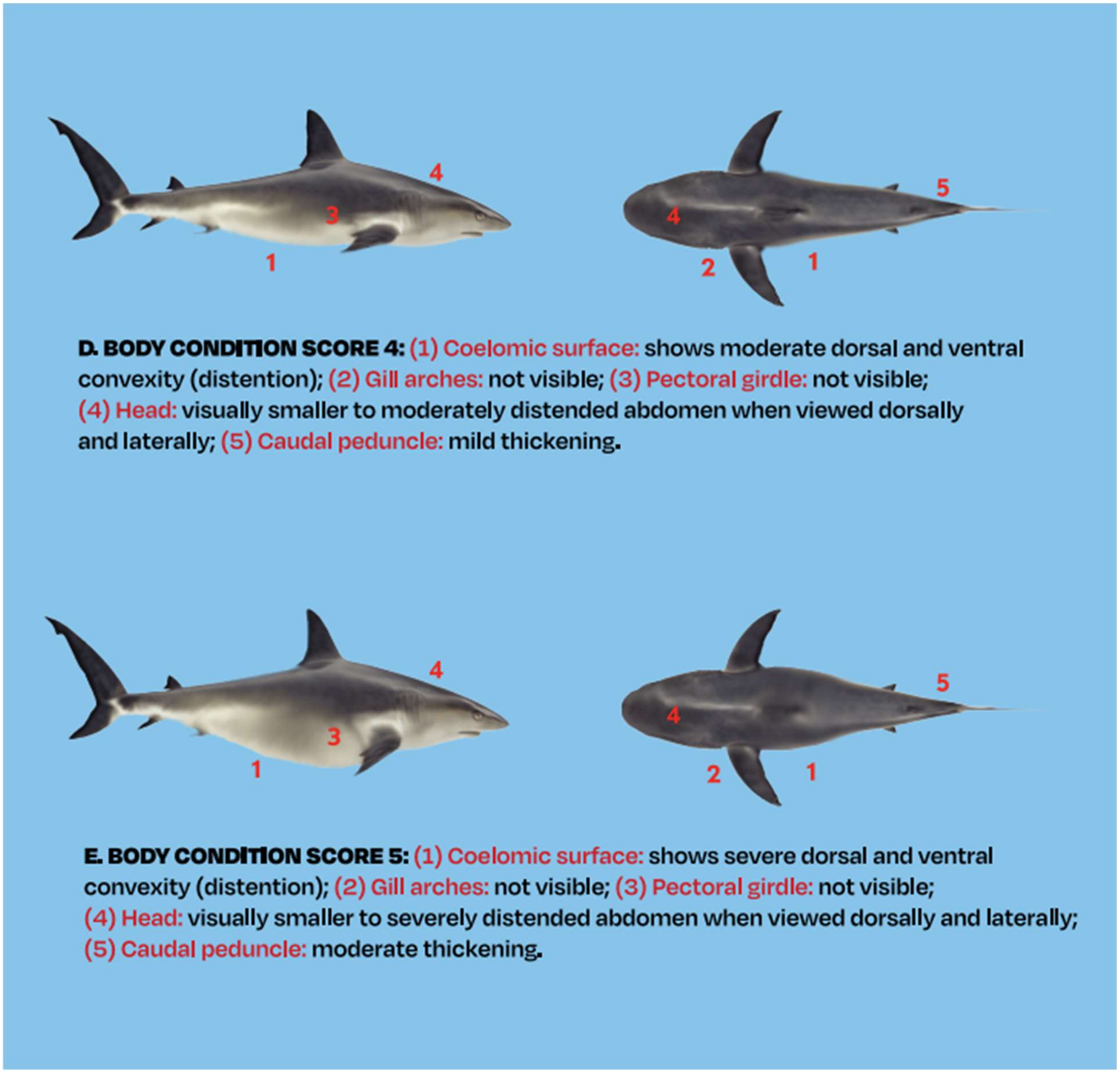


Figure 1b. Shark Body Condition Scoring utilized a carcharhinid model to illustrate body proportions and key anatomical features viewed laterally and dorsally. Scores 4 and 5 shown.

References

Clark, T. S., Pandolfo, L. M., Marshall, C. M., Mitra A. K., & Schech, J. M. (2018). Body condition scoring for adult zebrafish (*Danio rerio*). *Journal of the American Association for Laboratory Animal Science, 57*(6), 698-702. http://doi.org/10.30808/AALAS-JAALAS-18-000045

Kamerman, T. Y., Davis, L., & Capobianco, J. (2017). Development of a body condition scoring tool for the spotted eagle ray, *Aetobatus narinari*. In M. Smith, D. Warmolts, D. Thoney, R. Hueter, M. Murray, & J. Ezcurra (Eds.), *The Elasmobranch Husbandry Manual II*: *Recent Advances in the Care of Sharks, Rays and their Relatives* (pp. 147-151). Ohio Biological Survey, Inc.

STIRRING THE POT:
A NOVEL METHOD FOR REARING MARINE INVERTEBRATES

Mariah (Riah) Evin, Senior Biologist, mevin@calacademy.org
Margarita Upton, Larval Program Manager, mupton@calacademy.org

California Academy of Sciences, Steinhart Aquarium, 55 Music Concourse Drive, San Francisco, CA 94118

Culturing invertebrate marine larvae presents various challenges when considering their care and complex life cycle. These delicate larvae are often incompatible with traditional ways of creating water movement via aeration or flow-through, or when using static cultures. Air bubbles can damage larvae irreparably, flow-through systems risk damage via immobilization and abrasion on screens, and the lack of water movement in static cultures lends itself to stagnation, starvation, and opportunistic benthic pests. Historically, these problems have been solved through the use of large motor-driven stirring armatures such as the Strathmann Apparatus (Hodin, 2019). These large structures require a significant amount of in-house fabrication and have a limited ability for modular fine tuning as cultures change through time. Rather than building a large apparatus, we approached the issue by utilizing small, off the shelf items. The setup we describe here can be used with as little as one culture jar, or can be scaled up to as many as space, cost and/or time allows, thus allowing for incredible modularity.

Materials and Methods

In 2022, Steinhart Aquarium began to take a more active role in invertebrate rearing. We focused primarily on tropical urchins for algae control and temperate sea stars for display. We chose to test different rearing methods with *Patiria miniata.* Our preliminary collection of gametes from *P. miniata* were placed in an insulated plastic cooler and we observed very poor fertilization rates. Under microscopic examination, eggs appeared to be damaged or broken. Our next collection was performed in a Pyrex glass bowl and fertilization was achieved at nearly 90% with healthy and undamaged eggs. Therefore, all gametes and larvae were primarily kept in glass containers in successive projects.

We began with several experimental designs to raise *P. miniata*, utilizing the spawning procedure documented by Morrow (2021). We set up three commonly used conditions: 2 traditional static cultures and 1 flow-through culture. The static culture was replicated as Morrow (2021) observed the most success with this method. We also set up two experimental cultures to test if different methods of circulation would work: one using a magnetic stir bar at the bottom of a glass jar and another utilizing an automatic pot stirrer - the StirMate® Variable Speed Smart Pot Stirrer- Gen 3 (https://www.stirmate.com/). All cultures were housed in 10 L glass jars with the exception of a flow-through, aerated 75 L fiberglass black round tub (BRT). The BRT was utilized to test if invertebrates could be successfully reared in a fish propagation vessel (Fig. 1).

Figure 1. Initial setup of *P.miniata* rearing strategies. From left to right: 75L BRT with flow-through and air-agitation, static glass jar, magnetic stir bar in glass jar, StirMate® on glass jar, static glass jar. R.Evin © California Academy of Sciences

The StirMate® is an electric automatic pot stirrer that comes with a 12-hour backup rechargeable battery. The speed of stirring is easily controlled by a hand dial. The device clamps on to most any container and the adjustable rotor allows for use on containers with diameters ranging from 6 to 13 inches. This modularity allows for a great range of diversity in flow dynamics and vessel type. We elected to pair it with a glass candy jar that had a flat area around the rim where we could easily attach the StirMate® (Fig. 2). These jars were chosen due to the volume, availability, low cost, and ease of cleaning. We calibrated the volume of the jar by measuring and marking the outside with a paint pen. Jars were kept in a water bath to maintain temperature (Fig. 3).

Figure 2. StirMate® setup equipment. M. Upton
© California Academy of Sciences

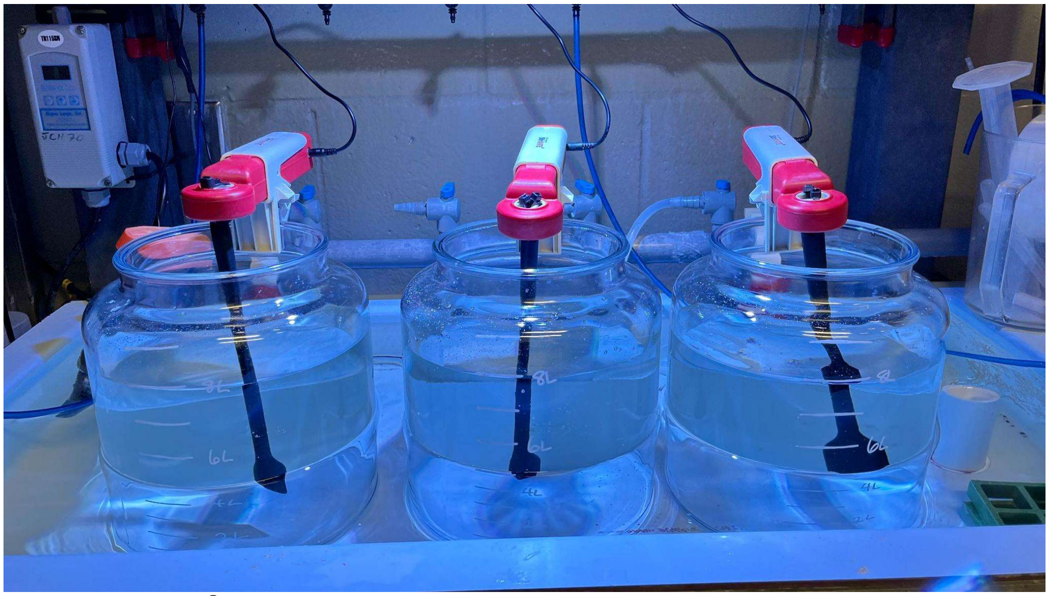


Figure 3. StirMate® jars in a water table to maintain temperature. M. Upton © California Academy of Sciences

The StirMate® comes prepackaged with a 'stirring wand', however this contraption is intended to drag along the bottom and has many abrasive surfaces that can damage delicate larvae. Therefore, we opted to equip our StirMate® units with long, one-piece silicone jar spatulas (Patelei Silicone Jar Spatula, ASIN B08QMVDNX4) (Fig. 4). Jar spatulas or other stirring components can be easily installed into the StirMate® with zip ties. Jar spatulas have a longer, more narrow profile than traditional spatulas and thus offer less damaging surface area. Adjusting the StirMate® so that the spatula sits at an angle relative to the bottom of the jar minimizes the creation of a stagnant area where larvae and detritus can accumulate (Fig. 5).

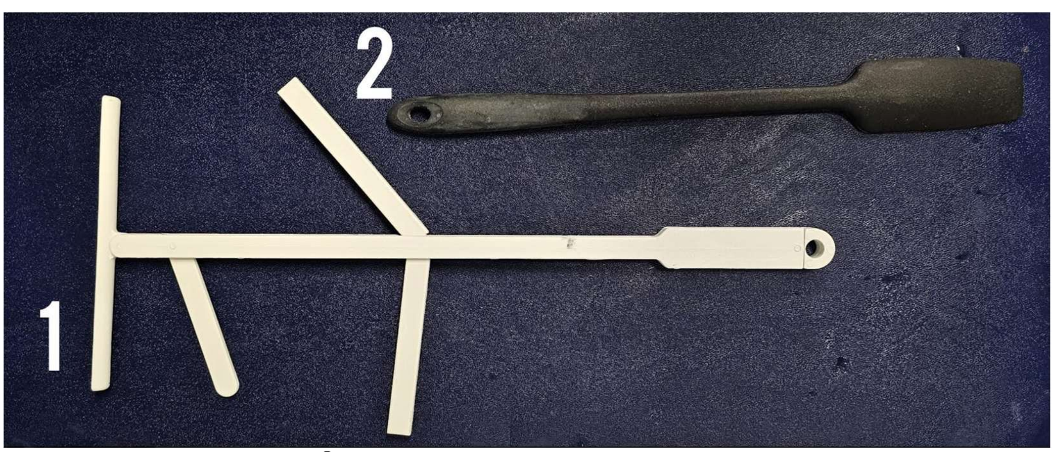


Figure 4. 1. Included Stirmate® stirring wand 2. Silicone jar spatula, 28 cm. R.Evin,© California Academy of Sciences

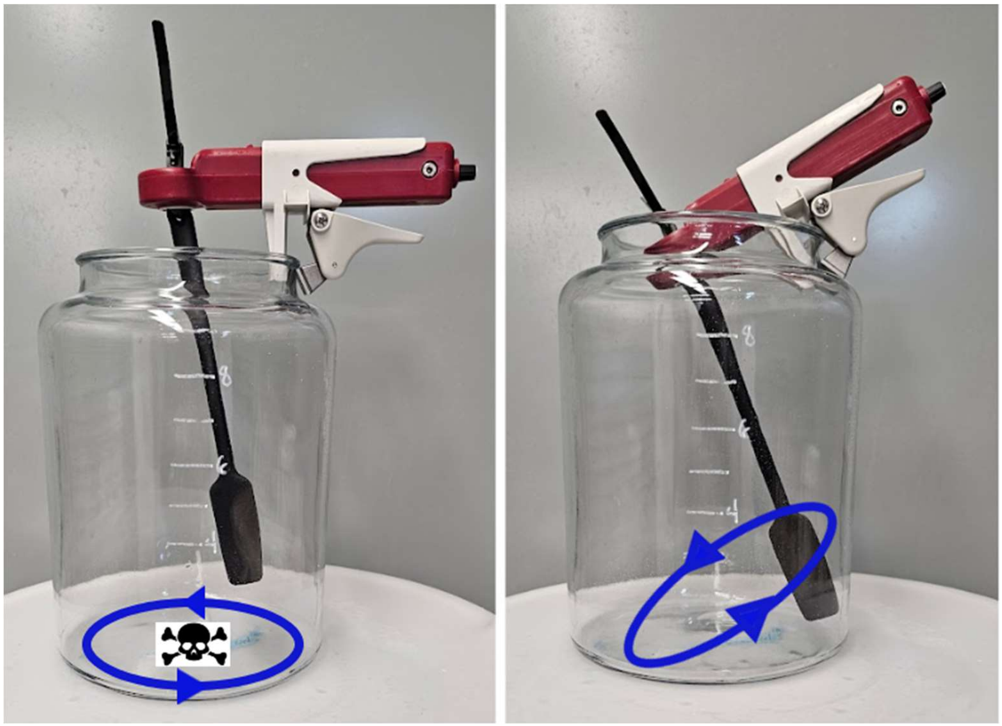


Figure 5: Angle of the spatula will impact flow dynamics and can be adjusted to prevent stagnation. R. Evin © California Academy of Sciences

Larvae were primarily fed primarily *Rhodomonas lens*, though *Nannochloropsis oculata* and *Isochrysis galbana* (T) were utilized in the absence of *Rhodomonas*. Larvae were examined regularly to ensure food was present in their digestive tract. Feeds were increased or decreased as deemed necessary by these observations (Fig. 6).

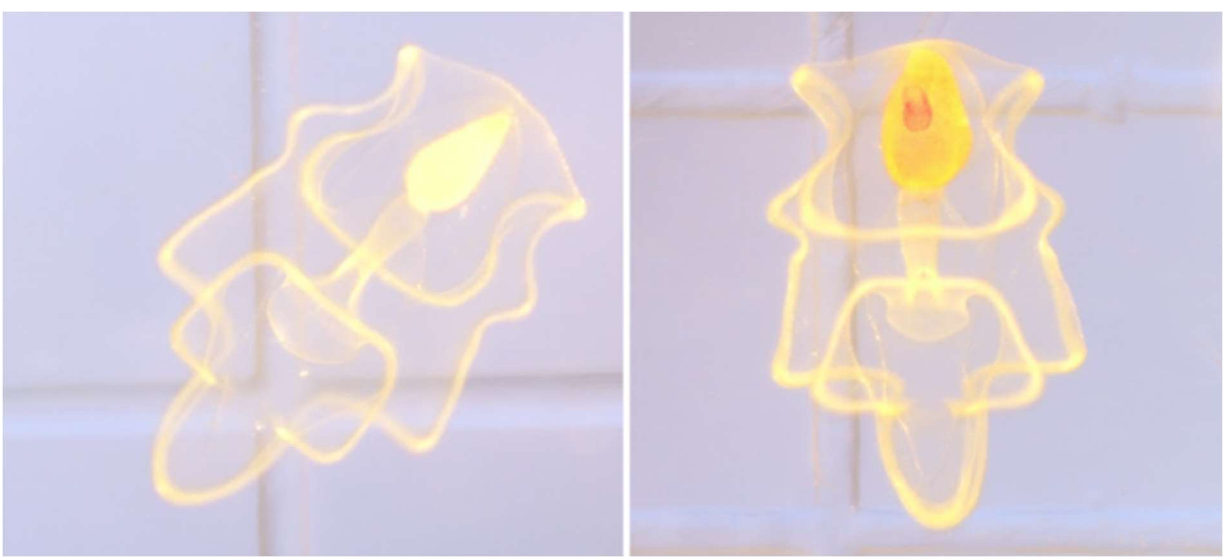


Figure 6. Larvae of *P.miniata.* Left: larvae with very little food present in the digestive tract - note lack of red coloration. Right: larvae with dark red pigmentation of gut tract indicates consumption of *Rhodomonas lens.* R. Evin © California Academy of Sciences

Weekly maintenance on all glass jars included two 50% water changes and one 100% water and jar change. Jars were sterilized with hydrochloric acid between use. Silicone spatulas were cleaned with hydrochloric acid or reverse osmosis water and melamine sponges during water changes. This maintenance regimen was used to eliminate the build up of benthic pests, debris and maintain water quality within the jars. BRT maintenance primarily consisted of changing the flow-through screen.

Results

The *P. miniata* flow-through BRT and static jars yielded few larvae settling into juveniles. The magnetic stir bar culture proved lethal to *P.miniata* as no larvae survived to settlement. The StirMate® jar had the most larval settlement - ultimately leading to several hundred settled stars in 2022 (Table 1).

Table 1. *P. miniata* settlement utilizing different rearing techniques.

Experimental Design	Number of Settled Larvae
BRT	10-20
Magnetic Stir Bar	0
StirMate®	>300
Static Jar A	10-15
Static Jar B	10-20

The StirMate® setup success was repeated in 2023 with thousands of juvenile stars settling out within each culture jar. The flexibility of this system allowed us to study the density of *P. miniata* within our cultures. The densities varied from 5 larvae/mL water to 10 larvae/mL water. The higher density was as effective at producing large numbers of newly settled stars as the lower density. However, higher density cultures took longer to reach settlement with the lower density settling 15 days earlier.

The preliminary success with *P. miniata* spurred us to experiment with tailoring this setup to propagate a variety of other species (Table 2). These included the urchins *Strongylocentrotus purpuratus*, *Lytechinus variegatus*, *Tripneustes gratilla* and *Mespilia globulus*, and the sand dollar, *Dendraster excentricus*, utilizing the spawning protocol documented by Hoden (2019). Opportunistic egg production from the nudibranch *Melibe leonina* and the peppermint shrimp, *Lysmata boggessi,* also allowed us to use this rearing method with non-echinoderm larvae. We achieved the most success with the echinoderms *S. purpuratus*, *L. variegatus, M. globulus* and *D. excentricus* yielding hundreds to thousands of settled juveniles for each jar. *T. gratilla* was successful in settlement, though at a much lower yield, possibly due to larval diet. As observed in the *P. miniata* cultures, when larvae were kept at a higher density, the larval stage was prolonged and settlement occurred at smaller sizes. The outcome of the density difference was most notable in *M. globulus* where the lower density cultures had visibly larger larvae, began settlement 14 days earlier and ultimately had larger juveniles.

The non-echinoderm larvae we attempted were less successful, though we believe the system may still work with modifications. *Melibe leonina* were successfully kept alive with this method up to 51 days though ultimately we were unable to settle juveniles from the larvae. *Lysmata boggessi* were kept alive for 21 days nearly to their last instar but we were unable to settle juveniles.

Table 2. Species reared and time to settlement using the StirMate® setup.

Species	Temp (°C)	Density (Larvae/ mL water)	Diet	Days to First Settlement
Patiria miniata	15.6	5	*Rhodomonas lens*	49
Patiria miniata	15.6	10	*Rhodomonas lens*	64
Strongylocentrotus purpuratus	10	5	*Rhodomonas lens*	37
Strongylocentrotus purpuratus	10	10	*Rhodomonas lens*	36
Dendraster excentricus	10	unknown	*Rhodomonas lens*	29
Dendraster excentricus	10	10	*Rhodomonas lens*	36
Dendraster excentricus	15.6	10	*Rhodomonas lens*	25
Lytechinus variegatus	79	10	*Rhodomonas lens, Isochrysis galbana (T)*	21
Tripneustes gratilla	79	10	*Nannochloropsis oculata, Rhodomonas lens*	31
Mespilia globulus	79	10	*Isochrysis galbana (T)*	33
Mespilia globulus	79	3-5	*Isochrysis galbana (T)*	19
Melibe leonina	10	unknown	*Isochrysis galbana (T)*, *Nannochloropsis oculata*, *Rhodomonas lens*	No Settlement, Larvae viable to day 19
Melibe leonina	10	unknown	*Rhodomonas lens*	No Settlement, Larvae viable to day 51
Lysmata boggessi	79	0.025	Rotifers, *Artemia, Parvocalanus crassirostris, Isochrysis galbana (T)*, *Nannochloropsis oculata*	No Settlement, Larvae viable to day 21

Discussion

The StirMate® setup performed well when rearing echinoderm species. Success is likely due to the ability to keep larvae suspended in the water column without abrasion. The speed of stirring can be adjusted as larvae grow to ensure they remain in suspension. The contained nature of the system allows for long term contact with food. One challenge this method can present is labor intensive weekly maintenance, dependent on number of replications, culture density, and pest load.

System modifications for non-echinoderm larvae may provide success with larval settlement. As *Lysmata* larvae molted and their appendages became larger, they were sensitive to jar changes. Later instars also needed a faster rate of stirring to keep them suspended in the water column. We will attempt *Lysmata* again but modify the water changes to siphoning only without moving the animals, in addition to maintaining them at a lower density. *Melibe* larvae have a complex development that may require settlement cues. Future culture attempts with *Melibe* can be adapted to include the addition of macroalgaes or marine plants introduced at metamorphosis. Additional non-echinoderm species that could potentially be reared with this system include bivalves, gastropods, and anemones.

The true success of this study has been the ease of setup and modularity (Fig. 7). With such a small footprint, the StirMate® setup can be utilized in even the tightest of back of house spaces. A small platform in a sump, a corner of a water table or even an ambient culture on a desk are all achievable. The setup also lends itself to easily duplicating studies and/or producing a wide variety of variables to test. Utilizing off the shelf components with an initial hardware investment of under $150 USD (at the time of this publication) can make culturing marine invertebrates accessible to aquariums or institutions that previously may not have had the funding or space to do so. This simple method can contribute to sustainable aquarium collections by utilizing cultured animals for display, diet and research, while minimizing the overall carbon footprint and environmental pressure associated with wild collected organisms.

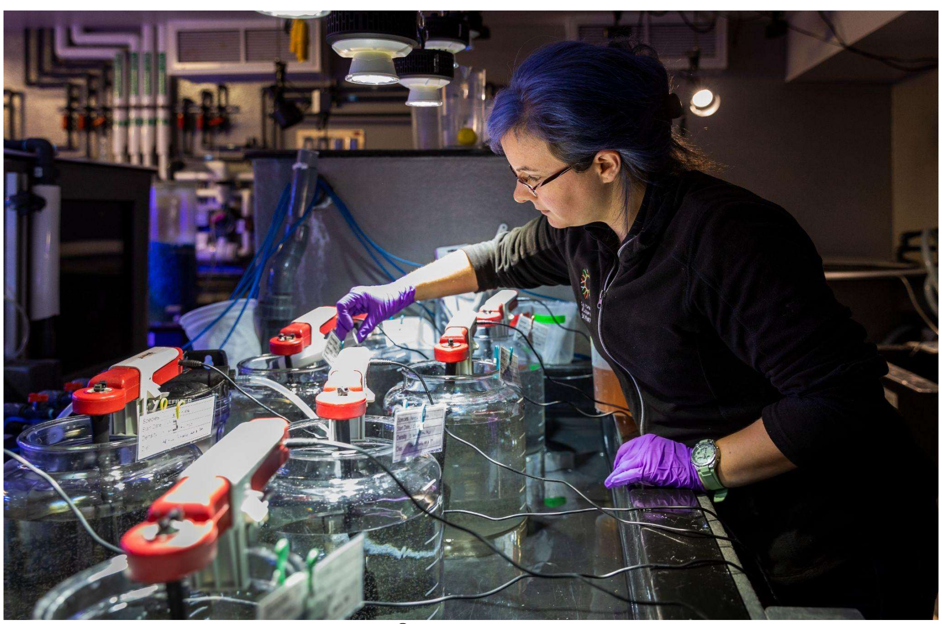


Figure 7. Modularity of the StirMate® setup. G. Laird © California Academy of Sciences

Acknowledgements

The authors would like to share special thanks to Nick Yim, Kylie Lev, Julian Plamann, Jessica Witherly, and Charles Delbeek. Additionally, we would like to thank the CAS Animal Health and Aquarium Teams at the Steinhart Aquarium for their dedication to animal care and innovation.

References

Hodin, J., Heyland, A., Mercier, A., Pernet, B., Cohen, D.L., Hamel, J-F, Allen, J.D., McAlister, J.S., Byrne, M., Cisternas, P. and S.B. George. 2019. Culturing echinoderm larvae through metamorphosis. *Methods in Cell Biology 2019*:150:125-169. doi: 10.1016/bs.mcb.2018.11.004.:, p.125-169.

Morrow, B. 2021. Aquaculture of *Patiria miniata*. *Drum & Croaker. 52*:3-10.

https://www.stirmate.com/shop

REMEMBERING DAVE POWELL

Ken Peterson

Monterey Bay Aquarium

Dave Powell was a giant in the public aquarium profession, a visionary whose work transformed the range of marine life that aquariums could bring to the public and who shaped both the culture and look of Monterey Bay Aquarium.

Dave passed away in August 2023 at the age of 96. He leaves an enduring legacy throughout the global aquarium community and in the hearts of those he touched personally and professionally. His influence will reach future generations of guests who visit aquarium exhibits that were shaped by his creativity and passion for the ocean environment.

He told much of his story in his autobiography, *A Fascination for Fish: Adventures of an Underwater Pioneer* – required reading for anyone in the aquarium profession: his upbringing in South Africa and England, his early adventures scuba diving in the 1950s, his life in southern California, and his passion to bring the world below the surface to the public – first at Marineland of the Pacific, then at SeaWorld San Diego, then Steinhart Aquarium at San Francisco's California Academy of Sciences, and as the original and visionary animal care director at the Monterey Bay Aquarium. He consulted for aquariums around the world, including Two Oceans Aquarium in South Africa.

David dedicated his life to understanding the secrets of fish and inventing methods to collect all types of aquatic creatures – unharmed and healthy – to thrive in exhibits that replicate their natural habitats. Upon encountering an interesting fish or invertebrate, he'd ask, "How can we safely collect these so others appreciate their beauty and unique behaviors?" In his relentless pursuit, he inspired everyone to push the boundaries and do whatever it took, no matter that it hadn't ever been done before.

In Monterey, he shaped the design of both public and behind-the-scenes areas, assuring that his aquarists had both physical space and time in their schedules to learn how to raise species that had never been exhibited. And he was equally attentive to the life-support systems that are essential to keeping new and challenging species on exhibit.

Think of the successes in Monterey: creating the first living kelp forest exhibit anywhere; expanding the possibilities of exhibiting delicate jellies after propagating them through a complex life cycle of free-swimming, sexually reproductive adult medusae through a sessile polyp phase that led to the asexual reproduction of new generations of baby jellies); and introducing touch pools where guests could have hands-on encounters with intertidal animals and plans.

He went deep with the first large-scale exhibit of deep-sea organisms. He went big with an open ocean exhibit that housed the only tunas outside Japan and – eventually – a half-dozen juvenile white sharks that navigated the exhibit, took food from the animal care staff, and months later were successfully returned to the wild.

As a beloved mentor, Dave motivated his staff of aquarists with his perseverance to invent and problem-solve. While modest and sharing credit, he fostered a sense of awe in everyone who worked with him. He did so with his unique sense of humor.

There are as many stories as there are dimensions to Dave Powell. Here are a few told by his friends and family. His mother, for example, often shared the now classic story of him sleeping with his first-caught fish under his pillow! And he would himself recount how he met and wooed

Betty, his wife of 68 years, by inviting her up to his apartment…to see his octopus, one that he collected and kept in a home aquarium.

Decades later, he and Betty kept another unusual ocean animal – a rescued sea otter pup – in their bathtub at home when the Monterey Bay Aquarium team was first trying to figure out how to raise orphaned pups for exhibit.

Monterey Bay Aquarium Executive Director Julie Packard recalls that, "When our founders first envisioned a new kind of aquarium on Monterey Bay, we had great stories to tell about the bay's amazing ocean life but knew next to nothing about how to bring this vision to reality. Dave thought we were pretty crazy at first – especially our desire to create a living kelp forest – but soon joined our team. We would not be the aquarium we are today without his many remarkable contributions. He had a brilliant mind for exhibit design, creating most of the signature designs in the original galleries that set our experience apart from the usual rows of rectangular tanks in dark hallways so typical of aquariums at the time.

"Dave was passionate, creative, risk-taking, and not afraid to buck the rules. He wasn't always easy to work with but stuck to his convictions and earned unconditional respect from all who worked with him. We owe him such an enormous debt of gratitude."

His grandson Norman Powell remembers as a child visiting the aquarium in Monterey that "I never realized the significant role my Baba played in curating such a magical place. I just knew he knew a lot about fish, who knew other cool people who knew a lot about fish. When you get to meet the jellyfish lady and feed otters in bathtubs you don't think much of it as a kid, besides it being pretty cool. Looking back, it was an amazing experience being able to see behind the scenes of a world-famous aquarium, especially being led around by a famous fish guy. But to me, he was just Baba. A man who loved all things water-related, had an interesting collection of fish and other under-water creatures – all preserved – scattered around his house. I'll miss my Baba dearly. "

His nephew Jeff Brownow remembers Dave as someone "who loved fish and the environment they lived in. He loved the sea and marine life. But I also know he loved humans because he wanted to share with as many people as he could, in the best way he knew how, his love of the water and aquatic life."

"Dave was a warm and kind man, respectful of those he met," Jeff says. "And he had a warm and kind handshake. You can tell a lot about a person from their handshake. He was kind and always offered a helping hand to me and to others I saw him interact with. I admired his soft shyness, and I loved his quiet, tucked-in form of giggling, his body shaking with joy at anything funny. I remember he loved limericks, which he would recite from memory, and would end each one with his giggle thing when speaking the last line, which was always the punch line."

Dave also had a "damn the torpedoes – full speed ahead" rule-breaking attitude that produced great results – and interesting stories, too. John Hewitt, his colleague at SeaWorld, remembers driving with Dave from San Francisco to Los Angeles to pick up a new Boston Whaler.

"Dave, with absolutely no previous experience in boat hauling or loading took charge!!" John said. "All was well until about halfway to San Diego when it was noticed that the new vessel was loaded without tiedown straps of any kind, and it was moving aft on the trailer by almost half a boat length. No problem when this was brought to Dave's attention. He proceeded to build up speed on the interstate, headed down a more-or-less-straight off ramp, and slammed on the brakes at approximately 60 miles a hour and hope it would move forward as a result. Two or three such maneuvers and we made it home."

"When asked why he didn't apply more tiedowns, Dave replied he didn't have any and he was not about to use his prized 'Hippie Belt' for such a menial task. Glad we all survived!"

Jon Hoech, originally an aquarist and now vice president of animal care at Monterey Bay Aquarium, says his memories revolve around "Dave's mentorship style, his staunch devotion to sharing the marine environment, and his passion for distilling his messages into remarkable simple terms. I learned a lot from Dave."

"Some of my early memories of Dave." Jon says, "come not from my direct experiences, but from the stories. I recall hearing about Dave's philosophy on exhibit service areas. During the early design stages of the Monterey Bay Aquarium, Dave was known for his rigorous advocacy for large work areas. There is always competition for building footprints when weighing the balance of a guest's experience, the need for animal support areas, and other crucial infrastructure. Dave was a firm believer in the idea that if you give aquarists room to work in, make it easy for them to store tools, give them plenty of room to move and get dirty, they will be that much more motivated because of it.

"'Ultimately,' Dave would say, 'give the people room to do their job, and the payoff will be reflected in the quality of the guest experience.' I'm not entirely sure people really got it at the time, but Dave's legacy lives on. He was right!

Jon also reflected on how Dave advocated for creating exhibits that would appeal to visitors - "the people we strive to inspire. It's common that aquarium scientists are more driven by our own biases - what we want to display and what we are excited about. We often forget about what the public interest might be. I think it's easy for us to forget.

"I still hear Dave's voice in my mind. The one that would say things like: 'We need more wolf eels in the Kelp Forest Exhibit. I was out on the floor this morning and heard a young boy say to his parents, *I can't find the wolf eel*. We need more wolf eels.' I remember another time, a morning after I had spent several days combing the tidepools and diving in the local reefs searching for and collecting a diverse array of nudibranchs for one of our exhibits. That morning, Dave walked up to me and showed me a note from our visitor suggestion box that read, '*love the nudibranchs*!' Dave simply showed me the note and said, 'They notice,' and walked away. To the point, effective, meaningful. Dave's compliments weren't always direct, but impactful.

"Dave had a simplistic wit about him. Sometimes it would come across as offensive, but almost always Dave would leave me with something to reflect upon: he would cause me to think about how I might over-complicate things, teach me something about the elegance of simplicity, or point out the benefit of close and detailed observation.

"I recall a time just after we'd stocked our new Open Sea Exhibit with bluefin and yellowfin tuna. For most of us, seeing tuna in an aquarium was a new experience, and many of us would rush to the front of the exhibit during feeding time. It was fast, furious, and exciting. One time, one of my colleagues said, 'Jeez, I don't get it. These guys are bolting around in a 30-mile-an-hour frenzy and I don't understand what keeps them from eating their own [poop].' Without missing a beat, and with a frown of disappointment, Dave turned and responded: 'If I handed you a hotdog in one hand, and a turd in the other, I bet you'd figure it out." I don't believe that question ever came up again."

"Dave wasn't one to allow someone to go too far astray, but neither was he eager to solve your problems for you. I remember working on a plumbing project, a dynamic water-motion device.

"Whatcha up to?" Dave asked when he saw me. After hearing my answer, he muttered a simple 'Hmmm,' took a drag off his cigarette, turned, and said 'Good luck.' Hours later when he came by again and saw that I had completely altered my strategy, he smiled, and said, 'Great job.' It was his way of encouraging you to experiment and learn on your own. He believed experience was one of the greatest teachers.

“Dave was an innovator, and he encouraged exploration. He wouldn’t stifle creativity by inserting doubt. He might not think you were headed down the right path, but he was willing to let you try. If you failed, that was part of your education. If you succeeded, that was worthy of celebration.”

Weedy Seadragon, Bruce Koike

COMMENTS ON "WATSON AND THE SHARK" BY JOHN SINGLETON COPLEY: HISTORY ALWAYS MATTERS

Pete Mohan

Editor, Drum and Croaker

Introduction

Most of us have seen the image featured on this year's cover. I encountered an "original" (there are at least several versions) while visiting the Detroit Institute of Arts. My first impression was "cool shark!" It seemed like an image you would all enjoy, so without further thought or investigation, I requested and received permission to reproduce it here.

Biologists have noted the rather crude depiction of the shark. The presence of an external ear on the National Gallery of Art's original, bright pink lips on a copy (Metropolitan Museum of Art, New York), odd-shaped fins, and water spouting from its nares as from a whale's blowhole, all suggest Copley had never seen an actual intact shark, alive or dead. But this was an improvement over earlier artists' renderings that looked more like crosses between alligators, salamanders and whales.

The depicted scene is one of chaos, with the possibilities of both rescue and disaster frozen in time. Centuries of analysis and speculation have added to the confusion. Art historians have all kinds of opinions on the style, composition and influences that are reflected in this painting. Critics comment on issues with the waves, styles of ships, demeanor of the rescuers, etc. Not my thing, but Arthur Marks' recent review covers it all if you're up for a very long read. There is perhaps some social commentary going on here as well. I'll get to a bit of that later.

Truths Revealed – Uh-oh.

Being a bit of a history geek, I started digging into the story behind the painting. Initial skimming revealed that this is a record of an actual event that occurred in 1749. Fourteen-year-old Brook Watson went for a swim while his ship was moored in Havana harbor. He lost his lower right leg, but was rescued after the shark was eventually fended off with a boat hook. Watson survived and became the Lord Mayor of London. The artist was perhaps the most famous American painter from the colonial era, and the well-known portrait of Paul Revere is his work. His shark attack imagery launched a successful run as a painter of historical depictions. Copley Square in Boston, bears his name. Wow, success stories all around! Unfortunately, the details are far murkier and more complicated.

The painting was completed in 1778, during the early years of the American Revolution. Copley however, was not in America at that time, having been forced to evacuate from Boston to England a few years earlier due to his in-law's strong loyalist ties and a couple of close encounters with patriot mobs at their homes. Copley himself was said to be apolitical and his views at that time appear to have been more sympathetic to both the colonists and reconciliation. He painted Samuel Adams in the aftermath of the Boston Massacre, showing him pointing to documents demanding the expulsion of British troops from the city. His famous portrait of Revere included a tea pot, in a not-so-subtle nod to the recent Boston Tea Party. This was especially notable as the tea in question was being shipped to Copley's father-in-law, Richard Clarke! Dinner table conversation was likely minimal that week. The artist had "married up" due to his status as a respected artist. Along with the rich wife, came several enslaved persons. When Watson and the Shark was painted (in the UK), the abolitionist movement was gaining momentum in England. This is significant as it may relate to the addition of the black rescuer to the apex of the final painting, replacing a white sailor in the draft sketch. Was Copley showing his true (or revised) sympathies, as he did in the painting of Revere? Was he simply aware that black sailors were well represented on West Indies crews? Theories abound. We can only hope, but must add him to the list of prominent colonial Americans with this particularly abhorrent biographical detail.

While Copley may have had a few positive characteristics, Watson turned out to be "a real piece of work." In 1772 Watson helped found Lloyds of London, then a major insurer of ships transporting enslaved Africans (covered as "goods") on the Middle Passage. In a strange twist, he also dealt with the transport of tea by the East India Company, including the particular shipment destined for: Copley's father-in-law, the subsequent bath in Boston Harbor, and memorialization in Copley's painting of Revere. During the Revolutionary War he was an agent for the crown, and was charged with overseeing the transport of the captured American officer, Ethan Allen (of Green Mountain Boys fame) to prison in England. The shipboard conditions were filthy, and Allen later wrote that he "was put under the power of an English Merchant from London, whose name was Brook Watson: a man of malicious and cruel disposition". Watson's presence on the ship and demeanor was not a coincidence, as he was fleeing after being identified as a possible British spy. Watson later served in Parliament, where he was said to be a "warmonger." He also voted against the abolishment of the slave trade as it would place economic hardships on both those transporting slaves, and the Newfoundland fishers supplying the poor-quality salt fish provided to enslaved communities. One may also assume that the threat of a loss of income to Lloyds may have been another consideration.

Watson used the shark attack for self-promotion throughout his life. His coat of arms (Figure 1) features his severed leg under an image of Neptune. He may have actually commissioned the original painting to commemorate the event. The now eponymous title evolved from the original "A boy attacked by a shark, and rescued by some seamen in a boat; founded on a fact which happened in the harbour of the Havannah." He bequeathed the painting to Christ's Hospital (a public school), with the hope that it would prove "a most useful lesson to youth". This is the first version, and now resides in the National Gallery in Washington, D.C. It formerly hung in the dining hall of the school, probably evoking some humorous commentary by the students. I think we can all agree that the shark, presumably a tiger, gets points for attempted predation.

Reflections

Damn. I just wanted to tell you an interesting story about a shark painting. While I might admire the art, I'm put off by the background history. How is Watson and the Shark relevant to our community, other than it's a cool picture of a badly painted shark? Directly, of course, it's not. It's just an example of the warts you'll unexpectedly find while digging into the past. Things are not always what they seem, and you might not like what you find. When you encounter an issue with husbandry and dig into the relevant literature, including the earliest editions of *Drum and Croaker*, you will occasionally find some husbandry practices that were normal or even ground-breaking back in the day, but are unacceptable when viewed from 2024. I have not hidden these in the D&C archives. Take the opportunity to learn from the mistakes of the '50s, '60s and '70s. As you read, you'll note we "reinvent the wheel" repeatedly. As Churchill said, "Those that fail to learn from history are doomed to repeat it." Use your research to evolve beyond the status quo. Find and test solutions. Reveal and correct hidden problems. Most importantly, document your work in print for future husbandrists. And don't swim in Havana Harbor.

Figure 1. Brook Watson's Personal Coat of Arms.
That's Neptune above the shield. Note the severed right leg at the upper left.
The moto translates to "under God's protection," and the ravens are symbols of divine providence. The moon symbolizes serenity.
Self-promotion, indeed.

References and Further Reading

Marks, Arthur S. 2022. Brook Watson, A Boy, and A Shark: Appropriation, Academic Matters, and the Americanizing of a John Singleton Copley Painting. University of North Carolina at Chapel Hill. 278 pp.
https://www.academia.edu/75583103/Brook_Watson_A_Boy_and_A_Shark_Appropriation_Academic_Matters_and_the_Americanizing_of_a_John_Singleton_Copley_Painting

Watson and the Shark. National Gallery of Art, Washington, DC.
https://www.nga.gov/collection/highlights/copley-watson-and-the-shark.html
(1778. Full sized original, approximately 6 x 7.5 ft, purchased by Watson)

Watson and the Shark. The Met (Metropolitan Museum of Art), New York, NY.
https://www.metmuseum.org/art/collection/search/10554

(1778. This was a smaller format, rapid study that Copley copied from the original and used to prepare for later versions.)

Watson and the Shark. Museum of Fine Arts Boston, Boston, MA.
https://collections.mfa.org/objects/30998
(1778. Full sized copy of original, painted for Copley himself)

Watson and the Shark. Detroit Institute of Arts, Detroit, MI.
https://dia.org/collection/watson-and-shark-41300
(1782. Smaller format copy, featured by permission in this issue of D&C)

Watson and the Shark. Wikipedia.
https://en.wikipedia.org/wiki/Watson_and_the_Shark

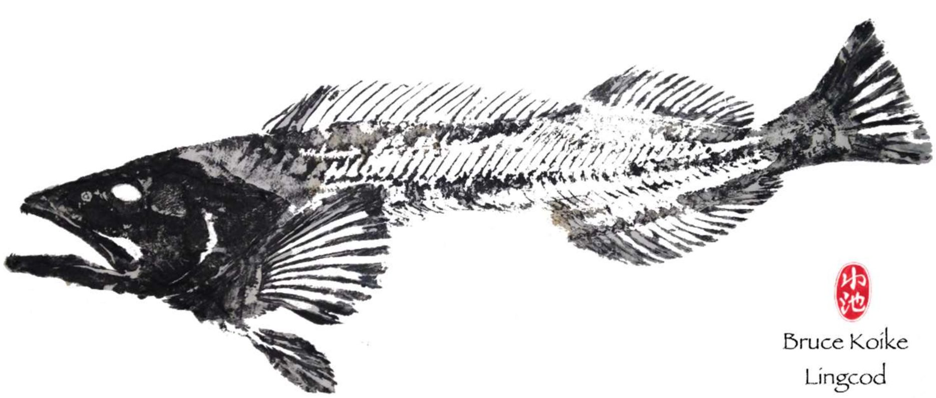
John Singleton Copley. Wikipedia.
https://en.wikipedia.org/wiki/John_Singleton_Copley

Ethan Allen. Wikipedia.
https://en.wikipedia.org/wiki/Ethan_Allen

The Life and Times of Sir Brook Watson. The Bank of England Museum.
https://www.bankofengland.co.uk/museum/online-collections/blog/the-life-and-times-of-sir-brook-watson

Lloyds of London Archives Show How Important the City Was to the Transatlantic Slave Trade. Katie Donington, The Conversation.
https://phys.org/news/2023-11-lloyds-london-archives-important-city.html

Lessons in Looking. Rethinking Watson and the Shark. Craig McDaniel. The Montréal Review, August 2023.
https://themontrealreview.com/Articles/Rethinking_Watson_and_the_Shark.php



STURGERY: SURGICAL FOREIGN OBJECT REMOVAL FROM *Acipenser transmontanus* AT WONDERS OF WILDLIFE

Briana Schmidt, Lead Aquarist bischmidt@wondersofwildlife.org

Wonders of Wildlife National Museum & Aquarium,
500 W. Sunshine, Springfield, MO 65807

Introduction

In early January 2022, it was noted that a white sturgeon (*Acipenser transmontanus*) by the house name of "Roger", had begun swimming with difficulty through his 131,000 gallon "Community Pond" home. Based on immediate observations, it was surmised that the animal had swallowed something large and heavy that was causing an issue in buoyancy (Figure 1). Roger was an animal that had arrived at Wonders of Wildlife in 2016, prior to the facility's grand re-opening in September of 2017. The sturgeon was originally placed in a 33,000 gallon "Under River" exhibit but his rapid growth ultimately compelled his move to the larger exhibit a year later where he became an impressive ambassador for his species and the star of the regularly performed dive show.

Figure 1. "Roger" with a belly full of rocks from the public view.

Observations and Treatments

Because of the original observation of this animal's physical condition, and issues with staying steady while swimming, Roger was caught and brought to the surface so that in-house veterinarian Dr. Michael Stafford could perform an ultrasound to help pinpoint the issue. After the first ultrasound and abdominal palpation, it was determined that the animal must have ingested substrate (river stones) and would need to fast during targeted feeds to encourage regurgitation. This method proved successful in correcting this ingestion of rocks and again in April when it happened again.

In June of 2022, a very large amount of substrate was ingested, causing more of a disturbance in the animal's swim pattern than before. Due to the continued trend with this animal and his taste for substrate, the decision was made to remove all ingestible rocks from the exhibit to prevent further events. With the substrate removed and the animal fasting from targeted feeds, it was presumed that the rocks would be regurgitated one final time.

June gave way to July and Roger still had a belly full of rocks. Because this exhibit also houses a collection of other freshwater species, the previous fasting attempts had only been during targeted feeds (during the dive show) and the exhibit's broadcast diet had not been halted. Due to the elapsed time and no progress in removing the rocks still in Roger's stomach, the broadcast diet was halted and the entire exhibit was fasted for a week in attempt to encourage regurgitation.

This fasting event did not work as planned and it was noted that Roger's behavior was changing. This animal had regularly come up during the show dive (even when not being fed) to interact with divers and always possessed a strong feed response but had recently begun to act avoidant of the divers and did not react voraciously to mouth stimulation as before.

At the end of July, it was decided to catch, sedate, and attempt a manual removal of the foreign objects. Roger was sedated MS222 (at 80 ppm) but still showed signs of resistance to the drug so the dosage was increased to 100ppm. Dr. Stafford placed the animal in dorsal recumbency and passed his arm through the animal's mouth and into his esophagus (Figure 2) but ultimately the animal was too long and manual removal of the rocks was not successful. While sedated, radiographs confirmed a "copious amount" of rocks in the stomach (Figure 3). It was decided to place Roger back on exhibit to recover and schedule a surgery for removal of the rocks.

Surgery

As Roger's buoyancy and behavior remained unchanged post manual removal attempt, he was removed from the exhibit again on August 10, 2022 for surgical intervention. A summary of Dr. Stafford's post-op notes on the surgery are as follows:
"Animal was anesthetized with MS-222 at 150 ppm for surgery. Placed in dorsal recumbency, MS-222 pumped over gills 150 ppm at 200% oxygen saturation. Dam placed caudal to gills to prevent water from contaminating surgery site (Figure 4). […] A one-inch incision was made on the ventral mid-line in the skin and extended into the coelom, the incision was extended 4 inches cranially and 2 inches caudally via operating scissors. The coelom was explored, stomach and rocks found cranially. The stomach was partially exteriorized, packed off with lap sponges and a 3-inch incision was made through an avascular area over a rock, the rocks were manually manipulated through the stomach and out the incision for removal (Figures 5 and 8). The stomach was closed in two layers, mucosa was closed with 3/0 webmax in an imbricating fashion, outer

mucosa was closed with 2/0 webmax with a 0 webmax oversew, both imbricating. Coelom was flushed with saline and the peritoneum was closed with 0 webmax incorporating the subcut layers in a simple continuous fashion, the outer skin was closed with 0 Nylon in a SI pattern (Figures 6 and 7). Heart was beating normally on ultrasound post op. No contamination of coelom noted during surgery. Actual surgery time approximately one hour."

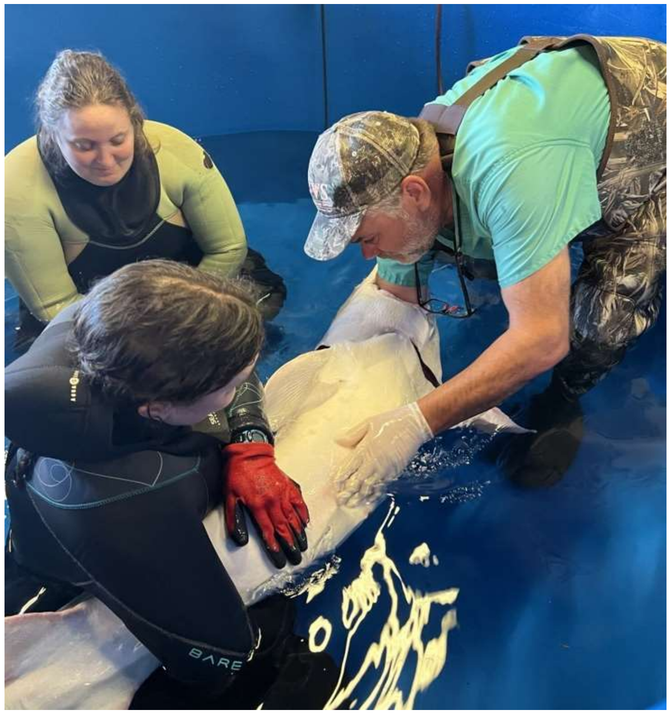


Figure 2. Dr. Stafford attempts manual removal of the rocks.

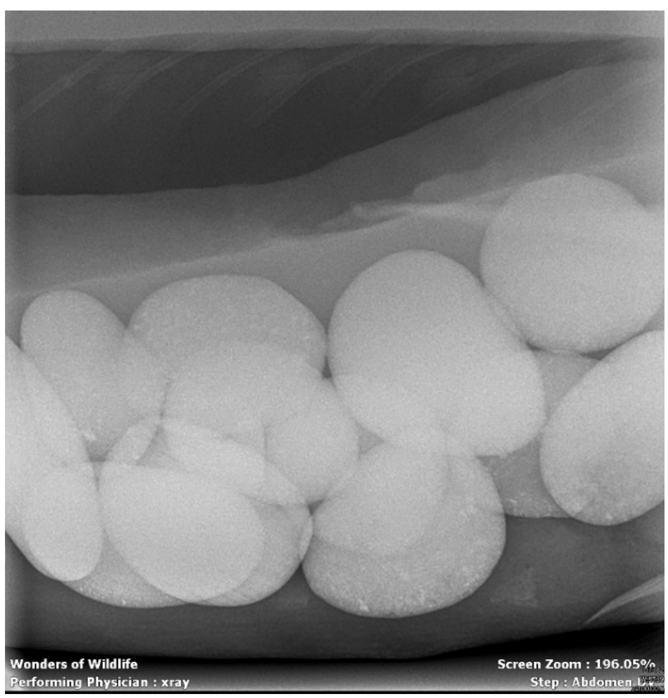


Figure 3. Radiographs showing a large collection of stones in the stomach.

Figure 4. Roger being anesthetized with dam in place.

Figure 5. Rocks being removed.

Figure 6. Dr. Stafford adding the final sutures.

Figure 7. Final product.

Figure 8. Eighteen river stones weighing 4.56lbs were removed from Roger's stomach.

Recovery

Roger was moved to Aquatic Holding post-surgery to heal and recover off exhibit. From there it was observed that he seemed to be doing well and was showing signs of positive recovery. It was noted that he appeared to be very strong and active. He was on injections on enrofloxacin and meloxicam every 72 hours for the first week. It was noted that the suture line appeared to be healing well with occasional redness but no signs of dehiscence.

Aquatic holding staff began offering Roger food during the week of August 22nd. It was noted that he was making passes at food and appeared to be eating some of it, but was regularly spitting it out. Feeding method was changed from broadcast to hand feedings with one staff member in the water as it was surmised that that is what the animal had previously been accustomed to.

Suture removal was attempted on August 29th (Figure 9) but the skin gapped after removal of the central suture so the procedure was halted. Suture removal was attempted again two weeks later on September 14th (Figure 10) where it was discovered that the suture line was dehisced almost 100% with the exception of the two proximal sutures, exposing the coelomic contents to tank water. Dr. Stafford removed the sutures and reclosed the skin in one layer (Figure 11). He then administered 400 mg of enroflaxin and prescribed it for five days. After the first five days at 400 mg the dosage was changed to 200 mg for five more.

Figure 9. Picture of the suture line on 8/29/22.

Figure 10. Picture of the suture line on 9/14/22.

Figure 11. Dr. Stafford reclosing the incision on 9/14/22.

Careful monitoring of the animal continued for the next month and it was noted that Roger's appetite slowly began to decrease. As per Dr. Stafford's direction – all handling was minimized so as to give the incision a chance to heal. All visual observations of the incision line indicated signs of improvement with no other concerns raised at the time so Roger was left unbothered to continue his recovery.

Result

On November 2nd Roger was caught for an exam of the suture line (Figure 12). There was a 100% dehiscence of the suture line and the coelomic contents were exposed, leaving no opportunity for repair. Because of this, the decision was made to euthanize Roger with 250 ppm MS-222 for 45 minutes. The following necropsy showed that the stomach suture had healed well but there was no evidence of improvement in the abdomen.

Figure 12. 100% dehiscence of the suture line on 11/2/2022 exposing coelomic contents to tank water.

Concluding Remarks

Although this animal had been in this exhibit for over four years with the same substrate with no prior issues, the decision was made to not replace the substrate in the exhibit and continue with a “bare bottom” approach for the remaining sturgeon. It was assumed that this issue happened because of an increase in broadcasted amounts accounting for larger and growing animals, while also giving the opportunity for the sturgeon to forage along the bottom (although regularly hand and target fed). Roger was the largest sturgeon on exhibit at Wonders of Wildlife and he had an impressively strong suction response during feedings. It is assumed that by keeping the bottom of this exhibit free from any swallowable objects that we can avoid a repeat of this situation in the future.

Acknowledgments

I would like to take the opportunity to thank the entirety of the 2022 vet team, natives and amazon team, aquatic holding team, and aquatic curatorial team as well as the herd of past and present volunteers and interns who jumped at the opportunity to move many vats of water, run to prop open doors, and end up covered in copious amounts of sturgeon slime. With some of the

challenges we faced through exhibit design and proximity of the exhibit to the vet clinic, these catches and moves of this animal took a great deal of strength, tenacity, and collective brain storming to make it go as quickly and smoothly as it did.

White Crappie, Bruce Koike

IN MEMORIAM: ALAN DAVID HENNINGSEN (24 DECEMBER 1959 – 29 JULY 2021)

Emily Anderson Kelly, Senior Aquarist ekelly@aqua.org
Allan Kottyan, Senior Aquarist akottyan@aqua.org
Elizabeth Schneble Claus, Senior Aquarist eclaus@aqua.org
Lindsey Fitzpatrick, Aquarist lfitzpatrick@aqua.org
Jennie D. Janssen, Assistant Curator jjanssen@aqua.org

National Aquarium, 501 East Pratt Street, Baltimore, MD, USA

I have fought the good fight, I have finished the race, I have kept the faith. 2 Timothy 4:7-8 NIV
Photo courtesy of National Aquarium

Biography

The ever humble, kind, and softspoken Alan David Henningsen passed away from Lou Gehrig's Disease on Thursday, July 29, 2021, at the age of 61, at his home surrounded by his family and pets in Parkville, Maryland, USA. As the Fishes Research Specialist at the National Aquarium (Baltimore, MD), he served as the resident elasmobranch expert for 32 years. He was a legend and a pioneer in elasmobranch husbandry and research, inspiring countless aquarists, researchers and members of the general public. Alan was an incredibly humble and modest man, never one to talk about himself. He was a loving and devoted husband, father, grandfather, and best friend to his family and friends. To his colleagues and coworkers, he was a mentor, friend, dive buddy, and graduate advisor. Above all, he was dedicated to Jesus Christ, and he lived by the motto *Soli Deo gloria* – Glory to God alone.

Alan was born on Christmas Eve, 1959, in Greensburg, IN to Alfred R. and Jean A. (Morgan) Henningsen. His older brother, Tracy H. Henningsen, maintains that Alan was his "Christmas present." Growing up in Indiana, he had a love for animals early on. He showed sheep as part of local clubs and fairs, and he even had aspirations of becoming a veterinarian. He graduated from Greensburg High School in 1978 and set off to the University of Miami to pursue a career in the marine and biological sciences (Greensburg Daily News, 2015, 2021).

Career

Alan began his studies by double majoring in Marine Science and Biology with a minor in Chemistry. He graduated *magna cum laude* in 1982. These bachelor's degrees were followed by a Master of Science in Biology and Living Resources in 1989, all from the University of Miami, Rosenstiel School of Marine and Atmospheric Sciences (RSMAS) in Miami, FL, under the tutelage of Dr. Samuel "Sonny" Gruber (aka "Doc"). His master's thesis focused on juvenile lemon sharks (*Negaprion brevirostris*) (Figure 1) in the Bahamas (Henningsen, 1989). From 1994 to 2001, Alan pursued a Ph.D. from the University of Maryland studying Marine, Estuarine, and Environmental Science, focusing on the reproductive physiology of elasmobranchs. He completed all but his dissertation.

Alan and Doc worked together for many years before Doc founded the Bimini Biological Field Station on South Bimini in the Bahamas, affectionately known as "the Shark Lab." Alan was fortunate to later participate in several research cruises to Bimini. When remembering his longtime mentor, Alan recounted that he had the pleasure of learning from Sonny as a volunteer, work-study student, research assistant and graduate student. He considered himself blessed to work with and for Sonny at RSMAS and locations all over the world, including the Florida Keys, the Bahamas, Egypt and the Red Sea in Israel. While working on his master's thesis, he utilized the database of lemon sharks tagged in the Florida Keys and Bahamas to determine several parameters of population dynamics, which were later refined to estimate biological productivity of three age classes of lemon sharks in the North Sound of Bimini. He also suggested ways to census the lemon shark population in the same area.

Fellow RSMAS graduate students, Bradley M. ("Brad") Wetherbee (now University of Rhode Island, Kingston, Rhode Island) and Enric Cortés (now National Oceanic and Atmospheric Administration (NOAA) Fisheries, Panama City, Florida) fondly remember that there was a big rock nicknamed "Henningsen's Reef" at the entrance of a canal in South Bimini where Alan had crashed one of the whalers on his way back to the dock after some night sampling. Also remembered as an animal lover, Alan was always willing to put himself on the line. Whether he was attempting to wrangle the feral cats hanging around the shark tanks at the RSMAS lab or longlining for sharks in Bimini, he always maintained a surprising amount of calm, even when a 6-foot nurse shark (*Ginglymostoma cirratum*) bit down on the tip of his finger when he was removing a hook from its mouth. Cortés regaled, "*Most people would have instinctively yanked their finger out the shark's mouth if they had their finger bitten, but Alan sat there calmly and told us that the shark had a hold of his finger and he couldn't get his finger out. He wanted us to get a* *screwdriver and pry open the shark's mouth so that he could remove his finger. That's what we did and there was only a faint mark on his finger where the shark was biting him. Very calm Alan.*"

From 1986 to 1988, Alan lived in the Florida Keys and worked at Dynasty Marine Associates (Marathon, FL) as their Hatchery Manager. There, he bought a boat (Figure 2) and was at home on the ocean. When Alan moved to Maryland in 1989 and took a position as an Aquarist at the National Aquarium, he embarked on a career that would span over 30 years. Soon after moving to Maryland, he met his beloved wife, Nanci. They were introduced through a dating agency, and Nanci remembers vividly, "*They would mail you the person's name and phone number. I noticed when I got his, they had the wrong number for me. I called him and he did not even receive my information. We talked for 3 hours, and he asked for my number*." They were married six months later on March 17, 1990. Together, they raised two sons, Ashby ("AJ") and Avery. Alan and Nanci's love was incredibly evident to all staff who knew him. Former National Aquarium coworker Dr. Caitlin Hadfield (now Senior Veterinarian at Seattle Aquarium) would *"sometimes see Nanci and Alan walking in together, always hand-in-hand. I remember hoping that everyone could experience a bond like theirs."*

In the mid-90's, Alan became a Senior Aquarist and further expanded his career by taking on additional responsibilities as the Volunteer Dive Coordinator and later becoming a Research Associate. In 2004, he assumed the role of Fishes Research Specialist. In this role, he focused much of his time on mentoring several graduate students, editing various periodicals, and serving on and leading a variety of elasmobranch conservation committees and initiatives. During his 32 years at the National Aquarium, he conducted elasmobranch research, published several articles and book chapters, and presented at numerous conferences (see Appendix). One of his primary focus areas was the reproductive behavior, biology, and husbandry of sand tiger sharks (*Carcharias taurus*) under human care (Figures 3 & 4). His last paper (Claus et al., 2021) was a collaboration with National Aquarium Senior Aquarist Elizabeth "Beth" Schneble Claus. It was published in the Journal of Zoo and Aquarium Research just days before his passing.

For the Love of Sawfish

While it was clear that Alan loved sharks, he had a more profound love and appreciation of sawfish (Figure 5). His wife Nanci described his love of sawfish as originating from his love of the ocean and that he loved sawfish because they were so different and critically endangered. He was an integral part of introducing two largetooth sawfish (*Pristis pristis*) to the *Shark Alley* exhibit at the National Aquarium in 2004 (Figure 6). His love and devotion to these two individual sawfish were plain to see by the meticulous way he cared for them. Some might even say they were spoiled… just a bit.

Alan's devotion to sawfish went above and beyond excellent daily care. He led and participated in supporting and crafting plans and policies to conserve these enigmatic, critically endangered species and their habitats. From 2015 to 2020, he proudly served as the Vice Coordinator of the Association of Zoos and Aquariums (AZA) Sawfish Species Survival Plan (SSP). In that role, he co-founded International Sawfish Day in 2017, which is celebrated annually on October 17th. This day was established to highlight endangered sawfish and the challenges they face, and it was brought about through collaboration between the AZA Sawfish SSP, the European

Figures 1-5. In Memoriam: Alan D. Henningsen: Figure 1. Alan releasing a lemon shark (*Negaprion brevirostris*), Bimini, Bahamas (*c.* 1984). Courtesy of Enric Cortés; Figure 2. Alan in his boat, Marathon, Florida (*c.* 1986). Credit: Bradley M. ("Brad") Wetherbee; Figure 3. Alan releasing a sand tiger shark (*Carcharias taurus*) after a routine exam, *Shark Alley* exhibit, National Aquarium, Baltimore, Maryland (*c.* 2003). Courtesy of National Aquarium; Figure 4. Alan feeding sand tiger sharks (*Carcharias taurus*) in the *Open Ocean* exhibit (now *Shark Alley*), National Aquarium, Baltimore, Maryland (*c.* 1999). Courtesy of National Aquarium; Figure 5. Alan diving with one of his beloved largetooth sawfish (*Pristis pristis*) in the Shark Alley exhibit at National Aquarium, Baltimore, Maryland (c. 2018). Courtesy of National Aquarium.

Association of Zoos and Aquaria studbook program, and research and conservation organizations worldwide. Paula Carlson, Director of Husbandry at the Dallas World Aquarium (Dallas, TX) and Chair of the AZA Marine Fishes Taxon Advisory Group (MFTAG), fondly remembers when "*Alan, Stacia* [White]*, Katy Duke and I were working on the International Sawfish Day planning in 2016 and 2017 and again at the SEZARC* [South-East Zoo Alliance for Reproduction & Conservation] *meetings at White Oak. We had many phone calls, emails, etc., and Alan was so energized and had so much to offer in the way of information, advice and guidance based upon his many years of experience. He was always so positive, gracious and humble, never wanting to* *take full credit or be recognized in any grand manner."* Tonya Wiley, President of Havenworth Coastal Conservation, remembers Alan as a remarkable man, a tremendous colleague and mentor, and a key figure in her professional success. She recalls, "*In 2003 at the American Elasmobranch Society* [AES] *meeting in Manaus, when I was in my early years as both a sawfish researcher and AES member, Alan graciously and eagerly shared his experiences and expertise in sawfish handling."* Alan generously gave of his time and expertise, traveling to Florida to relay those hands-on techniques in-person—something that stood out in her memory.

In 2022, in honor of Alan's passion for sawfish, the National Aquarium and members of the sawfish research and conservation community came together to form the Alan D. Henningsen Memorial Sawfish Conservation and Research Grant Fund (National Aquarium, 2022). This fund collects donations through the MFTAG to support research that increases our understanding of sawfish to advance their conservation (https://give.seattleaquarium.org/MFTAG). Grant applications are reviewed annually by the Institutional Representatives of the AZA Sawfish SSP. Those who knew Alan could think of no better way to memorialize his legacy.

What Would Alan Do?

Many have fond memories of working with Alan throughout his career at the National Aquarium. Former National Aquarium coworker Dr. Leigh Clayton (now Director at Wildlife Conservation Society's New York Aquarium, Brooklyn, NY) describes Alan as "*a good partner for a veterinarian and he clearly delighted in talking to people about elasmobranchs. That enthusiasm came through all the time. We had a little private joke about fish gills vs lungs… one of my first days in a post, I casually said lungs instead of gills, the look of shock and perhaps fear on his face, and maybe growing trust when I said I was joking. He certainly didn't hold that against me, but we joked around that for years."* Alan genuinely loved being able to mentor and teach anyone he came across at the aquarium and was always willing to take a moment to speak to children and adults alike. Dr. Caitlin Hadfield remembers Alan as *"the most passionate supporter of elasmobranchs I have ever met and a terrific teacher."* Through mentoring numerous interns, volunteers, and co-workers, Alan's knowledge spread throughout the industry. Kelli Cadenas (former National Aquarium coworker, now Curator at SEA LIFE Orlando Aquarium, Orlando, FL) fondly remembers, "*When I came to National Aquarium, I had already heard of Alan Henningsen. He was well respected for his elasmobranch research, and I was excited to have the opportunity to work with him. Little did I know that I would not only be privileged enough to have Alan as a mentor, but truly as a friend. Whenever someone had a question about sharks, I always answered with, 'I know a guy.' Alan was the guy. Alan was the 'shark guy' that actually preferred rays."*

He is remembered as always willing to make time for a phone call or text message to answer a question about elasmobranchs. Kelli Cadenas noted him as always having *"steady and thoughtful advice."* National Aquarium Senior Aquarist Allan Kottyan had a similar experience while working at Jenkinson's Aquarium (Point Pleasant Beach, NJ). "*I was looking for some information about a leopard shark under my care, and my co-workers told me that I should get in touch with Alan Henningsen because Alan could answer any questions about sharks. This shows how respected Alan was in this field because even people who had never worked with Alan knew he could and would be more than happy to help anyone. I was lucky enough to work alongside Alan later and get to know what a great person he was."*

When National Aquarium Senior Aquarist Emily Anderson Kelly applied for an internship at the Aquarium, she *"was really hoping to be placed with the world-known shark guy, so I emphasized my love of sharks and that I hoped my future career would be working with them in some manner. To my delight, I was placed with Alan and was so excited to be learning and working with sharks. He was and continues to be a vital part of my career."* To those under his former tutelage, his teachings continue to have an impact, especially regarding the care of the elasmobranchs and his former exhibit, *Shark Alley.* So much so that even now, there are moments where the Aquarists question "What would Alan do?" National Aquarium Aquarist Lindsey Fitzpatrick remembers, *"The day after I heard the news of Alan's passing, I went into Shark Alley and took a moment to myself to remember all of the things I learned from him as one of his former interns and additionally as a teammate. I wanted to make him proud as I continued to care for the exhibit and animals as he had done for so many years. I think about Alan often, especially while diving with his beloved sawfish."*

To the authors and many others who worked closely with Alan over the years, he was both a mentor and a friend. He was always at the ready with a silly pun or to talk about any sporting event. Jack Cover, General Curator at the National Aquarium, could always tell when that day's pun or "dad joke" was about to be unleashed. A half-smile would emerge on Alan's face just before he delivered it. One such moment was during a Facebook Live interview with the Washington Post (Washington Post, 2017), as he was explaining the interesting reproductive habits of sand tiger sharks (*Carcharias taurus*) to the reporter. The reporter responded by explaining she heard that chickens are often known to do something similar. Without hesitation, at timestamp 10:31, Alan quipped: "*Well that's fowl."* The reporter chuckled awkwardly before realizing Alan's joke and laughing in earnest. To all of us watching, it was a classic Alan moment, one that remains one of our favorites.

Snack of the Day

Alan and his wife, Nanci, were known to make cheesecakes and were constantly testing out a variety of flavors or toppings. It was always a special treat when he came to work with small tin foil packages that he would offer out to people during lunch, eagerly waiting to hear their feedback on the latest creation of specialty cheesecake. Alan even won first place in a baking contest at the Aquarium with one of his and Nanci's cheesecake creations. For special events or birthdays, Alan would go so far as to make a custom-order cheesecake for coworkers to bring home to share with their families (or not).

Alan was also a connoisseur of sweet things and coffee. He was affectionately known as the "CCO" or "Chief Coffee Officer"—a title and honor that came complete with a badge he proudly wore. He could always be trusted to have some coffee grounds on hand or candy or some sweet confection hidden away. Former coworker Katie DiCioccio has a fond memory: *"For Alan's thirty-year work anniversary, we knew he would not want to be made the center of attention, but we wanted to honor him in some way. We came up with the idea to drop off small gifts of Alan's favorite things throughout the day. We secretly told people throughout the Aquarium, so it seemed like a fun coincidence instead of an orchestrated effort. By the end of the day, Alan's desk was overflowing with Reese's Pieces, Peeps, cheeseballs, coffee, Krispy Kreme doughnuts, Cheetos, and Zebra Cakes. Alan, being the selfless man he was, shared his snacks with us over the next few* *days. He brought out one type of snack each day, which we called 'Snack of the Day'. Alan got a big kick out of hearing us asking him what the snack of the day would be, although we never did see those Zebra Cakes again."* This sweet surprise for Alan and the development of "Snack of the Day" resulted in some of our most fond memories that we continue to share.

During his lunch hours, he often skipped lunch, opting for a run around the Inner Harbor of Baltimore City. He also repeatedly participated in races and marathons and encouraged some of the other aquarists or other National Aquarium colleagues to compete as a team. Events such as the Sole of the City and the Baltimore Running Festival were among the most popular events in which the team "Running Water" participated (Figures 7 & 8). Alan also loved running in the River Valley Run alongside his sons and former coworker Katie DiCioccio. Although these were individual events, Alan always supported Katie and proudly sent updates of her finishing time with photos to the rest of their colleagues.

Kiss a Shark

During Alan's time at the National Aquarium, he was able to re-visit his roots of working in the field with sharks. Alan joined many field operations for shark tagging and collecting. Former coworker Andrew Pulver (now Vice President of Animal Care at the John G. Shedd Aquarium, Chicago, IL) remembers, "*Arriving at the boat the first day, I saw a wild, thin, pirate-like figure with a bandana and ragged pants standing on the stern of the boat, only to realize that it was Alan! It was quite the contrast from the Aquarium Alan I had come to know. In that setting, many stories would emerge from his memory of sea faring adventure from his time at University of Miami to working with Dynasty Marine and packing coffee grounds in his lip when aboard to meet his daily caffeine needs."* Former coworker Andy Dehart (now President and CEO at Loggerhead Marinelife Center, Juno Beach, FL) remembers another important rule on the boat. *"We had a rule on board the vessel: 'Be kind to animals, kiss a shark.' Very few sharks went overboard, tagged and on their merry way, without a kiss from Alan. He was always a champion of sharks, and his passion was infectious."*

On many of our shark fishing trips, we would have guest researchers on-board, including Dr. Jennifer Wyffels (Researcher at University of Delaware, Newark, DE), who joined us on many occasions. Jen remembers, *"In 2016, Alan helped me get a spot on the National boat fishing again, and that year the crew caught a YOY* [young-of-the-year] *thresher. It was a BEAUTIFUL little shark; purple-blue hues sparkled off its skin at the surface. Alan did most of the work up as everyone on the boat snuck over to get a good look. It was the first and so far, only time I have seen a thresher. It was stunning in form and color. They scare easily, so there was not time for everyone to get a close encounter before wanting to release it. Alan gave that beautiful fish a kiss on the snout, and we watched it swim off. Afterward, the first thing he did was apologize for "hogging" the thresher. So thoughtful and kind, one of the most genuine, helpful, curious, and kind persons I had the pleasure of knowing professionally and personally."* To this day, when we are out on the Aquarium's Research Vessel *Nani* (formerly R/V *Carcharhinus*) (Figure 9), Alan is remembered through our demonstration of the skills he taught us, and if a shark is fortunate

Figures 6-9. In Memoriam: Alan D. Henningsen: Figure 6. Alan kissing a juvenile largetooth sawfish (*Pristis pristis*) being introduced into the National Aquarium *Open Ocean* exhibit (now *Shark Alley*) in 2004. Courtesy of National Aquarium; Figure 7. Team "Running Water" at the Sole of the City race in Baltimore, Maryland (c. 2018). From left to right: Alan D. Henningsen, Holly Richardson, Katie DiCioccio, Jaime Webster, Elizabeth Claus. Courtesy of Jaime Webster; Figure 8. Team "Running Water" at the Baltimore Running Festival in Baltimore, Maryland. (c. 2017). From left to right: Jaime Webster, Elizabeth Claus, Katie DiCioccio, Alan D. Henningsen. Courtesy of Jaime Webster; Figure 9. Pulling in a long-line set aboard National Aquarium's shark fishing vessel R/V *Carcharhinus* (c. 2011). From left to right: Alan D. Henningsen, Ryan Dumas, Rick Harabin, Allan Kottyan, Colleen Newberg, Andrew Pulver. Courtesy of National Aquarium.

enough, it gets a good luck kiss before it goes on its way, for Alan. Throughout Alan's career, he was instrumental in many scientific research-based organizations. He was a member of the Northwest Atlantic Regional International Union for Conservation of Nature (IUCN) Shark Specialist Group, Vice Coordinator of the AZA Sawfish SSP, on the Editorial and Steering Committee for the development of the Elasmobranch Husbandry Manual, and Co-Leader of the AZA Saving Animals From Extinction (SAFE) Shark & Ray International Census of Chondrichthyans in Human Care (aka ChrondroCensus) (Table 1). Alan launched the ChondroCensus with National Aquarium colleague Jennie D. Janssen in 2017, and she remembers sheepishly introducing herself to the already renowned Alan at the 1996 AES meeting in New Orleans. *"He managed to keep one foot in elasmobranch research while keeping the other in caring for these animals in aquariums and zoos. His ability to bridge those two sometimes separate worlds is a testament to how loved and respected he was in both communities. Given all that, what is still most striking is the contrast between his inherent notoriety in the field with his of humility of person, presumably founded in his deep respect for others' skills and contributions. His was a living example of humility (praise that he would also likely eschew)."*

Table 1. Professional Affiliations of Alan D. Henningsen.

1985-2020	American Elasmobranch Society member
1999-2004	Editorial and Steering Committee for the development of the Elasmobranch Husbandry Manual
2002-2020	Member, Northwest Atlantic Region IUCN Shark Specialist Group
2015-2020	Vice Coordinator, AZA Sawfish SSP
2017-2020	Co-Leader, AZA SAFE Sharks & Rays International Census of Chondrichthyans in Human Care (aka ChondroCensus)

Most notably in his years of service, Alan was heavily involved in AES for the majority of his career (Table 2). Program Director for the Pacific Shark Research Center (Moss Landing, CA), Dr. David Ebert, remembers that Alan "*was at the meeting in 1983 where Sonny, Don, and Leonard first came up with the concept of AES."* Senior Scientist at Mote Marine Laboratory (Sarasota, FL), Dr. Carl Luer, who has done extensive research on skate reproductive physiology and embryonic development, met Alan frequently at AES meetings (Figures 10 & 11) and recalls: "*We became immediate friends when we discovered that we were both huge baseball fans. Whenever we met at subsequent AES meetings, we always chatted about the latest baseball news. During one annual meeting, I remember talking with Alan about tonic immobility. Alan was conducting a comparative study with numerous elasmobranch species to see how readily immobility was induced and how long the immobility endured. I told him that the clearnose skate had the most "uncooperative" response among the species I had worked with. Alan ended up publishing his results in 1994 in a very nice paper in Zoo Biology. In this paper, Alan confirmed that among the species he studied that exhibited the tonic immobility response, clearnose skates had the longest induction time and the shortest duration of immobility*" (Henningsen, 1994).

Table 2. Alan D. Henningsen's service to the American Elasmobranch Society.

1991-2007	Northeast Regional Coordinator for annual census of captive elasmobranchs
1993-2005	Regional Coordinator for Japan for annual census of captive elasmobranchs
1994	Nominating Committee
1995-1996	Grant Committee
1996-2000	Student Affairs Committee
2001	Chair, Nominating Committee
2003-2008	Board of Directors
2016-2020	Co-Chair, Captive Elasmobranch Census Committee

Alan is also remembered for always thinking of others, even at conferences. Florida Atlantic University (Boca Raton, FL) Professor Dr. Steven Kajiura recalls flying into Baltimore *"intending to carpool with Alan to the AES conference in Pennsylvania. Unfortunately, my suitcase ended up on a different flight, so I had nothing but the clothes I was wearing. Upon arriving at the conference venue, Alan was surrounded by his many friends and colleagues, who invited him to socialize with them. Nonetheless, he declined meeting with his friends to drive me around looking for a Walmart to get some clothes. I was very appreciative of his attentiveness to my needs above his own."*

Figure 10. In Memoriam: Alan D. Henningsen - Alan at American Elasmobranch Society (AES) meetings, courtesy of AES: Figure 10a. American Museum of Natural History, New York, NY 1991; Figure 10b. Champaign-Urbana, IL 1992; Figure 10c. Austin, TX 1993; Figure 10d. Guelph, Ontario, Canada 1998; Figure 10e. La Paz, Baja California Sur, Mexico 2000; Figure 10f. Manaus, Brazil 2003; Figure 10g. Norman, OK 2004; Figure 10h. Tampa, FL 2005.

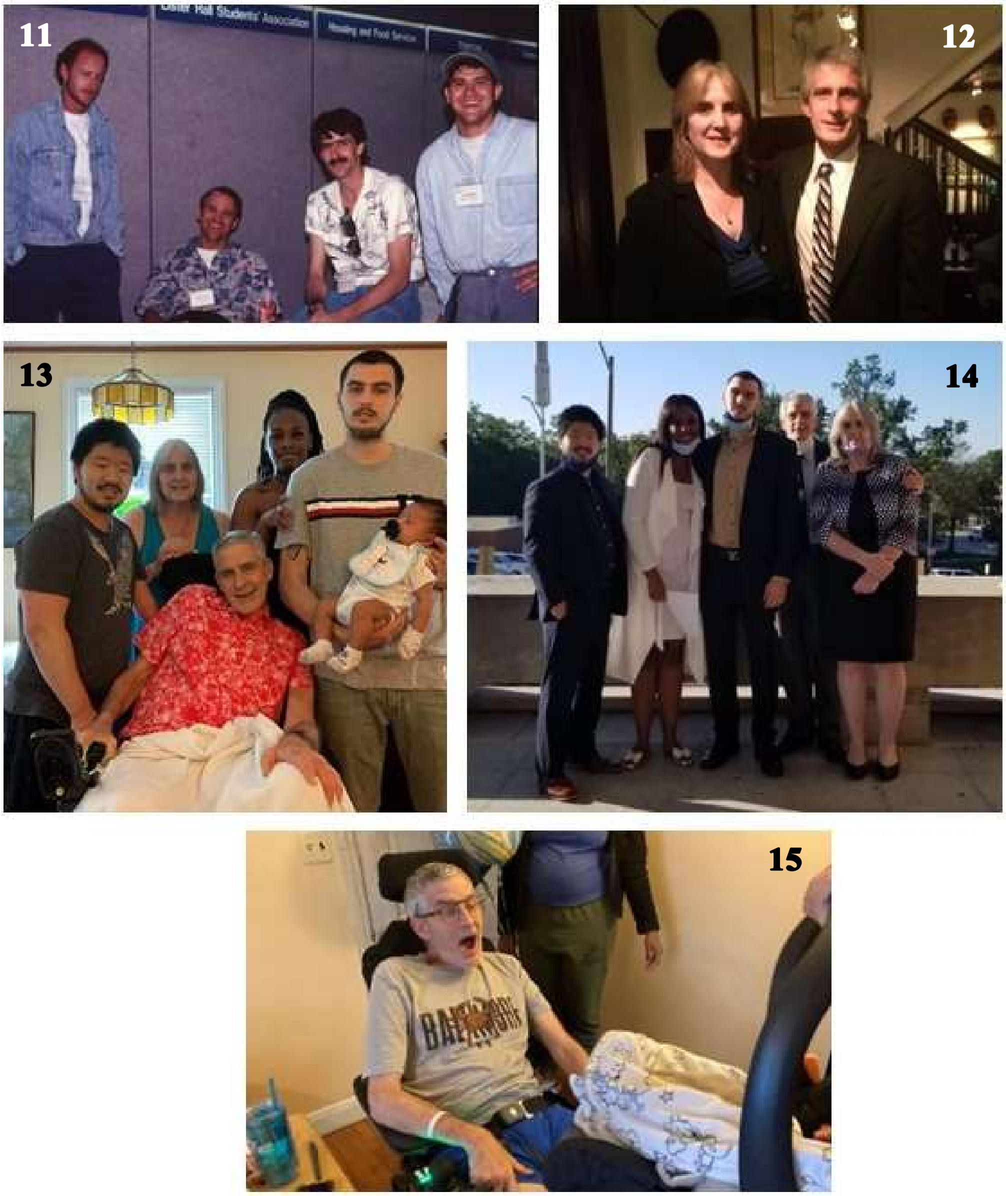
A Family Man First and a Scientist Second

Alan was a dedicated family man and his fatherly ways extended into the workplace. Senior Aquarist Emily Anderson Kelly thought of Alan as a "work dad." To her, he wore many hats: intern mentor, coworker, thesis advisor, and work dad. But knowing Alan, he would have been most humbled by work dad. A favorite memory of hers was of her master's thesis defense. Alan, having mentored and advised her on her project, was present. Emily recounts, *"I was very nervous, which Alan knew, but as I was successfully defending my work, Alan took a moment to take off his 'thesis-advisor' hat and put on his 'work dad' hat and subtly took pictures of me presenting my work. The pictures were blurry and poor quality since he was trying to not be obvious to me, but the sentiment behind that gesture was of a proud dad. It meant so much to me."*

Alan was willing to go above and beyond to aid or guide any of his colleagues, as it was just in his nature to help others. He saw it all, us at our best and worst. He taught us everything we could know about elasmobranchs, gave us advice, laughed with us, and cried with us; above all, he was abundantly kind, generous, and exceptionally modest. He would never brag about the time he gave Brad Pitt and Angelina Jolie a private tour of *Shark Alley* or spent the day teaching one of then-President Obama's daughters all about sharks above the shark exhibit, apart from her Secret Service detail. For milestones like weddings, births, deaths, or any other personal or work experiences, he offered a warm embrace, a shoulder to cry on, and an offer to pray for you. Those who work in our industry know that you develop a high level of trust with your colleagues due to the unknowns of working with animals, some being potentially dangerous. Alan's calm demeanor was soothing in moments of high stress, as we trusted Alan with our safety to guide us through uncertain circumstances many times. We created bonds over mundane and serious tasks alike, from food prep and cleaning the kitchen to handling large sand tiger sharks or major medical cases.

While Alan was passionate about sharks, his wife and sons remained at the forefront (Figures 12-14). Andrew Pulver remembers, *"Throughout those years and over field expeditions, I got to know Alan better, and something that I came to cherish about him was how important his family was to him. Every day, I woke early in our rented houses to get the coffee pot going and rouse the tired crew still trying to sleep off their boat bruises from the day before, and I'd find Alan already having been awake for some time. His reason for rising so early was that he felt guilty being out on our adventure while leaving Nanci alone at home to handle all the routine for their boys; he was waking whenever they had to at home and checking in every day. Now, with a family of my own, I often reflect on that memory. When checking in on my own kids and asking what is happening at home, I feel that same separation anxiety Alan felt and understand why he was so often hesitant to join many of our later expeditions. Then that memory always leads to another, when I must laugh about the morning Alan decided to throw his wet shark fishing sneakers in the drier of the rental house at 4 a.m. and wake the whole house up! He was one of a kind, and we surely miss him."*

Alan frequently spoke with pride about the milestones and accomplishments of his sons, AJ and Avery, and it was encouraging and instructive for those of us without children yet to see the care and respect he held for his. Colleague and shark geneticist at The Field Museum (Chicago, IL), Dr. Kevin Feldheim, warmly remembers, "*He spoke a lot about his kids. This was at a time when my wife and I were thinking about having kids ourselves. It really stuck with me that here was a really well-respected scientist who seemed like a family man first and a scientist second. I* *think those interactions with Alan were part of the reason I always try to put my kids ahead of my work!*" In May 2021, Alan became a proud grandfather to his first grandson, born to son Avery and his wife Giya, named Asaiah Alan in honor of him (Figure 15). After Alan's passing, a second



Figures 11-15. In Memoriam: Alan D. Henningsen: Figure 11. American Elasmobranch Society meeting in Edmonton, Alberta, Canada, July 1995. From left to right: Christopher ("Chris") Lowe, Gerald ("Jerry") Crow, Alan D. Henningsen and James L. ("Jay") Bradley. Credit: Steven ("Steve") Kajiura; Figure 12. Alan and his wife Nanci celebrating their 25th wedding anniversary at the Oregon Grille in Hunt Valley, Maryland, March 2015. Courtesy of Nanci Henningsen; Figure 13. Celebrating Father's Day with a theme of ocean and sharks, June 2021. From left to right: AJ Henningsen, Nanci Henningsen, Alan D. Henningsen, Giya Henningsen, Avery Henningsen, Asaiah Henningsen. Courtesy of Nanci Henningsen; Figure 14. Celebrating Avery and Giya's marriage, Towson, Maryland, October 2020. From left to right: AJ Henningsen, Giya Henningsen, Avery Henningsen, Alan D. Henningsen, Nanci Henningsen. Courtesy of Nanci Henningsen. Figure 15. Alan meeting his first grandson, Asaiah Alan, for the first time, Parkville, Maryland, May 2021. Courtesy of Nanci Henningsen.

grandson was born in July 2022, again to Avery and Giya. They named him Ashtyn David as another homage to him, as David was his middle name. At the National Aquarium, a newly acquired sand tiger shark (*Carcharias taurus*) was also named "David" in Alan's honor. His third grandson, Amari was born to Avery and Giya in October of 2023.

Those who knew Alan, knew his true passion, beside his family, was his love for Jesus Christ. You could always tell when Alan was the previous driver of the Fishes Department van because the radio would be set to the local Christian radio station in Baltimore. Alan embodied a spirit of giving, always keeping food and water in his car to provide for people experiencing homelessness, often giving his own lunch away. If he had nothing to offer, he simply shared kind words, taking the time to talk and listen to their personal stories. He considered himself blessed to teach children in Sunday School and substitute teach at a local Christian school. Alan and his family also volunteered with various groups aiding the less fortunate and participated in numerous mission trips to Central and South America, spreading the word of Jesus Christ.

As of this writing, Alan continues to be honored by his colleagues and community. In early 2024, he will be inducted into the Greensburg High School Hall of Fame. His enthusiasm for and knowledge of elasmobranchs have been an inspiration to the co-authors and so many other professionals. Beyond that, many of the same feelings and sentiments were repeated by numerous contributors as we compiled remembrances for this paper. Words such as *kind, generous, selfless, gentle, thoughtful,* and *friend* were repeatedly appreciated. We hope this paper can give a small glimpse into all the different ways he touched the lives of those he interacted with. As Dr. Steve Kajiura aptly summarized, *"Almost any positive attribute could be applied to Alan…He was, and always will be, my friend."* In a final act of giving, Alan chose to donate his skin to The Living Legacy Foundation of Maryland, whose mission is: "To inspire our community to save and enhance lives through organ, eye, and tissue donation while honoring the legacy of donors and their families." As Alan shared in a 2015 interview with the *Daily News* of his hometown of Greensburg, IN, *"I see our roles as people charged by our God to have dominion over creation, but to be wise stewards of the resources that He gave us*."

Acknowledgements

The quotes attributed to Alan herein were gathered from personal emails, online sources, and authors' memories. We would like to thank all those who shared their professional and personal memories with us. This includes colleagues from both academia and aquaria. While it was impossible to include all the stories, it was incredibly moving to read and hear how Alan had touched the lives of so many. We would like to especially thank Alan's family for their support in our drafting this publication, in addition to sharing in our lives as Alan's friends and colleagues.

Literature Cited

Claus, E., A. Henningsen, M. Shivji, and B. Wetherbee. 2021. Sexual conflicts in sand tiger sharks *Carcharias taurus* (Rafinesque, 1810) in an artificial environment. Journal of Zoo and Aquarium Research 9(3): 161-169. https://doi.org/10.19227/jzar.v9i3.528

Greensburg Daily News. 2015. Shark Expert Henningsen is from Decatur County. https://www.greensburgdailynews.com/news/local_news/shark-expert-henningsen-is-from-decatur-county/article_85278088-3c3e-5c3a-b021-765b26fac3b5.html?mode=jqm (accessed 29 November 2023).

Greensburg Daily News. 2021. Obituary for Alan David Henningsen. https://obituaries.greensburgdailynews.com/obituary/alan-henningsen-1082939709 (accessed 29 November 2023).

Henningsen, A.D. 1989. Annual production of the juvenile lemon shark, *Negaprion brevirostris*, in a shallow Bahamian lagoon. Unpublished M.S. thesis, University of Miami, Miami, Florida.

Henningsen, A.D. 1994. Tonic immobility in 12 elasmobranchs: Use as an aid in captive husbandry. Zoo Biology 13:325-332. https://doi.org/10.1002/zoo.1430130406

National Aquarium. 2022. National Aquarium Celebrates International Sawfish Day: The Alan D. Henningsen Conservation Research Grant will fund sawfish research and conservation efforts. https://aqua.org/contact-us/newsroom/press-releases/2022-10-20-national-aquarium-celebrates-international-sawfish-day (accessed 12 December 2023).

Washington Post. 2017. Shark Week is a sham. But sharks are still really cool. https://www.washingtonpost.com/news/speaking-of-science/wp/2017/07/26/shark-week-is-a-sham-but-sharks-are-still-super-cool/ (accessed 29 November 2023).

APPENDIX

Journal Articles by Alan D. Henningsen

Claus, E., A. Henningsen, M. Shivji, and B. Wetherbee. 2021. Sexual conflicts in sand tiger sharks *Carcharias taurus* (Rafinesque, 1810) in an artificial environment. Journal of Zoo and Aquarium Research 9(3): 161-169. https://doi.org/10.19227/jzar.v9i3.528

Curran, S.S., A.J. Phillips, R.M. Overstreet, G.W. Benz, and A. Henningsen. 2016. *Austrobdella* cairae n. sp., an Oioxenous Marine Leech (Clitellata: Piscicolidae) from the Banded Guitarfish, *Zapteryx exasperata,* in the Northeastern Pacific Ocean. Journal of Parasitology 102(2): 179-186. https://doi.org/10.1645/15-829

Feldheim, K. A., A. Clews, A. Henningsen, L. Todorov, C. McDermott, M. Meyers, J. Bradley, A. Pulver, E. Anderson, and A. Marshall. 2017. Multiple births by a captive swellshark *Cephaloscyllium ventriosum via* facultative parthenogenesis. Journal of Fish Biology 90(3): 1047–1053. https://doi.org/10.1111/jfb.13202

Gruber, S.H., C.A. Brown, and A.D. Henningsen. 1985. Age and growth of the lemon shark, *Negaprion brevirostris* (Poey), as determined by mark/recapture data and the examination of tetracycline labeled vertebral centra [Abstract]. American Zoologist, 25(4), 106A.

Henningsen, A.D. 1989. Annual production of the juvenile lemon shark, *Negaprion brevirostris*, in a shallow Bahamian lagoon. Unpublished M.S. thesis, University of Miami, Miami, Florida.

Henningsen, A.D. 1994. Tonic immobility in 12 elasmobranchs: Use as an aid in captive husbandry. Zoo Biology 13:325-332. https://doi.org/10.1002/zoo.1430130406

Henningsen, A.D. 1996. Captive husbandry and bioenergetics of the spiny butterfly ray, *Gymnura altavela* (Linnaeus). Zoo Biology 15:135-142. https://doi.org/10.1002/(SICI)1098-2361(1996)15:2<135::AID-ZOO4>3.0.CO;2-C

Henningsen, A.D. 1998. Review. Levels of recirculating reproductively-related steroid hormones in female elasmobranchs. Implications for reproduction in a captive environment. Aquarium Science and Conservation 2(3):97-116. https://doi.org/10.1023/A:1009688826898

Henningsen, A.D. 2000. Notes on reproduction in the southern stingray, *Dasyatis americana*, (Chondrichthyes: Dasyatidae) in a captive environment. Copeia 2000(3): 826-828. https://doi.org/10.1643/0045-8511(2000)000[0826:NORITS]2.0.CO;2

Henningsen, A.D., and S.H. Gruber. 1985. Assessment of two lemon shark, *Negaprion brevirostris* populations, by multiple mark procedures. Florida Scientist, 48(suppl. 1), 32.

Henningsen, A.D., and R.T. Leaf. 2010. Observations on the captive biology of the southern stingray. Transactions of the American Fisheries Society 139: 783-791. https://doi.org/10.1577/T09-124.1

Henningsen, A.D., F.L. Murru, L.E.L. Rasmussen, B.R. Whitaker, and G.C. Violetta. 2008. Serum levels of reproductive steroid hormones in captive sand tiger sharks, *Carcharias taurus* (Rafinesque), and comments on their relation to sexual conflicts. Fish Physiology & Biochemistry 34(4):437-46. https://doi.org/10.1007/s10695-008-9202-9

Henningsen, A.D., B.R. Whitaker, K.E. Kight, D.I. Hess, C. Hadfield, and Y. Zohar. 2015. The use of a gonadotropin releasing hormone antagonist in captive sand tiger sharks, *Carcharias taurus*, and the serum levels of the antagonist and reproductive steroid hormones. Journal of Zoo and Aquarium Research 3(3): 107-115. https://doi.org/10.19227/jzar.v3i3.120

Henningsen, A.D., B.R. Whitaker, and I.D. Walker. 2005. Protrusion of the valvular intestine in captive smalltooth sawfish and comments on gastrointestinal anatomy and intestinal valve types in pristids. Journal of Aquatic Animal Health 17(3): 289-295. https://doi.org/10.1577/H04-063.1

Vaudo, J.J., B.M. Wetherbee, G.C.M. Harvey, J.C. Harvey, A.J.F. Prebble, M.J. Corcoran, M.D. Potenski, K.A. Bruni, R.T. Leaf, A.D. Henningsen, J.S. Collie, and M.S. Shivji. 2017. Characterisation and monitoring of one of the world's most valuable ecotourism animals, the southern stingray at Stingray City, Grand Cayman. Marine and Freshwater Research 69(1):144-154. https://doi.org/10.1071/MF17030

Book Chapters

Henningsen, A., E. Claus, D. Littlehale, J. Choromanski, I. Gordon, and K. Willson. 2017. Reproduction of the sand tiger sharks, *Carcharias taurus*, in aquaria: a framework for a managed breeding program. p. 375-390. *In:* Elasmobranch Husbandry Manual II: Recent Advances in the Care of Sharks, Rays, and their Relatives. M. Smith, D. Warmolts, D. Thoney, R. Hueter, M.J. Murray, and J.M. Ezcurra (eds.). Special Publication, Ohio Biological Survey, Columbus, Ohio.

https://drive.google.com/file/d/1X3npInqWwT3JmnMS_KQAgS7gTL393nfQ/view

Henningsen, A.D., M.J. Smale, R. Garner, and N. Kinnunen. 2004. Reproduction, embryonic development, and reproductive physiology of elasmobranchs. p. 227-236. *In:* Elasmobranch Husbandry Manual: Captive Care of Sharks, Rays, and their Relative. M. Smith, D. Warmolts, D. Thoney, and R. Hueter (eds.). Special Publication, Ohio Biological Survey, Columbus, Ohio. https://drive.google.com/file/d/1wbiwQ70y1ic56mqoalCdHEGZN0vptPNf/view

Henningsen, A.D., M.J. Smale, I. Gordon, R. Garner, R. Marin-Osorno, and N. Kinnunen. 2004. Captive breeding and sexual conflict in elasmobranchs. p. 237-248. *In*: Elasmobranch Husbandry Manual: Captive Care of Sharks, Rays, and their Relatives. M. Smith, D. Warmolts, D. Thoney, and R. Hueter (eds.). Special Publication, Ohio Biological Survey, Columbus, Ohio. https://drive.google.com/file/d/1wbiwQ70y1ic56mqoalCdHEGZN0vptPNf/view

Smale, M. J., R.T. Jones, J.P. Correia, A.D. Henningsen, G. Crow, and R. Garner. 2004. Research on elasmobranchs in public aquariums. p. 533-541. *In:* Elasmobranch Husbandry Manual: Captive Care of Sharks, Rays and their Relatives. M. Smith, D. Warmolts, D. Thoney, R. Hueter (eds.). Special Publication, Ohio Biological Survey, Columbus, Ohio. https://drive.google.com/file/d/1wbiwQ70y1ic56mqoalCdHEGZN0vptPNf/view

Conference Presentations

*Carlson, P., K. Duke, A. Henningsen, and S. White. 2018, June 3-8. International Sawfish Day-Collaborating for Conservation [Abstract]. Sharks International & the 34th Annual Meeting of the American Elasmobranch Society. João Pessoa, Brazil https://www.sharksinternational.org.br/arquivos/Sharks_International_Conference-Abstract_Book_2.pdf

Clark, S., G. Violetta, *A. Henningsen, V. Reischuck, P. Mohan, and J. Keyon. 2003, June 26-July 1. Growth in captive smalltooth sawfish, *Pristis pectinata* [Abstract]. 83rd Annual Meeting of the American Society of Ichthyologists and Herpetologists, 19th Annual Meeting of the American Elasmobranch Society. Manaus, Brazil. https://elasmo.org/meetings/abstracts/abst2003/

*Duke, K., P. *Carlson, S. White, and A. Henningsen. 2016, September 25-29. Making Connections for Sawfish: The Role of Public Aquaria in Sawfish Biology and Conservation [Abstract and presentation]. International Aquarium Congress. Vancouver, British Columbia, Canada. https://www.animalprofessional.com/iac-2016-elasmonbranch-conservation.html

Gruber, S.H., C.A. Brown, and A.D. Henningsen. 1986. Age and growth of the lemon shark, *Negaprion brevirostris* (Poey), as determined by mark/recapture data and examination of tetracycline labeled vertebrae [Abstract]. 2nd Annual Meeting of the American Elasmobranch Society. Victoria, British Columbia, Canada.https://elasmo.org/meetings/abstracts/abst1986/

Harrison, L.R., J. Carlson, M. Diop, M. Ducroq, C. Duffy, S. Fowler, S. Fordham, A. Henningsen, H. Koldeway, M. McDavitt, S. Norton, G. Sant, C. Simpfendorfer, and N.K. Dulvy. 2011. Towards a species conservation strategy for sawfishes. Sawfish in Peril Educator Workshop. Gainesville, Florida.

Henningsen, A.D. 1993, May 27-June 3. Tonic immobility in elasmobranchs: Preliminary results on 12 species and its use as an aid in their captive husbandry [Abstract]. 73rd Annual Meeting of the American Society of Ichthyologists and Herpetologists, 9th Annual Meeting of the American Elasmobranch Society. Austin, Texas. https://elasmo.org/meetings/abstracts/abst1993/

Henningsen, A.D. 1995, June 15-19. Biology of the spiny butterfly ray, *Gymnura altavela,* in a captive environment [Abstract]. 75th Annual Meeting of the American Society of Ichthyologists and Herpetologists, 11th Annual Meeting of the American Elasmobranch Society. Edmonton, Alberta, Canada. https://elasmo.org/meetings/abstracts/abst1995/

Henningsen, A. D. 1997, June 26-July 2. Observations on the reproductive biology of southern stingrays, *Dasyatis americana* [Abstract]. 77th Annual Meeting of the American Society of Ichthyologists and Herpetologists, 13th Annual Meeting of the American Elasmobranch Society. Seattle, Washington. https://elasmo.org/meetings/abstracts/abst1997/

Henningsen, A. 2002, July 3-8. Age and growth in captive southern stingrays, *Dasyatis americana* [Abstract]. 82nd Annual Meeting of the American Society of Ichthyologists and Herpetologists, 18th Annual Meeting of the American Elasmobranch Society. Kansas City, Missouri. https://elasmo.org/meetings/abstracts/abst2002/

Henningsen, A.D. 2005, July 6-11 Boys will be boys: Sexual conflicts in captive sand tiger sharks, *Carcharias taurus* [Abstract]. 85th Annual Meeting of the American Society of Ichthyologists and Herpetologists, 21st Annual Meeting of the American Elasmobranch Society. Tampa, Florida. https://elasmo.org/meetings/abstracts/abst2005/

Henningsen, A.D., and S.H. Gruber. 1985. Assessment of two lemon shark, *Negaprion brevirostris*, populations by multiple mark procedures. Florida Scientist. 48(1):32. Annual Meeting of the Florida Academy of Sciences. St. Leo, Florida. https://www.jstor.org/stable/24319853

Henningsen, A.D., and S.H. Gruber. 1985. Assessment of two lemon shark, *Negaprion brevirostris*, populations by multiple mark procedures [Abstract]. 65th Annual Meeting of the American Society of Ichthyologists and Herpetologists, 1st Annual Meeting of the American Elasmobranch Society. Knoxville, Tennessee. https://elasmo.org/meetings/abstracts/abst1985/

*Henningsen, A.D., and S.H. Gruber. 1988. Annual production of the juvenile lemon shark (*Negaprion brevirostris)* in a tropical lagoon [Abstract]. 68th Annual Meeting of the American Society of Ichthyologists and Herpetologists, 4th Annual Meeting of the American Elasmobranch Society. Ann Arbor, Michigan. https://elasmo.org/meetings/abstracts/abst1988/

Henningsen, A.D., B. Hecker**,** and C. Andrews. 1992. Fish Breeding Program at the National Aquarium in Baltimore: A Case Study of Cichlid Fauna of Lake Victoria [Poster]. Symposium on Conservation Genetics and Evolutionary Ecology. Columbus, Ohio.

Henningsen, A.D., B. Hecker, P. Hampton, and R.T. Jones. 1996, June 13-19. Survey of large coastal sharks in the Delaware Bay by longline [Abstract]. 76th Annual Meeting of the American

Society of Ichthyologists and Herpetologists, 12th Annual Meeting of the American Elasmobranch Society. New Orleans, Louisianna. https://elasmo.org/meetings/abstracts/abst1996/

Henningsen, A., and *J. Janssen. 2018, May 14-18. AZA SAFE International Census of Chondrichthyans in Human Care – Who has cartilaginous fishes, and where [Abstract and presentation]? Regional Aquatics Workshop. Tampa, Florida.
https://www.animalprofessional.com/raw-2018-shark-conservation--partnerships-abstracts1.html

Henningsen, A., and J. Janssen. 2018, June 3-8. AZA SAFE International Census of Chondrichthyans in Human Care – Who has cartilaginous fishes, and where? Sharks International & the 34th Annual Meeting of the American Elasmobranch Society, Business Meeting. Presented by Paula Carlson. Sharks International, João Pessoa, Brazil.

Henningsen, A., and *J. Janssen. 2018, September 24-27. One Shark, Two Shark, Flat Shark, Ghost Shark - SAFE Shark and Ray Project: International Census of Chondrichthyans in Human Care. Association of Zoos and Aquariums. Seattle, Washington.

Henningsen, A.D., M. Schmale, I. Gordon, R. Garner, R. Marin-Osorno, and N. Kinnunen. 2001, October 3-7. Captive breeding and sexual conflict. 1st International Elasmobranch Husbandry Symposium. Orlando, Florida.

*Henningsen, A.D., J.M. Trant, S. Ijiri, and S. Kumar. 2000, June 14-20. The short-term *in vitro* response of stingray trophonemata to exogenous agents: a preliminary report [Abstract]. 16th Annual Meeting of the American Elasmobranch Society. La Paz, Baja California Sur, Mexico.
https://elasmo.org/meetings/abstracts/abst2000/

*Henningsen, A.D., J.M. Trant, and A.R. Place. 1999, June 24-30. Preliminary results on the size of proteins and protein concentration in histotroph from three species of batoids [Abstract]. 79th Annual Meeting of the American Society of Ichthyologists and Herpetologists, 15th Annual Meeting of the American Elasmobranch Society. State College, Pennsylvania.
https://elasmo.org/meetings/abstracts/abst1999/

*Henningsen, A., E. Schneble, D. Littlehale, J. Choromanski, I. Gordon, and K. Willson. 2013, November 11-13. Sand tiger shark, *Carcharias taurus*, reproduction: A framework for a captive breeding program [Abstract and presentation]. 2nd International Elasmobranch Husbandry Conference. Monterey, California.
https://www.animalprofessional.com/iehs-2013-sustainability-abstracts.html

*Janssen, J., and A. Henningsen, and T. Niemann. 2019, May 12-17. #ChondroCensus 2019: Roll Call for Chondrichthyans. Regional Aquatics Workshop [Abstract and presentation]. Columbus, Ohio. https://www.animalprofessional.com/raw-2019-elasmobranch.html

Janssen, J., and A. Henningsen, and T. Niemann. 2019, July 24-28. #ChondroCensus 2019: Roll Call for Chondrichthyans. 34th Annual Meeting of the American Elasmobranch Society, Business Meeting. Presented by Paula Carlson. Snowbird, Utah.

Michels, R., A. D. Henningsen, and R. T. Jones. 1996, June 13-19. A trawl survey of sharks in the Delaware Bay [Abstract]. 76th Annual Meeting of the American Society of Ichthyologists and Herpetologists, 76th Annual Meeting of the American Society of Ichthyologists and Herpetologists, 12th Annual Meeting of the American Elasmobranch Society. New Orleans, Louisianna. https://elasmo.org/meetings/abstracts/abst1996/

Smale, M., J. Correia, R.T. Jones, A.D. Henningsen, and R. Garner, and G. Crow. 2001, October 3-7. Research on elasmobranchs in public aquaria. 1st International Elasmobranch Husbandry Symposium. Orlando, Florida.

White, S., K. Duke, P. Carlson, A. Henningsen, K. Hunter, R. Preziosi, J. Rountree, G. Hill, and J. Fitzpatrick. 2016, July 7-10. Making Connections for Sawfish: The Role of Public Aquaria in Sawfish Biology and Conservation [Abstract]. 83rd Annual Meeting of the American Society of Ichthyologists and Herpetologists, 19th Annual Meeting of the American Elasmobranch Society, Sawfish Symposium. New Orleans, Louisiana.
https://elasmo.org/meetings/abstracts/aes-2016-abstracts/

Wyffels, J.T., A.B. Bodine, T.R. Scott, R.S. Rodgers, C.A. Luer, C.J. Walsh, and A. Henningsen. 1995, June 15-19. Preparation and specificity of monoclonal antibodies against IgM of the Atlantic nurse shark, *Ginglymostoma cirratum* [Abstract]. 75th Annual Meeting of the American Society of Ichthyologists and Herpetologists, 11th Annual Meeting of the American Elasmobranch Society. Edmonton, Alberta, Canada. https://elasmo.org/meetings/abstracts/abst1995/

*Wyffels, J., F. Bulman, C. Coco, H. Fatzinger, A. Henningsen, J. Jewell, S. Martin, M. McKnight, C. Schreiber, P. Sloan, and L. Penfold. 2016, March 22-25. Development and dissemination of tools to access reproductive status of sand tiger sharks (*Carcharias taurus*) and advance the field of assisted reproduction in sharks [Abstract and presentation]. Regional Aquatics Workshop. New Orleans, Louisiana.
https://www.animalprofessional.com/raw-2016-sharks--sand-tiger-shark-round-table.html

Young, F.A. and A.D. Henningsen. 1988. An occurrence of the royal gramma *Gramma loreto*, in the Florida Keys. 68th Annual Meeting of the American Society of Ichthyologists and Herpetologists. Ann Arbor, Michigan.

Lectures

Age and Growth of the lemon shark, *Negaprion brevirostris*. 1984. Florida Marine Aquarium Society Meeting. Miami, Florida.

Biology and systematics of chondrichthyans (some of my best friends are sharks). 1994. National Aquarium in Baltimore. Baltimore, Maryland.

GnRH and reproductive cycles in fish. 1995. The University of Maryland at Baltimore, Department of Physiology, Center of Reproduction. Baltimore, Maryland.

Reproductive modes and levels of recirculating reproductively related steroid hormones in female viviparous elasmobranchs. 1996. University of Maryland, Horn Point. Cambridge, Maryland.

Reproductive physiology of elasmobranchs. 1998. National Aquarium in Baltimore. Baltimore, Maryland.

The role of aquaria in sawfish conservation and research. 2004. Sawfish Recovery Team Meeting. Mote Marine Laboratory, Sarasota, Florida.

Growth in captive smalltooth sawfish, *Pristis pectinata*. 2004. Sawfish Recovery Team Meeting. Mote Marine Laboratory, Sarasota, Florida.

Reproduction, captive breeding, and sexual conflicts. 2008, 2010, 2011. Lecture as part of Elasmobranch Husbandry Course, Aquatic Sciences Program at Oregon Coast Community College, presented via teleconferencing.

The who, what, and where (or biology and conservation status) of sawfishes. 2012. National Aquarium, Baltimore. Baltimore, Maryland.

Research Grants
Institute of Museum and Library Services. 1992. Induced spawning of two species of marine ornamental fish, the high hat, *Equetus acuminatus*, and the smallmouth grunt, *Haemulon chrysargyreum*. In collaboration with Dr. Yonathan Zohar, Center of Marine Biotechnology, Marine Biotechnology Institute and Maryland Agricultural Experiment Station, University of Maryland System, Baltimore, Maryland.
Total budget granted: $25,000.

National Aquarium in Baltimore. 1999. Recirculating levels of reproductively-related steroid hormones in captive sand tiger sharks, *Carcharias taurus*, with notes on their relation to pre-copulatory behavior. In collaboration with Frank Murru, Sea World of Florida; Dr. L.E.L. Rasmussen, Oregon Graduate Research Institute; and Dr. Brent Whitaker, National Aquarium in Baltimore.
Total budget granted: $3,000.

Bernice Barbour Foundation. 2005. Investigation on the levels of reproductively-related hormones in captive sand tiger sharks, *Carcharias taurus*, and their relation to sexual conflicts.
Total budget granted: $30,000.

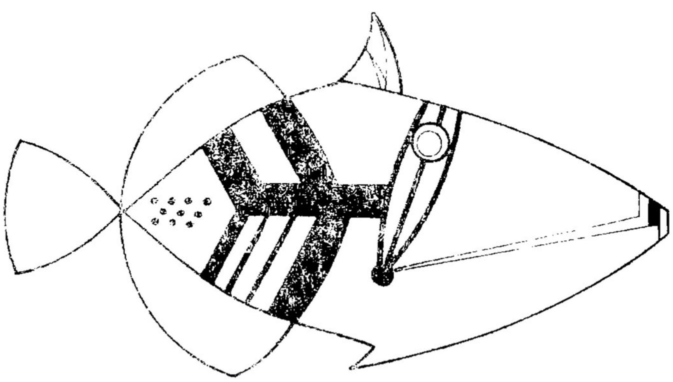
Peer Review
Journal of Aquaculture Research & Development
National Fish and Wildlife Foundation
ICES Journal of Marine Science
Environmental Biology of Fishes
North American Journal of Aquaculture
Elasmobranch Husbandry Manual, 1st and 2nd editions.
Marine Ecology Progress Series
Zoo Biology
Comparative Biochemistry and Physiology

Fisheries Research
The International Journal of Tropical Biology and Conservation "Revista de Biología Tropical"
International Journal of Comparative Psychology
General and Comparative Endocrinology

Graduate Committees
Cole, J. 1996. Visual physiology of elasmobranchs. University of Maryland, Baltimore County. Non-research thesis. Chair, Tom Cronin, Ph.D.

Schneble, E. 2014. A review of reproductive behavior of the sand tiger shark (*Carcharias taurus*) with special emphasis on captive sharks at the National Aquarium, Baltimore. Nova Southeastern University.

Anderson, E. 2017. Mating system in a captive population of a dasyatid ray, the southern stingray (*Hypanus americanus* Hildebrand and Schroeder 1928), at the National Aquarium, Baltimore, Maryland. Towson University.



INTERANNUAL VARIATION OF APPETITE AND REPRODUCTIVE BEHAVIOR IN SEA TURTLES (*C. caretta* AND *C. mydas*) ON EXHIBIT AT NEW ENGLAND AQUARIUM

Matthew Samara, Aquarist II msamara@neaq.org
Michael O'Neill, Manager moneill@neaq.org

New England Aquarium, Central Wharf, Boston MA 02110

Introduction

The seven species of extant sea turtles are all recognized as "Endangered", "Critically Endangered", or "Vulnerable" by the International Union for the Conservation of Nature (IUCN). Studying them provides valuable information that can help inform conservation and management strategies to ensure survival (Eckert et al. 1999). Sea turtles living in captivity provide a unique and highly accessible way to examine their biology and behavior. They are popular exhibit animals in zoos and aquaria due in large part to rescue and rehabilitation programs and networks which have increased in number and become more refined over the years (Wood, 2022). Stranded turtles occasionally suffer incapacitating injuries which prevent them from being released back to the wild and will live out their lives at an institution under human care. Food intake and nutrition are foundational aspects of animal care, and reduced food intake can often be cause for concern across many species. Understanding how natural and artificial factors impact food intake and other behaviors has beneficial implications for the management of animal welfare in captive individuals and wild populations. Studying behavioral variation is particularly intriguing in long-lived and difficult to track taxa like sea turtles. Reviewing long-term resident sea turtles in zoos and aquariums provides an opportunity to examine extended fine-scale behavioral records that are impossible to collect in wild populations. These datasets can contribute complimentary information to aspects of sea turtle biology studied in the wild creating a more wholistic understanding of species life histories and population dynamics.

There are several sea turtle behaviors tied to seasonal changes in their natural environment, including foraging and fasting (Miller et al. 2014). Sustained behavior changes like a months-long decrease in appetite are difficult to document in free-ranging sea turtles. The highly migratory and cryptic habits of sea turtles make it difficult to track not only the turtles, but the potential prey items and food ingestion as well (Marn et al. 2017a; Fouda et al. 2023). Periods of inappetence (fasting) in sea turtles have been attributed to brumation (Hawkes et al. 2007) and reproductive cycle (Ceriani et al. 2015; Marn et al 2017 a., Fouda et al. 2023). Brumation is defined as a period of winter dormancy unique to ectotherms and is analogous to hibernations undertaken by certain mammalian species (Wilkinson et al. 2017). Since ectothermic animals cannot regulate their internal body temperature, this state of torpor or inactivity is thoroughly influenced by the environmental temperature. The benefit of brumation in highly migratory animals like sea turtles, however, is unclear since migration itself is an adaptation to avoid cold temperatures (Hawkes et al. 2007). Nonetheless, several studies have suggested that sea turtles may hibernate or enter winter dormancy (Felger et al. 1976; Carr et al. 1980; Ogren and McVea 1995; Godley et al. 2002; Velez-Rubio et al. 2017). These studies were based in water observation (Carr et al. 1980), epibiont density (Velez-Rubio et al. 2017), and secondhand accounts and observations (Felger et al. 1976). As a result of the randomness of brumation events and difficulty of detection, brumation has not been confirmed in North American sea turtle populations ion the decades since this question was initially investigated (Hochsheid et al. 2005, Hawkes et al. 2007) Indeed, it remains unclear whether sea turtles truly brumate, and brumation may be unnecessary for sea turtles that inhabit tropical and subtropical waters where cold temperature are less of a concern (Moon et al. 1997).

Although brumation may cause free-ranging sea turtles to enter a fasting period, other mechanisms are likely involved with fasting in captive sea turtles that are not exposed to low environmental temperatures. In captivity, it may be more likely that reproductive cycles influence feeding behavior. In general, vertebrates can be characterized as income breeders or capital breeders. (Sainmont et al. 2014). Income breeders utilize resources immediately for reproductive activities while capital breeders build up a reserve that is used for reproduction at a later time. Capital breeding is believed to be widespread among marine turtles (Hays et al. 2002, Deem et al. 2009, Hawkes et al. 2009, Plot et al. 2013, Perault et al. 2014, Ceriani et al. 2015; Marn et al. 2017 a; Fouda et al. 2023) In-water observations, gut-content analysis, clinical pathologic data, and isotope tracking have been used to better understand this subject. There are, however, documented exceptions to the capital breeding strategy in turtles. Hays et al. (2002), studied green sea turtles at Ascension Island and found that they were true capital breeders with females fasting for the entire nesting season, but green sea turtles in Cyprus grazed on sea grass during the inter-nesting period. So, while capital breeding is likely not universally utilized among sea turtles, there is strong evidence connecting fasting to the reproductive cycle.

The general stages of the reproductive cycle in a sea turtle are foraging, migration, mating, and nesting. Sea turtles initially spend time in foraging areas to build up a reserve of resources to fuel their migration to breeding areas, mating, and nesting, which are very energetically expensive. After migration begins, a period of inappetence is believed to occur, lasting until females complete their entire reproductive cycle. After arriving at nesting beaches, turtles can spend up to a few months there laying multiple clutches of eggs. Between egg-laying events, females linger in nearby waters until it is time to exit and lay another clutch, known as the inter-nesting period. Due to the costly nature of reproduction, female sea turtles do not repeat this cycle annually. Females, after returning from nesting grounds to their foraging areas, enter a quiescent period where they recover from reproduction and migration. This inactive period can last up to a few years, and once concluded, the cycle begins anew. (Miller et al. 2014).

In this study, we followed three sea turtles that have been multi-decadal residents of the New England Aquarium. This project focuses on exploring the phenological characteristics of appetite fluctuations in these turtles and the onset of reproductive activity by a reproductively mature loggerhead turtle. We hypothesize that a confluence of natural and artificial phenomena impacts the timing of both foraging and reproductive activity.

Methods

Animals

The sea turtles studied include “GREEN”, a female green sea turtle (*Chelonia mydas*), “LOGGER1” the older of two female loggerhead turtles (*Caretta caretta*), and “LOGGER2” the younger of the two loggerhead turtles. “GREEN”, the green sea turtle, arrived at the New England Aquarium in 1970 from the now closed Provincetown Aquarium. Records of her origin prior to exhibit at the Provincetown Aquarium are unavailable, but anecdotal evidence suggests she originally came from a population of sea turtles in the Florida Keys and was collected in the mid-late 1960's. Sea turtle growth rates are highly variable and the relationship between age and other physical metrics including weight and carapace length are still unclear (Braun-McNeill et al. 2008). This makes an accurate determination of the exact age of the turtles in this study difficult. Accession records from her arrival at New England Aquarium in 1970 indicate her weight was over 90 kg, and it was estimated that she was between 20 and 40 years old. Following her 53 years on exhibit GREEN is now 225.4 kg and has a straight carapace length (SCL) = 109 cm and is estimated to be between 73 and 93 years old. GREEN receives a daily diet that is approximately 1% body weight, comprised of 80% vegetation and 20% fish and squid. She has consistently displayed a preference for the protein items in her diet meaning she rarely declines fish or squid when offered and is used by animal care staff for vitamin and nutrient supplementation delivery.

Both loggerhead sea turtles came through the New England Aquarium's sea turtle rescue and rehabilitation program. LOGGER1 was found stranded and cold-stunned on November 23, 1987 in Brewster, MA. She arrived in moribund condition and it was determined that she had significant ocular damage resulting in partial-to-full blindness. She was not considered a candidate for release and became a full-time exhibit resident on January 12, 1988. Based on her size at stranding (SCL = 44.5 cm and 14.1 kg) and the additional years in human care, her current age is estimated to be between 39 and 42 years old (current weight = 88.5 kg). LOGGER2 was also a cold-stunned turtle rescued after she was found stranded in Wellfleet, MA on December 23, 1999, at a weight of 28.8 kg and SCL = 57.1 cm. She was behaviorally evaluated during rehabilitation and deemed non-releasable based on an inability to forage for food indicating some degree of neurological damage and partial visual impairment. She became a full-time exhibit resident at the New England Aquarium on December 2, 2000. Her current age is estimated to be between 30 and 35 years old and her current weight is 72.1 kg. Like GREEN, both loggerheads receive a daily diet equivalent to 1% body weight, however it is comprised entirely of fish and squid. They are target trained to attend a diver-mediated feeding session upon hearing an auditory cue underwater.

All three sea turtles discussed in this study reside in the Giant Ocean Tank (GOT), a 200,000-gallon cylindrical exhibit filled with filtered Boston Harbor water and showcasing a taxonomically diverse collection of tropical and sub-tropical Western-Atlantic fish species. At the center of the exhibit is a two-story artificial reef structure replicating typical reef-building Caribbean coral species. Exhibit conditions during the 23 years reviewed in this study can be split into two dominant eras delineated by a major exhibit renovation that was executed over a 9-month period from the fall of 2012 to the spring of 2013. From 2000-2012, the exhibit was maintained at 24-25.5°C year-round and illuminated by metal halide lights with a simple on/off daily photo period of 8-9 hours. Following the renovation's conclusion in 2013, the GOT was maintained at 23-24°C year-round. A more dynamic LED lighting array was installed with sunrise and twilight transitional periods as well as periodic intensity fluctuations to simulate daytime cloud cover. While more complex in operation, the post-renovation lighting schedule was also consistently maintained year-round with regard to time of sunrise and sunset, and a full-intensity daytime photoperiod of 8 hours. The inclusion of the transitional sunrise and twilight phases extended the total period of exhibit illumination to 14 hours per day.

Data Collection

We retrospectively reviewed daily husbandry records from 2000-2023 and gathered observations on fasting periods and reproductive events. Information that was vague, illegible, missing, or inconclusive was discarded from the analysis. Interpretation of turtle appetite was different between species, but the method of data collection had been consistent year-to-year for each of the three animals. Fasting behavior in GREEN was characterized by reduced food intake as opposed to outright inappetence. Fasting was defined as a reduction in the consumption of vegetation of at least 50% or more for at least 3 consecutive days. The criteria for resolution of fasting were 3 consecutive days with vegetation consumption greater than 50%.

Fasting for the loggerhead turtles was defined as not consuming any amount of food for three consecutive feeding days. Because of the episodic shifts in appetite, there were periods when the loggerheads were offered food daily and other periods where they were offered every other day to minimize food waste. For the latter scenario, data was carefully evaluated as to only include three consecutive food-offering days when determining the start and stop criteria for a fast.

Data visualizations and statistical analysis of fasting activity focused on duration and phenological characteristics. To visualize each documented fast in a continuous linear dumbbell plot format, start and stop dates were assigned a numerical day-of-year (DOY) from 1-365 for each day of a calendar year and fasts that began in the second half of a previous year were converted to the negative value of (365-DOY) for that date. For example, a fast that started on December 1, 2001, and ended on April 15, 2002, would start on – 30 DOY and end on DOY 105 for the year 2002. Exploratory analyses of potential influences on this behavior based on species biology, life history, and natural or artificial phenomena were conducted using the open-source software R (R Core Team, 2021). The distribution of fasting durations for each turtle was analyzed for normality using a Shapiro-Wilks test and a comparison of the distributions across all turtles was made using a Kruskal-Wallis test. A pair-wise comparison was then made via Dunn's test with a Bonferroni correction to assess whether there were significant differences between each turtle's fasting duration. To measure the effect of a major exhibit renovation in 2012, shifts in fasting days pre- and post-renovation were compared. Beyond phenological changes, a comparison of the duration between fasts for the GREEN turtle was examined based on anecdotal reports that the seasonal fasting of this animal was diminishing over time. Mean durations without fasting before and after the 2012 exhibit renovation were compared. Non-fasting periods that occurred mid-renovation while GREEN was in a different enclosure were removed, and extremely short periods between fasting ($n \leq 12$ days) were also discarded as outliers. The distributions and variances of non-fasting periods pre-and post-renovation were compared using a Shapiro-Wilks test and a Levene's test respectively to ensure the assumptions of further analyses were met. Based on those findings, a one-way Analysis of Variance (ANOVA) was performed to compare the significance of the changes in the mean duration of non-fasting periods pre and post exhibit renovation by GREEN.

For characterization of reproductive status, data for egg laying were collected from daily records for individual animals based on animal care observations. The developmental condition and number of the eggs laid was documented in each daily record observation and assembled across all study years for review.

Results

Each turtle exhibited some degree of periodic fasting, but the consistency and individual characteristics of each animal's fasting pattern varied. Most fasting took place in the first half of each year though all three turtles had previously started fasting in the fall or winter and continued into spring of the following year (Figure 1).

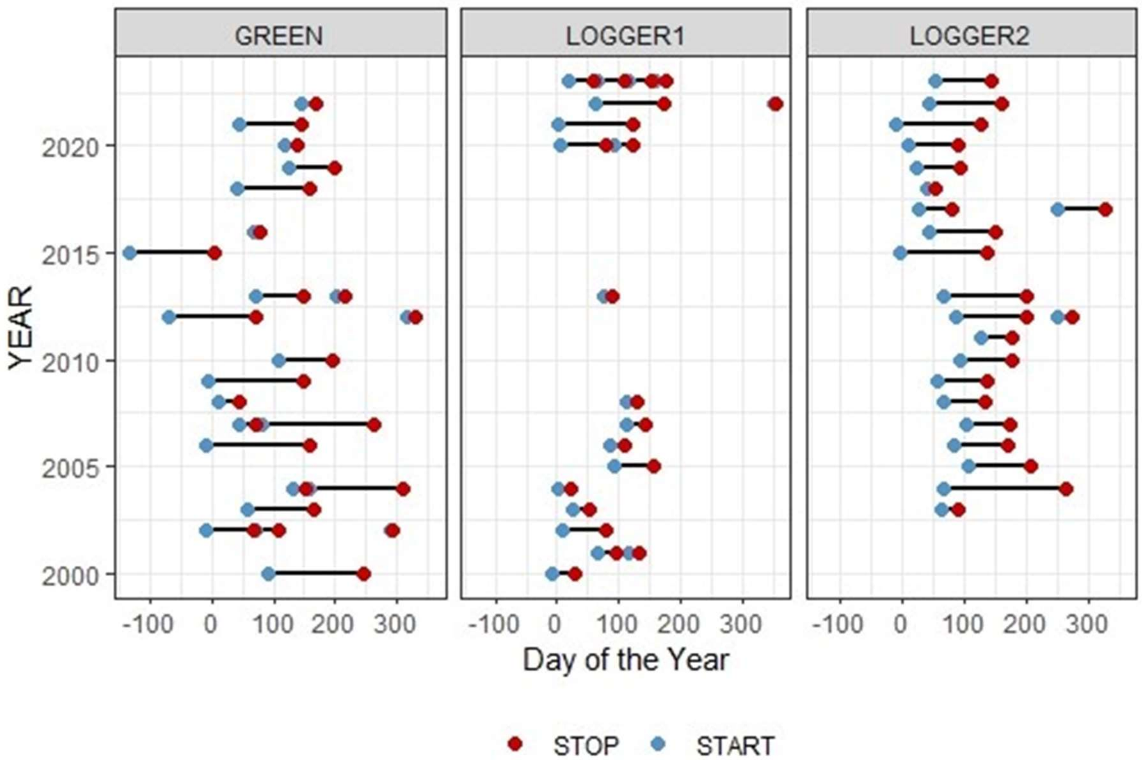


Figure 1. Appetite reduction (fasting) start and stop dates by day-of-year for each of the three resident sea turtles from 2000-2023.

Fasting Duration by Turtle

A summary of the distribution statistics of each turtle's fasting duration is illustrated in Table 1 and visually represented in Figure 2. The Shapiro-Wilk tests for normality of fasting durations for each turtle revealed that GREEN and LOGGER1 fasting durations were significantly different from the normal distribution (p-value GREEN = 0.027; LOGGER1 = 0.004). The fasting duration distribution of LOGGER2 had a p-value = 0.653 indicating the likelihood of a normal distribution. A comparison of the duration distributions for all three turtles using a Kruskal-Wallis test confirmed a significant difference (p-value = 0.009) among the group. A pair-wise comparison for each turtle using Dunn's test was made with a Bonferroni correction and revealed the significant difference between the duration distributions was only between LOGGER1 and LOGGER2 (adjusted p-value = 0.008).

Table 1. Sea turtle fasting duration parameters from fasting activity between 2000-2023.

TURTLE	n	Min (days)	Max (days)	Median	Mean	sd	ci
GREEN	24	3	182	78.5	81.7	59.4	25.1
LOGGER1	20	6	121	31.5	42.4	31.1	14.6
LOGGER2	22	16	198	82.5	88.5	43.3	19.2

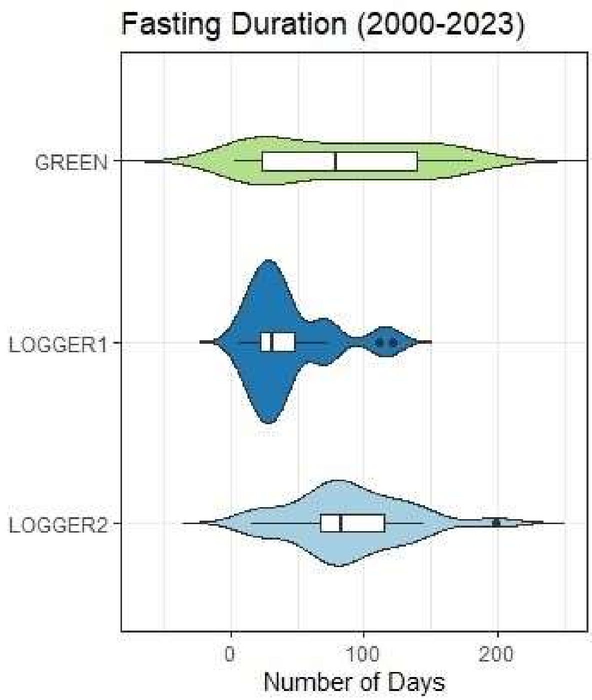


Figure 2. Violin plots illustrate the distribution of fasting durations for each turtle residing in the Giant Ocean Tank from 2000-2023. Colored regions indicate the kernel probability density of the duration data. Overlaid are box and whisker plots with the box marking the median and first and third quartiles (25% and 75% percentiles). Whiskers extend to the smallest and largest duration values within 1.5 times the inter-quartile range and outlying points beyond each whisker are individually plotted.

Phenology of Turtle Fasting

While the range and standard deviation of documented turtle fasting was substantial, the mean day of year (DOY) for the start of a fast across all years and turtles was remarkably similar, falling during the middle of March annually (DOY 74.3-81; March 15-22). The mean fasting resolution day was more variable, ranging from DOY 119 (April 29) for LOGGER1 to DOY 162 (June 11) for GREEN and LOGGER2 (Table 2).

Table 2. Summary of fasting start and stop Day of Year statistics by turtle residing in the Giant Ocean Tank.

TURTLE	START				STOP		
	Mean (DOY)	**Median (DOY)**	**SD (Days)**		**Mean (DOY)**	**Median (DOY)**	**SD (Days)**
GREEN	81	70.5	100		161.6	154	84.4
LOGGER1	77.6	71.5	80.2		119.05	116	70.6
LOGGER2	74.3	65.5	67		161.8	156	66.8

Visual representation of the start and stop days by turtle (Figure 3) revealed how diffuse the activity is by GREEN, which had the largest standard deviation for start and stop days as well as fasting duration across all years of the study. LOGGER2 had the most consistent start and stop days though the variation in duration exceeded that of LOGGER 1.

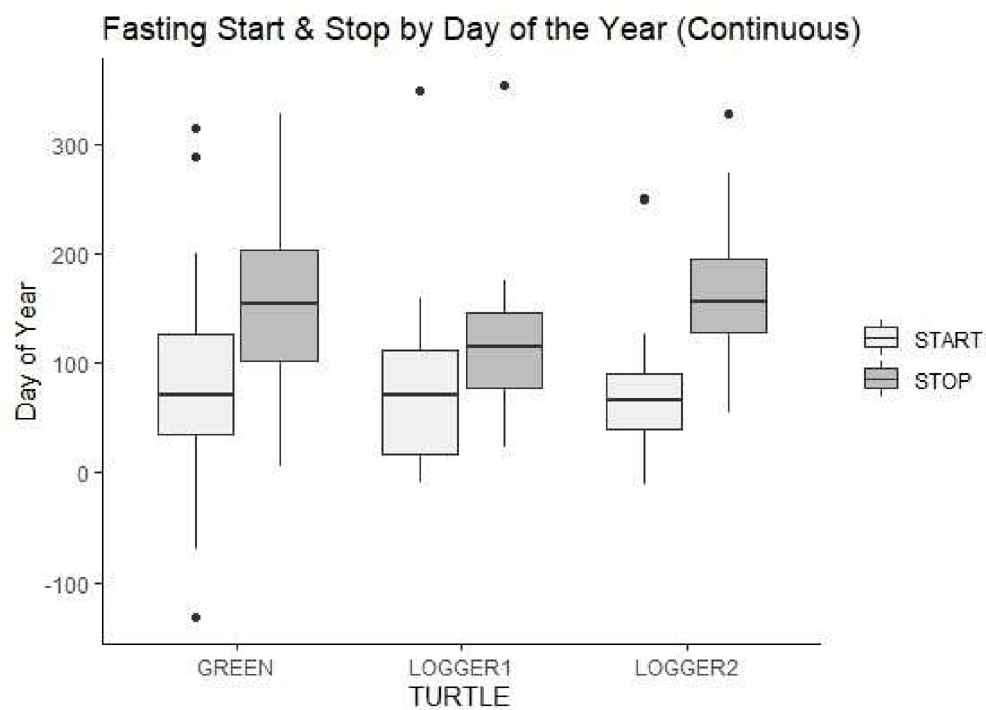


Figure 3. Box plots of fasting start and stop day of the year (DOY) by turtle. Boxes mark the median and first and third quartiles (25% and 75% percentiles). Whiskers extend to the smallest and largest duration values within 1.5 times the inter-quartile range and outlying points beyond each whisker are individually plotted.

Analysis of fasting start and stop day during the "Pre" (2000-2012) and "Post" (2013-2023) renovation years yielded mixed results (Table 3). Mean start days for GREEN and LOGGER1 shifted approximately 30 days later post-renovation though the distributions of those shifts were not distinct. The mean stop day for fasting by GREEN was little changed, shifting just six days pre- and post-renovation. In contrast, LOGGER2 exhibited a detectable shift in fasting activity approximately 20 days earlier for both mean start and stop days in the years post-renovation.

Table 3. Changes in fasting start and stop days by LOGGER2 before and after the 2012-2013 exhibit renovation.

TURTLE	RENOVATION STATUS	START			STOP		
		Mean (DOY)	Median (DOY)	SD (Days)	Mean (DOY)	Median (DOY)	SD (Days)
GREEN	PRE	67.8	64.5	89.3	164	156	87.3
	POST	99.5	93.5	116	158	153	84.8
LOGGER1	PRE	60.9	76	50.1	94.9	103	47.5
	POST	94.4	71	10.2	143	122	83.4
LOGGER2	PRE	84.9	83.5	22.3	172	175	47.6
	POST	65.4	40	89.3	153	140	80.5

Following the exploration of potential shifts in the timing of each turtle's fasting period, the duration of non-fasting periods by GREEN was compared pre- and post-renovation as a potential impact on behavior following exhibit changes. A Shapiro-Wilks test confirmed that non-fasting periods both pre- and post-renovation were normally distributed (p-value = 0.64 and 0.20 respectively). A Levene's test confirmed both data groups also had a similar variance (p-value = 0.96). With the necessary assumptions of normality and variance met, a one-way ANOVA resulted in a p-value = 0.073 indicating that at the 95% confidence interval, a significant difference could not be measured in the duration of time between fasts pre- and post-renovation by GREEN.

Egg Production

Since 2017, LOGGER1 produced eggs annually except for 2019 (Figure 4). The first two years in which eggs were detected (2017 and 2018) were characterized by the discovery of relatively few eggs in the exhibit water over periods of several weeks and months (the exhibit has no terrestrial nesting habitat). After a year with no egg production in 2019, LOGGER1 produced 3-5 clutches of eggs (≥25 eggs per laying event) annually with an average of 27 days between each clutch within a season. Egg production across all years peaked in April, though multiple clutches had been laid each month from February to July since 2019. In 2022, monthly ultrasound exams revealed the retention of several shelled eggs that were ultimately laid through medical induction in October of that year.

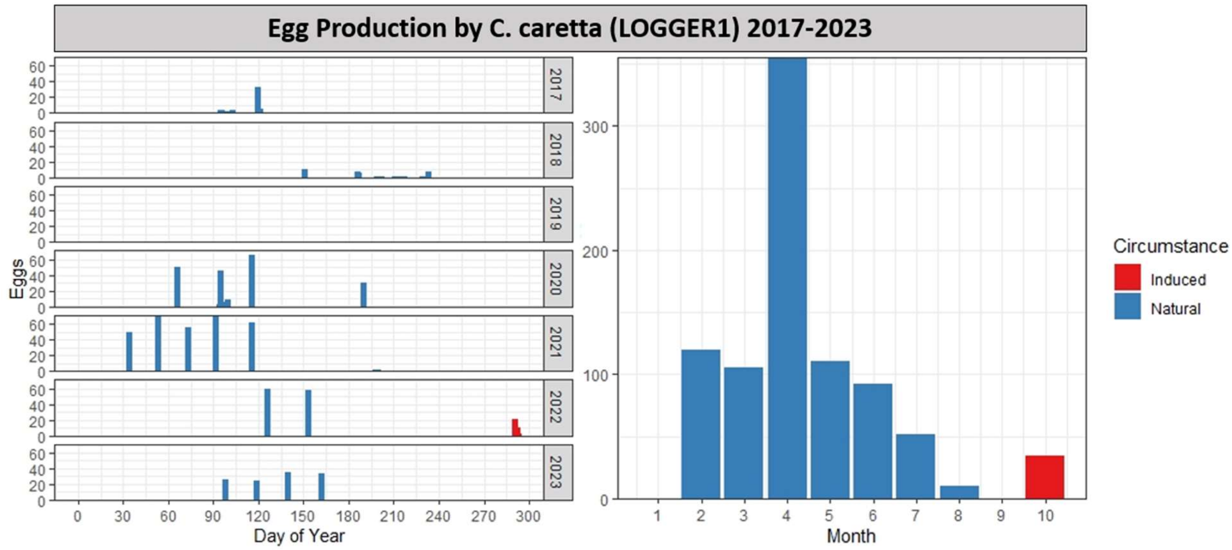


Figure 4. Number of eggs produced by LOGGER1 facetted by year (left) and total eggs produced in each moth across all years (right) (2017-2023).

Discussion

This study presents notable insights into the interannual timing and duration of fasting behavior by each sea turtle in the GOT over the past 23 years. The acquisition and analysis of daily observations spanning more than two decades reveals a record of animal behavior on a continuous and longitudinal scale rarely documented in members of the Cheloniidae. Across all turtles and years, fasting predominantly occurred in the first half of the year, with mean onset occurring mid-March and mean resolution occurring between the end of April and early June. Mean duration varied among the three turtles, ranging from 42 and 89 days. Major disruptions or changes to the exhibit appear to have mixed impacts on sea turtle behavior with phenological shifts in fasting moving later for GREEN and LOGGER1 on average, while LOGGER2 shifted earlier following the renovation. In addition to seasonal fasting behavior, LOGGER1 began producing eggs in 2017 and in the last four years of the study (2020-2023) produced 3-5 clutches of >25 eggs per clutch with peak egg production occurring in April. Fasting during these same four years also overlapped with the timing of egg production.

This project highlights the complexities in carrying out long-term animal behavior studies. In mining daily records for appetite information, different standards for fasting had to be developed to accommodate the distinct diet and feeding behaviors of green turtles and loggerhead turtles. The grazing behavior unique to GREEN in this study may have introduced inconsistencies in fasting characteristics based on the intermediate amounts of food consumed versus the more straightforward attendance or absence of a loggerhead sea turtle at a single daily targeted feeding. This aspect is compounded by evolving recordkeeping standards through time and inconsistencies in reporting amongst record keepers. All these components affect data quality over an extended retrospective study such as this one.

Consideration must also be given to the unique characteristics of each species, individual animal, and their specific life histories. Beyond differences in diet and foraging behavior by species, GREEN is a mature-to-geriatric female green sea turtle while LOGGER1 and LOGGER2 are both substantially younger and entering reproductive maturity. These differing stages of development have implications for the respective energy budget of each animal (Marn et al. 2017 b.). The observation of reproductive activity in LOGGER1 and its absence in the other two turtles may account for some of the fasting differences detected in this study. In wild populations, a series of interactions between exogenous and endogenous factors are responsible for reproductive activity in sea turtles (Miller et al., 2014). Changes in interannual population density estimates in the wild indicate foraging, migration, and nesting behaviors are highly variable year-to-year (Bovery and Wyneken, 2015). Similar interactions that expand to include the artificial exogenous factors of an exhibit (such as stable water temperature/chemistry, consistent photoperiod, regular access to food, etc.) along with endogenous factors of each sea turtle likely influence the observed seasonal activities in captive turtles. Due to the circumstances of their acquisition, each turtle in this study has varying sensory perception abilities that may affect the manifestation of foraging and reproductive behavior. The extensive vision impairment and neurological damage sustained by LOGGER1 and LOGGER2 might influence phenological aspects of their behavior in different ways. Alterations to exhibit photoperiod for example, may have a lesser impact on LOGGER1 versus LOGGER2 or GREEN.

In addition to the sensory perception of each turtle, environmental cues and conditions within the Giant Ocean Tank have varied throughout the decades and may have substantial influence on the timing of seasonal behavior. The 2012 renovation of the Giant Ocean Tank included a complete overhaul of the lighting system and its programing, shifts in exhibit water temperature, and the incorporation of new Life Support System (LSS) components among others. This major change in exhibit conditions appears to have had some impact on the onset and conclusion of fasting by LOGGER2 and perhaps an extension of the time between fasting periods by GREEN. In addition, throughout the decades of exhibit operation, husbandry techniques have evolved, and exhibit conditions manipulated to improve animal welfare. Environmental conditions such as water temperature, known to impact growth and reproductive rates in sea turtles (Marn et al., 2017 a.), may be intentionally stabilized or varied depending on desired exhibit outcomes which may impact interannual changes in animal behavior. There have also been periods in which other exhibit animals (marine fishes) have been exposed to pathogen outbreaks requiring manipulation of water chemistry (i.e. salinity) and/or the administration of medications. Resident sea turtles experience these perturbations and might change their behavior in response. These periods introduce yet another variable for consideration in influencing the observable appetites and behaviors of turtles in this study.

Despite living in artificial enclosures, exhibit animals are still exposed to varying degrees of natural phenomena that may influence seasonal behavior change. One natural condition that may impact exhibit turtles is the geomagnetic sensory perception that has been studied for many years in wild populations (Lohmann et al. 2001; Fuxjager et al. 2011; Fuxjager et al. 2014). Perhaps geographic location influences the timing of behaviors seen in captive sea turtles. Additionally, the Giant Ocean Tank operates as a semi-closed system, bringing in filtered saltwater from Boston Harbor and exchanging a small percentage of exhibit water with newly filtered harbor water each day through routine maintenance including filter backwashes and substrate vacuuming. The operation of this system may expose exhibit residents to detectable indicators of natural seasonal change including alterations in the chemical and biological components of the incoming harbor water such as those associated with spring plankton blooms. There is also evidence to support that natural lunar cycles can be detected by exhibit animals in the GOT regardless of exhibit lighting and influences fish spawning activity (unpublished data). It is worth considering the impact that geomagnetic fields, water-borne cues, and lunar cycles may have on animals within an exhibit and their potential contributions to the phenological characteristics of observed behavior. The influence of exhibit changes and pervasive natural phenomena on the behaviors of long-term resident exhibit animals is worthy of further investigation across several scientific disciplines.

Understanding how natural and artificial factors impact sea turtle behavior has beneficial implications for the management of animal welfare in captive individuals and wild populations. Reviewing long-term residences of sea turtles in zoos and aquariums provides an opportunity examine extended fine-scale behavioral records that are impossible to collect in wild populations. These datasets can contribute complimentary information to aspects of sea turtle biology studied in the wild creating a more wholistic understanding of species life histories and population dynamics. The information presented in this study serves as a starting point for future endeavors. Improvements in data quality and statistical analyses can be made with an expansion of study turtles to include others from collaborating institutions, mining additional years of observational records, data from medical examinations, and incorporating additional behavioral notes such as mating events or sustained swimming activity. Through the expansion of this work, the isolation of presumably confounding variables may result in a more accurate understanding of the factors that affect sea turtle fasting and reproductive behaviors in public aquaria and the wild.

Acknowledgements

The authors would like to thank the Fishes, Animal Health, and Environmental Quality Departments of the New England Aquarium for their contributions to this work. This project would not have come to fruition without the dedicated team of Giant Ocean Tank staff and volunteers responsible for the multidecadal care of our resident sea turtles and the diligent recordkeeping that made this paper possible.

References

Bovery, C. M., and J. Wyneken. 2015. Seasonal variation in sea turtle density and abundance in the Southeast Florida Current and surrounding waters. PLoS ONE 10(12): e0145980. https://doi.org/10.137/journalpone.0145980

Braun-McNeill, J., Epperly, S. P., Avens, L., Snover, M. L. and J. C. Taylor. 2008. Growth rates of loggerhead sea turtles (Caretta caretta) from the western north Atlantic, Herpetol. Conserv. Biol. 3(2): 273–281.

Carr, A., Ogren, L., and C. J. McVea. 1980. Apparent hibernation by the Atlantic loggerhead turtle off Cape Canaveral Floridae. Biol. Conserv. 19: 7-14.

Casale P, Mazaris AD, Freggi D. Estimation of age at maturity of loggerhead sea turtles Caretta caretta in the Mediterranean using length-frequency data. Endangered species research. 2011 Feb 3;13(2):123-9.

Ceriani, S. A., Roth, J. D., Tucker, A. D., Evans, D. R., Addison, D. S., Sasso, C. R., Ehrart, L. M., and J. F. Weishampel. 2015. Carry-over effects and foraging ground dynamics of a major loggerhead breeding aggregation. Mar. Biol. 162: 1955-1968. https://doi.org/10.1007/s00227-015-2721-x.

Deem SL, Norton TM, Mitchell M, Segars AL, Alleman AR, Cray C, Poppenga RH, Dodd M, Karesh WB. Comparison of blood values in foraging, nesting, and stranded loggerhead turtles (Caretta caretta) along the coast of Georgia, USA. Journal of Wildlife Diseases. 2009 Jan 1;45(1):41-56.

Eckert, K. L., Bjorndal, K.A., Abreu-Grobois, F. A., and M. Donnelly (Editors). 1999. Research and Management Techniques for the Conservation of Sea Turtles. IUCN/SSC Marine Turtle Specialist Group Publication No. 4.

Felger, R. S., Clifton, K., and P. J. Regal. 1976. Winter dormancy in sea turtles: independent discovery and exploitation in the Gulf of California by two local cultures. Science 191:283-285.

Fouda, L., Negus, S. R. B., Lockley, E. C., Fairweather, K., Lopes, A., Lopes, A., Correia, S. M., Taxonera, A., Schofield, G., and C. Eizaguirre. 2023. Productive foraging sites enhance maternal health and impact offspring fitness in a capital breeding species. Preprint. BioRxiv. https://doi.org/10.1101/2023.10.27.563439

Fuxjager MJ, Eastwood BS, Lohmann KJ. 2011. Orientation of hatchling loggerhead sea turtles to regional magnetic fields along a transoceanic migratory pathway. J. Exp. Biol. 214, 2504 – 2508. https://doi.org/10.1242/jeb.055921

Fuxjager, M. J., Davidoff, K. R., Mangiamele, L. A., and K. J. Lohmann. 2014. The geomagnetic environment in which sea turtle eggs incubate affects subsequent magnetic navigation behavior of hatchlings.

Godley, B. J., Richardson, S., Broderick, A. C., Coyne, M. S., Glen, F., and G. C. Hays. 2002. Long-term satellite telemetry of the movements and habitat utilization by green turtles in the Mediterranean. Ecography. 25: 352-362. https://doi.org/10.1034/j.1600-0587.2002.250312.x

Hawkes, L. A., Broderick, A. C., Coyne, M. S., Godfrey, M. H., and B. J. Godley. 2007. Only some like it hot – quantifying the environmental niche of the loggerhead sea turtle. Diversity Distrib. 13: 447-457. https://doi.org/10.1111/j.1472-4642.2007.00354.x

Hawkes, L. A., Broderick, A. C., Godfrey, M. H., and B. J. Godley. 2009. Climate change and marine turtles. Endang. Species Res. 7:137-154. https://doi.org/10.3354/esr00198

Hays, Graeme C., Broderick, A. C., Glen, F., and B. J. Godley. 2002. Change in body mass associated with long-term fasting in a marine reptile: the case of green turtles (Chelonia mydas) at Ascension Island. Can. J. Zool. 80:1299-1302. https://doi.org/10.1139/Z02-110

Hochscheid, S., Bentivegna, F., and G. C. Hays. 2005. First records of dive durations for a hibernating sea turtle. Biol. Lett. 1: 82-86. https://doi.org/10.1098/rsbl.2004.0250

Lohmann KJ, Cain SD, Dodge SA, Lohmann CMF. 2001 Regional magnetic fields as navigational markers for sea turtles. Science 294, 364– 366. https://doi.org/10.1126/science.1064557

Manire CA, Byrd L, Therrien CL, Martin K. 2008. Mating-induced ovulation in loggerhead sea turtles, *Caretta care*tta. Zoo Biol. May;27(3):213-25. https://doi.org/10.1002/zoo.20171

Marn, N., Jusup, M., Legovic, T., Kooijman, S. A. L. M., and T. Klanjscek. 2017 b. Environmental effects on growth, reproduction, and life-history traits of loggerhead turtles. Ecol. Model. 360: 163-178. https://doi.org/10.1016/j.ecolmodel.2017.07.001

Marn, N., Kooijman, S. A. L. M., Jusup, M., Legovic, T., and T. Klanjscek. 2017 a. Inferring physiological energetics of loggerhead turtle (*Caretta caretta*) from existing data using a general metabolic theory. Mar. Environ. Res. https://doi.org/10.1016/j.marenvres.2017.01.003

Miller, J. D., Limpus, C. J., and M. H. Godfrey. 2014. (Chapter 8) - Nest Site Selection, Oviposition, Eggs, Development, Hatching, and Emergence of Loggerhead Turtles.

Moon, D. Y., Mackenzie, D. S., and D. W. Owens. 1997 Simulated hibernation of sea turtles in the laboratory: I. Feeding, breathing frequency, blood pH, and blood gases. J. Exp. Zool. 278:372-380. https://doi.org/10.1002/(SICI)1097-010X(19970815)278:6<372::AID-JEZ4>3.0.CO;2-L

Ogren L., and C.J. McVea. 1995. Apparent hibernation by sea turtles in North American waters. In: Bjorndal KA (ed). Biology and conservation of sea turtles. Smithsonian Inst. Press, Washington, DC. 127-132

Parham JF, Zug GR. 1997. Age and growth of loggerhead sea turtles (Caretta caretta) of coastal Georgia: an assessment of skeletochronological age-estimates. Bulletin of Marine Science. ep 1;61(2):287-304.

Perrault JR, Wyneken J, Page-Karjian A, Merrill A, Miller DL. 2014. Seasonal trends in nesting leatherback turtle (*Dermochelys coriacea*) serum proteins further verify capital breeding hypothesis. Conservation Physiology. Jan 1;2(1): cou002.

Plot V, Jenkins T, Robin JP, Fossette S, Georges JY. 2013. Leatherback turtles are capital breeders: morphometric and physiological evidence from longitudinal monitoring. Physiological and Biochemical Zoology. Jul 1;86(4):385-97.

R Core Team (2021). R: A language and environment for statistical computing. Vienna, Austria. https://www.R-project.org/

Sainmont, J., Andersen, K. H., Varpe, O., and A. W. Visser. 2014. Capital versus income breeding in a seasonal environment. Am. Naturalist. 184(4): 466-476. https://doi.org/10.1086/677926

Velez-Rubio, G. M., Reyes, M. B., Monteiro, D., da Silva, A. P., Estima, S., Scarabino, F., and A Fallabrino. 2022. Mass stranding of overwintering green turtles *Chelonia mydas* in southern Brazil and Uruguay. Mar. Turtle Newsletter. 165: 28-32.

Wilkinson, A., Hloch, A., Mueller-Paul, J., and L. Huber. 2017. The effect of brumation on memory retention. Sci. Rep. 7, 40079. https://doi.org/10.1038/srep40079

Wood, L. D. 2022. Managing long-term wellness in captive sea turtles. Animal Welfare, 31: 423-432. https://doi.org/10.7/20/09627286.31.4.007

American Shad, Bruce Koike

RAW

POINT DEFIANCE ZOO & AQUARIUM

TACOMRAW2024

POINT DEFIANCE ZOO & AQUARIUM

Tacoma, Washington

Registration:

To register for the Regional Aquatics Workshop, click the link below and follow the instructions. https://whova.com/portal/registration/regio1_202405/

The registration link will show a price of $175 initially, but if you register before March 1, 2024, you will receive a discounted $150 early bird rate at checkout. Starting March 2, 2024, the price will be $175 as listed.

To pre-purchase a t-shirt for $25, click the link below and follow the instructions. Pre-paid shirts will be given out when you sign in at the registration booth during the event. A limited number of shirts will be available for purchase at the event, while supplies last.

The link for purchasing T-shirts will be available on the RAW website soon.

Contact:

If you have any problems or questions regarding registration or hotel reservations, reach out to Lisa Johnson at Lisa.Johnson@thezoosociety.org , 253-404-3651, or Chris Spaulding at Chris.Spaulding@pdza.org, 253-404-3675

Schedule:

The rough agenda will be as follows:
Sunday, May 5 – Aquatic TAG steering committee meetings
Monday, May 6 – Aquatic TAG public meetings, start of RAW presentations.
Monday, May 6 evening – Icebreaker at Point Defiance Zoo & Aquarium
Tuesday, May 7 through Thursday May 9 – RAW proper
*There will be evening events each night of the conference (trip to Seattle Aquarium, 7Seas Brewery downtown, and a lighting round of Tide Talks with dessert.

Program (in development)

Areas of focus during the meeting will include: Innovation in AQ sciences; Invertebrate Husbandry – with emphasis on diverse invert species; The Art of Displays – elements that make a "great" display (lighting, aquascaping, etc.); Husbandry of aquatic plants/algae (the non-nuisance kind); Aquarium research partnerships and collaborations; Mental wellness/ Employee wellbeing, Institutional/Industry Knowledge Retention/Transfer, and Life Support Systems. All submissions will be considered, but submissions are encouraged in these domains.

Conference Hotel:
Hotel Murano, $169 per night.
To reserve your room at the Hotel Murano use this link: https://be.synxis.com/?adult=1&arrive=2024-05-04&chain=21123&child=0&currency=USD&depart=2024-05-09&group=RAW2024&hotel=76784&level=hotel&locale=en-US&rooms=1 or call 253-238-8000 and ask for the block of rooms reserved for Regional Aquatics Workshop. If the initial block of rooms fills up, we will try and add more to meet demand, so let us know if you run into trouble.
(Overflow hotels include the Marriott Tacoma Downtown and the Courtyard by Marriott Tacoma Downtown, each within walking distance of the conference venue).

Conference Venue:
Conference space is provided at the Hotel Murano.

Airport Info:
Seattle-Tacoma International Airport (SEA-TAC)

Travel Estimates:
Flight Costs to SEA-TAC Airport from sample locations
(Priced on January 2, 2024 and based on Arrival May 5th and departure May 10th)

- Atlanta (ATL) ~$425+
- Boston (BOS) ~$325+
- New York (JFK) ~$375+
- Chicago (ORD) ~$325+
- Dallas (DFW) ~$428+
- Los Angeles (LAX) ~$235+

Driving Times to Tacoma from Selected Cities

- SeaTac Airport, WA 30 minutes
- Seattle, WA 45 minutes
- Portland, OR 2.5 hours
- Tri-Cities, WA (SE Washington, Pasco Airport) 3.5 hours
- Sacramento, CA 11.5 hours

ABSTRACTS from "RAW on the River," 2023
The Regional Aquatics Workshop, May 6-11.
National Mississippi River Museum & Aquarium, Dubuque, Iowa, USA.

May 6th and 7th
Coral School (separate registration was required)

May 7th
AZA Aquatic TAG Steering Committee Meetings

Monday, May 8th
Welcome and TAG Meetings

RAW Welcome, Ground Rules, etc.
Andy Allison

MFTAG Reporting Meeting
Paula Carlson et al.

FFTAG Reporting Meeting
George Brandy et al.

AITAG Meeting
Brian Nelson et al.

Tuesday, May 9th
Session 1: The National Mississippi River Museum and Aquarium – Past, Present and Future

Talks recorded by:
AnimalProfessional.com

Introductions and Welcome
Andy Allison

A Historic Society, a River, and a Dream – NMRMA's Past

Andy Allison, Aallison@Rivermuseum.com
National Mississippi River Museum & Aquarium,

What led to a county historic society deciding to create a world-class, AZA-accredited aquarium? Learn about the roots of the National Mississippi River Museum & Aquarium, and how we got to where we are.

Rivers to the Sea – NMRMA'S Present

Maia Davidson, mdavidson@rivermuseum.com
National Mississippi River Museum & Aquarium

Any facility will need some refreshing after 20 years and 3.5 million visitors. Learn about the latest and greatest aquarium renovation project at National Mississippi River Museum & Aquarium, Rivers to the Sea.

Paddling Ahead – NMRMA'S Future

Jacob Harmon, jharmon@rivermuseum.com
National Mississippi River Museum & Aquarium

What's next for the National Mississippi River Museum & Aquarium? Our Preserve the Wonder capital campaign includes plans to renovate some of our most visible aquariums.

Session 2: Animal Welfare/Wellbeing & Welfare/Wellbeing Assessment

Sponsor Presentation:
McRoberts, Inc.

Blood for Dummies: A No Frills Look at Blood Values in Fish and Elasmobranch Relating to Overall Health

Erika Pinney, ejpinney@wondersofwildlife.org
Wonders of Wildlife, Springfield, MO

Wonders of wildlife may be in the middle of country but we house a variety of fresh water, marine fish, invertebrates, sharks and rays. As part of our husbandry, we regularly catch our elasmobranchs to perform physicals. When assessing an animal's health many things need to be carefully looked at.

As technology advances and we learn more about our animals, we can take a more in depth look at our animals by performing a blood draw. In the public aquarium world, it is most often the job of the veterinary staff to look at the hematology samples taken from a fish or elasmobranch.

This presentation is a basic overview of values that are commonly tested when physicals are done on fish and elasmobranchs. The focus will mostly be on sharks, but the information will cover many marine fish and elasmobranchs. Blood gases, sodium, calcium, total protein and methods will be some of the key values we will take a closer look at when we run a blood test for elasmobranchs. We will discuss what these mean, what high/low levels can look like and how that may affect our animals. We can get a better snapshot of our animal's health with the understanding of basic hematology.

There is a lot of information hiding in that small vial of shark blood. We can evaluate reproductive status, organ health, injury healing, nutritional deficiencies and stressful events. I believe that with a basic grasp of blood levels and their meaning we can improve our understanding and thus husbandry for our aquarium animals.

It's Better Together: A Model for Advancing Behavioral Husbandry

Anaka Nazareth, anazareth@aqua.org
Jennie Janssen, jjanssen@aqua.org
National Aquarium

Historical industry data have shown that challenges to implementing or advancing positive reinforcement training into routine husbandry include staffing, available time, knowledge, tools, infrastructure, finances, and institutional support. At National Aquarium, behavior training has historically been a key component of animal care. In late 2021, our Ambassador Animal Programs team pivoted to become our Behavioral Husbandry (BH) team to support further advancement of behavioral husbandry for our animal collection at large. This led to a surge in our ability to make positive strides in existing training initiatives for much of our elasmobranch collection. Behavioral goals for these taxa were set collaboratively between the associated curatorial team and the veterinary and BH teams. The BH team then worked alongside aquarists to learn about each species' or individual's biology, ecology, medical and institutional history to develop realistic training plans toward these specific goals. At each animal training session, the BH team shared training theory, partnered in the act of training, built and shared mechanical skills, videoed sessions, kept records, and adjusted training plans as needed. BH's routine and active presence enabled us to conduct sessions otherwise impossible with current aquarist staffing, even keeping training sessions on track while maintaining consistency when aquarists were reprioritized elsewhere or short staffed. Given multiple recent successes with our elasmobranchs, this truly collaborative model could be useful at other facilities to advance training programs beyond diet management and feeding multispecies exhibits, as it addresses staffing/time, knowledge transfer, professional development, and of course and in effect, animal welfare.

Voluntary Cranial Flushes on a Porcupine Ray *(Urogymnus asperrimus)*

Amy Jo Li, Amy.j.esser@Disney.com
Epcot's The Seas with Nemo and Friends®, Walt Disney World Resort®

A female porcupine ray *(Urogymnus asperrimus)* at Disney's The Seas with Nemo and Friends presented with a raised area on her head due to bacterial infection. Treatment involved managing her in a 5.7-million-gallon environment with injections and oral medications. It became clear that more intensive treatment would be necessary. After transfer to a smaller environment, the veterinary team inserted a catheter into the area to allow daily antibiotic flushes. The husbandry team built on her previously established training of targeting and following a green acrylic square to assist in preforming the flushes. A table was designed to allow the ray to remain submerged while allowing the syringe to be removed from the water and a second trainer was introduced to allow flushes to be performed voluntarily during training. This helped to reduce the possible complications with repeated captures and anesthesia. After approximately 3 months of daily treatments, the catheter was removed. She was relocated back into her larger habitat and continues to thrive, thanks to the team's use of behavioral management.

What to do When Your GPO is Blue?
A Case Study of Protozoan Infections in *Enteroctopus dofleini.*

Katie Benya, kbenya@greensboroscience.org
Greensboro Science Center

Giant pacific octopus are a popular display choice among the cephalopod family as they are an iconic large species that are interactive towards guests and typically thrive in human care. Historically, these robust cephalopods received an observation quarantine period when brought into facilities. However, a recent individual at the Greensboro Science Center wasn't thriving as typical of the species, and upon investigation we discovered the coccidian, *Aggregata*.

Coccidia, particularly the genus *Aggregata*, is known to be one of the most widely distributed coccidian within the cephalopod family. This protozoan parasite not only infects the digestive tract of cephalopods bit also has a heteroxenous life cycle with its intermediate host being the gut of a crustacean. This lifecycle can pose a challenge when it comes to treatment as crustaceans are a staple in the diets of cephalopods and the infectious stage of *Aggregata* is passed through the fecal matter of cephalopods, releasing to the rest of the ocean or exhibit.

In early October 2021, the initial symptoms of our giant pacific octopus were loss of appetite and white coloration which progressed to denning up with no locomotion for several weeks. During this time, the individual had only been in our collection for 4 months and weighed 3kgs, ruling out early senescence. Over the course of 9 months, we were able to treat the *Aggregata* orally with several rounds of toltrazuril.

Saving the Night: Improving Animal Welfare by Decreasing Light Usage in an Aquarium

Leah Maurer, Kara Watts, Lauren Puishys, Wendi Fellner, Joseph Soltis
leah.m.maurer@disney.com
The Seas with Nemo and Friends, Walt Disney World Resorts

Disruption to a normal light/dark regime, specifically exposing animals to light at night, can have substantial influence on an animal's biological rhythm and behavior. Providing a period of darkness is important to ensure the best welfare, however, darkness can be unintentionally interrupted for animals housed indoors, especially for facilities that employ staff throughout the night. At Disney's The Seas, some unusual feeding behaviors of coral alerted aquarists to a potential environmental issue. Through careful investigation, it was discovered that lights were being turned on during the night unbeknownst to our animal care team. We then launched a light monitoring program in which light sensors were deployed to determine the extent to which lights were being turned on at night for cleaning and maintenance. All lights in the animal care areas were cataloged and mapped and then surveyed for usage after the facility closed to guests. These surveys and light sensors allowed us to discover how many lights were disrupting our animals' light/dark regime. Furthermore, a cost analysis of the lights in the animal areas was performed to understand the financial impact of keeping unnecessary lights off during the night. Conversations with our partners in the facility allowed us to implement changes to human behavior and improve electrical control accessibility, thus decreasing the disruptions to the light/dark regime. Ongoing monitoring of overnight lights in animal areas is now a permanent aspect of care in our facility.

Session 3: Animal Welfare/Wellbeing & Welfare/Wellbeing Assessment (continued)

Sponsor Presentation:
Piscine Energetics

Elasmobranch Ovariectomies for Management of Reproductive Disease

Charlene M. Burns, Charlene.M.Burns@Disney.com
Natalie Mylniczenko, Natalie.Mylniczenko@Disney.com
Disney's Animals, Science & Environment,

Aquariums often house female only elasmobranch groups to manage population size. The consequence of this is that some species develop reproductive disease, specifically, ovarian disease. Diagnosis is confirmed with ultrasound; associated bloodwork and coelomic fluid (Donnelly et al. 2019) can assist in determining severity. In advanced cases, treatment is difficult due to limited response to medical management. Surgical removal of the ovary (or ovaries) can be employed but most cases have a very large ovary which complicates a surgical procedure. Ovariectomies have been performed for permanent contraception in southern stingrays but have been advocated for specific developmental stages when the ovary is small and thus presents less risk (George et al. 2017) than those with advanced reproductive disease.

The removal of diseased ovary(-ies) involves a lengthy anesthesia and a sizeable incision. Tissues are fragile and tear easily, with moderate blood loss often occurring. Additionally, the epigonal is usually intimately associated, requiring complete removal or partial resection. Complications can include relative anemias, concurrent or resultant coelomitis, incomplete resection, and dehiscence of incisions.

Using balanced anesthesia and peri- and postoperative supportive care, outcomes can be markedly improved. A combination of immersion and injectable anesthesia, appropriate treatments (antiinflammation, analgesia and fluids) as well as delicate tissue handling diminishes complications and contributes to successful outcomes. Early diagnosis and intervention will reduce risk as well. Overall, these surgical procedures have resulted in the long-term health of the individuals and resolution of significant disease.

Cancer in Elasmobranchs: A Case Study

Dr. Brett De Poister, brett@theaquariumvet.com, +610430463314
Dr. Rob Jones, rob@theaquariumvet.com
The Aquarium Vet, Murchison, Victoria, Australia

It is a common misconception that elasmobranchs do not get cancer. Historically, there are limited reports documenting cancer in sharks and stingrays in the wild and in public aquariums. With increased veterinary investigation of elasmobranch health in the last decade there are more cases of cancer being documented.

An Australian Smooth Ray (*Bathytoshia brevicaudata,* previously *Dasyatis brevicaudata*) displayed in a public aquarium developed masses on the skin surface. Under anaesthesia, a biopsy was collected, and the histology resulted in a diagnosis of lymphosarcoma.

This ray was managed with prednisolone which resulted in regression of the masses for six months before euthanasia was required. Full details of the necropsy and histology will demonstrate how widespread the cancer had spread.

Unique Approaches in Resolving Buoyancy Issues in a Sand Tiger Shark

Josh Moline, joshua.moline@state.mn.us 952-431-9539
Christoph Noetzli, christoph.noetzli@state.mn.us
Minnesota Zoo, Apple Valley, MN

In 2018 the Minnesota Zoo received two juvenile female Sand Tiger Sharks (*Carcharius taurus)* from the Mystic Aquarium. One of these sharks was known to have a pre-existing buoyancy control issue. At the Minnesota Zoo, this shark (Osiris) struggled with buoyancy, often swimming in a 'spyhopping' position, and showing signs of breathing difficulty. Once this behavior was observed, the animal would be captured and placed in tonic immobility. Air would then be added to the stomach via an air cylinder and PVC pipe. While this procedure proved successful, it had to be repeated every few weeks and was stressful for both shark and staff.

As this process became more time consuming and labor intensive, different methods were explored to introduce and retain air in Osiris' stomach. Various treatment methods were attempted (including steroids, antibiotics, supplements, and even Alka Seltzer) over a two-year period with little success. After consulting with staff veterinarians and Dr. Rob Jones, a probiotic was recommended to boost the stomach's microbial load. In the end, the probiotic treatment proved successful. After ninety days of treatment, the time between manual inflation increased exponentially and currently stands at 15 months.

The correlation between the administration of probiotics and Osiris' improved condition shows that this could be a worthwhile approach for future buoyancy conditions in Sand Tiger sharks.

Session 4: Husbandry Techniques 1

Sponsor Presentation:
Dynasty Marine Associates, Inc.

Voluntary Tonic Immobility with the Bowmouth Guitarfish, *Rhina anclyostoma*

Tim MacKay, TMackay@georgiaaquarium.org
Georgia Aquarium

The Ocean Voyager habitat at the Georgia Aquarium is home to 1.2.0 critically endangered bowmouth guitarfish, *Rhina anclyostoma*. Over the course of several years, the Ocean Voyager team successfully station trained this species to offer a complete nutritional profile and visually monitor their physical health. As the animals matured, the team elevated their animal training program to better meet the needs of our female population and their offspring.

Utilizing the previous station training, the team approximated the animal to an inverted position to achieve a tonic immobility behavior for voluntary ventral presentation allowing for advanced diagnostics including ultrasound and morphometrics. With the introduction of a platform, aquarists were able to enhance control of the behavior and increase accessibility to the animal. Collaborative efforts with the Georgia Aquarium Veterinary team have allowed us to further our diagnostic efforts in parturition planning, as well as elevate our daily husbandry care of these individuals. This training continues as their husbandry needs evolve, furthering our support to this critically endangered species.

Stretcher Training of a 0.7 Southern Stingray (*Hypanus americanus*) Population at St. Louis Aquarium at Union Station for Improved Animal Husbandry

Hannah V. Roethemeyer, Abigale L. Stricker, Erin E. Harms
St. Louis Aquarium at Union Station, https://www.stlouisaquarium.com/

Training is an important tool used to improve animal husbandry under human care. Training of voluntary behaviors allows animal care professionals to better meet an animal's needs and reduce stress during husbandry activities. 0.7 southern stingrays (*Hypanus americanus*) residing within the Shark Canyon habitat at the St. Louis Aquarium at Union Station were trained using operant conditioning techniques to enter a stretcher voluntarily. The goal was to improve the care of these animals by encouraging voluntary participation in husbandries, such as feeds and veterinary procedures. A precursor to this training was that this group of rays recognized a specific area of the habitat as their feeding station. The rays were first trained to enter a custom-built feeding 'basket' one at a time at this station. Once each ray consistently entered the basket without assistance (or with minimal guidance), the stretcher and stretcher cover were slowly introduced for desensitization. Over six months, this training resulted in the rays gaining the ability to station and feed within the basket, as well as being able to station in the stretcher and then be removed from the enclosure for veterinary checkups and other animal care needs.

Eradicating *Acropora* Red Bugs
Using a Novel Application of the Ingredient Milbemycin Oxime

Emily Calhoun, Emily.c@sdaquarium.org
Shannon Kendrick, Shannon.k@sdaquarium.org
Butterfly House & Aquarium

Red bugs (*Tegastes acroporanus*) infest many species of *Acropora* coral. These bugs eat the mucosal layer of corals, which suppresses their immune function leading to inhibited growth and discoloration of tissue. In an aquarium setting, especially closed systems, an infestation of red bugs can decimate a coral population if not appropriately managed.

The most common regimen has historically been administering various flea treatments for dogs and cats with the active ingredient Milbemycin Oxime (M.O.). In our research, we tested a novel treatment of M.O. in a propylene glycol solution, with the intention of testing it against the efficiency and cost of other, more traditional treatments. Our consulting vet worked directly with a compounding pharmacy in creating a liquid milbemycin oxime solution that was administered directly to the system water. We started with a low dose of 0.157% M.O. administered twice weekly for 8 weeks, and with subsequent treatments increased the amount and concentration of M.O. By the fourth round of treatment, with a 0.5% concentration of M.O, we saw a complete eradication of red bugs within 4 weeks on one species of *Acropora.*

Our results showed that our novel solution was successful in eradicating red bugs from *A. yongei* frags in 4 weeks, and other *Acropora* species in 8 weeks. Further research is needed, but with our data, we conclude that this method of treatment has the potential to be an affordable and viable option for treating corals infested with red bugs.

Husbandry of Lesser Devil Rays *(Mobula hypostoma)*
at the St. Louis Aquarium at Union Station

Abigale L. Stricker, and Hannah V. Roethemeyer
St. Louis Aquarium at Union Station, https://www.stlouisaquarium.com/

Lesser devil rays (*Mobula hypostoma*) are not often in managed care due to the specialization required to keep them healthy and thriving. Identifying a method that efficiently delivers enough food to the filter feeder as they grow to adulthood is a challenge in a large, multi-species exhibit. 1.3 lesser devil rays have been under care at the St. Louis Aquarium at Union Station (SLAUS) since December of 2019. Since obtaining and following guidelines and suggested protocol from Dynasty Marine, we have transitioned from a bottle feed to broadcast feed that is more conducive with our exhibit design.

Several measures have been taken to ensure proper health for the rays. This includes disinfecting food containers between feeds, creating jump guards for the edge of the habitat, and creating and using beam covers for the back of house catwalk over the habitat. Veterinary exams are completed every three to six months to track the growth and health of each animal. While there is always more to learn, the protocols we have implemented thus far have resulted in successful care of our four lesser devil rays over the last 3 years.

Sponsor Presentation:
Abyzz

Session 5: Freshwater Mussels

Sponsor Presentation:
Advanced Aquarium Technologies

Freshwater Mussel Propagation for Conservation

Megan Bradley, megan_bradley@fws.gov
Genoa National Fish Hatchery

Freshwater Mussel Propagation for Conservation- Genoa National Fish Hatchery (GNFH) has been involved in the culture of freshwater mussels in the Upper Mississippi River Basin since efforts began in 2000. The program has expanded from working with a single species, using a single technique, to working with as many as 18 species from various river basins, and the techniques used have evolved and been made to suit the culture location or species-specific needs. From head-starting freshwater mussels to culturing them with our partners, to reintroduction and augmentation to prevent listing, or to move towards recovery, GNFH works with a diverse suite of organisms. In addition to the 18 species of freshwater mussels, the hatchery rears an endangered dragonfly, and around 17 species of fish.

Support Role of Zoos and Aquariums in Ex-Situ Head-Starting of Native Mussel Propagation for Restoration

Ben Minerich, ben.minerich@state.mn.us
Minnesota Zoo

Support role of Zoos and Aquariums in ex-situ head-starting of native mussel propagation for restoration: rearing, research, and reach. How the MN Zoo leverages its aquatic system design and maintenance, research methodology, and public engagement expertise to enhance unionid propagation efforts and improve public awareness and action in conservation science.

Population Genetics/Genomics Techniques Assessing Paternity and Parentage of Propagated Mussels

Dr. Kentaro Inoue, kinoue@sheddaquarium.org
John G. Shedd Aquarium

Captive propagation followed by release to natural habitats has become a common conservation practice for not only rare and endangered species to guard against the extinction but also various conservation and restoration projects. While propagation became an essential method for freshwater mussel conservation, the genetic impacts of the efforts are largely undocumented. Using population genetics/genomics techniques, we are now able to assess paternity and parentage of propagated juveniles and estimate the genetic diversity relative to the wild populations. Such information is necessary for the success of captive propagation followed by release to the natural habitats. Here, I use a case study of two common mussel species (*Lampsilis cardium* and *L. siliquoidea*) conducted in the Chicagoland area and discuss high rates of multiple paternity in a single female and potential alteration of genetic structure to the wild populations. I then discuss future research projects to investigate the population genomic structure of endangered mussel species that are propagated from the Genoa National Fish Hatchery and develop a guideline for genetic management.

The Iowa Mussel Blitz and Higgins Eye Pearly Mussel Recovery Efforts on Three Iowa Rivers

Scott Gritters, scott.gritters@dnr.iowa.gov
Iowa Department of Natural Resources

Since 2005, the Iowa Department of Natural Resources, U.S. Fish and Wildlife Service, and the U.S. Army Corps of Engineers have been researching the disappearance of native fresh-water mussels in Iowa. This includes searching for the federally endangered Higgins eye pearly mussel. Once ranging across most of the upper Midwest, this species has been eliminated from many of the rivers it once thrived. Efforts were made to restock the endangered mussels in three Iowa rivers, the Cedar, Iowa and Wapsipinicon. This effort utilized fish that were inoculated with glochidia at the Genoa National Fish Hatchery and stocked into these river sections. Blitz searches are coordinated by the Iowa DNR, but include many other conservation agencies, organizations and concerned citizens. The overall effort has been staggering with at least 28 river sections being surveyed with efforts branching out away from the original stocked areas at times. Blitzes have accounted for over 2,100 hours in the water and over 45,000 mussels of 36 species in various searches. The average Catch per hour is around 21 mussels. The highest collection rate recorded was in 2021 on the Winnebago River and the lowest collection rate was in 2022 on Lytles Creek. So far, a total of 85 of the stocked Higgins eye pearly mussel have been found in the Iowa and Wapsipinicon Rivers. A natural reproducing population of Higgins eyes was discovered in the Wapsipinicon River in 2015. This talk focuses on the Blitz effort and what potentially makes this endeavor so successful.

An Update on the Growth of tagged *Lampsilis siliquoidea* (Bivalvia: Unionidae) in Crooked Lake (Whitley County, Indiana).

Warren W. Pryor, wpryor@sf.edu
University of Saint Francis, Fort Wayne, IN

I reported at RAW 2019 that the growth rate of adult Fat Mucket mussels in Crooked Lake was about 0.88 mm/year. Subsequent data acquisition and analysis builds upon that foundation. Results from my examination of records from 240 tagged individuals since 2011 have allowed the calculation of von Bertalanffy growth function (VBGF) parameters, that help to describe the important role these mussels play in moving carbon from the plankton to the benthos. In addition, the VBGF parameters for this wild population provide a baseline for anyone caring for unionids in captivity. More broadly, the methods I will describe are applicable by aquarists to many other taxa – for instance, sharks, crabs and sea stars – to better understand their growth.

Sponsor Presentation:
Species 360

Water Quality Diagnostics Initiative Overview

Brian Nelson, BNelson@aqua.org
National Aquarium

Starting in 2018 the AZA began a collaborative coral rescue operation to preserve the genetic diversity of the Florida Reef Tract in the face of Stony Coral Tissue Loss Disease. We based our starting assumptions about water quality on the history of coral keeping influenced by the unique water quality of the waters of the Florida Keys. What we discovered is that these corals wanted something different. I'll be discussing a little history and some process along with what we found.

Coral Collections in Turbulent Times – How Wild Harvest Contributes to Conservation

Lyle V. Squire, Lyle.jnr@cairnsmarine.com
Cairns Marine, Queensland, Australia

Concerns over the health and resilience of reefs around the world has led to intense scrutiny of the harvest of corals from the Great Barrier Reef. Additionally, pressure from the governments of the UK and EU and the CITES Scientific Committee has influenced how Australia responds to satisfying non-detriment findings and to continuing the export of wild collected corals.

Dramatic changes have been made to the management of the coral fishery on the GBR, from a reduction of quota by over 70% for some species to the increased level of reporting of all coral catch.

Although wild harvest of corals is controversial and would not appear to align with reef protection efforts, collectors and fishery managers are working together to assess, develop and implement coral conservation and reef restoration on the GBR.

SAFE Under the Waves:
Collaborative Conservation for Aza's Aquatic Programs

Katey Leban[1], kleban@aza.org
Mitch Carl[2], mitchc@omahazoo.com
[1] Association of Zoos and Aquariums
[2]Omaha's Henry Doorly Zoo and Aquarium

AZA SAFE: Saving Animals From Extinction is a framework with a simple purpose: to help AZA members do more and better- meaning more impactful- conservation, and increase brand recognition among the public, scientific and nonprofit peers, and government agencies about the contributions of AZA members to conservation. By working together, AZA-accredited zoos and aquariums tap into the unique resources and expertise of the larger community; staff from animal care, education, communications, and more, and from facilities large and small, can contribute to the goals of SAFE species programs. AZA believes that as we do more conservation, we reaffirm our staff's personal commitment to this work, which may inspire even more conservation- creating a widespread conservation culture that more equitably integrates people into conservation solutions so that people and wildlife thrive together. Aquatic ecosystems experience both localized and wide-ranging threats. Achieving and sustaining success over the long-term, whether for a single species or wide-ranging taxa, requires more than species experts or a single facility. The five current aquatic-based SAFE programs acknowledge that challenge, and work together across the larger seascape, all while advancing their own relevant recovery plans. As we work toward shared goals, engage more of our collective resources in the efforts, and bring along people and communities under the SAFE framework, we're already seeing our community do more, and more impactful, conservation. We welcome more programs, organizations, and expertise to join.

The Journey to Today and Our Compass Heading to the Future

Beth Firchau, bfirchau@aza.org
Association of Zoos and Aquariums - Florida Reef Tract Rescue Project

In 2018, in response to the devastating effects of stony coral tissue loss disease (SCTLD) on Florida reefs, the State of Florida mounted an unprecedented effort to collect and archive the genetic identity of Florida coral species most susceptible to the disease. To that end, removal or rescue of sexually mature coral colonies from the reef began in earnest. The Florida Wildlife Conservation Commission (FWC) identified the Association of Zoos and Aquariums (AZA) and its over 230 accredited members as, "the only United States entity with the expertise, resources, and professionalism to take on this significant challenge…". Within months of AZA accepting FWC's invitation, Florida corals rescued ahead of the disease progression were on their way to aquariums in Florida and across the country. The AZA Florida Reef Tract Rescue Project was born.

Since 2018, a dynamic rescue coral care network of 25 facilities across the country has been forged. Even more significant than the collaboration creating this first-of-its kind network, is the wealth of information being gathered and shared about rescue coral species, the impressive advancement of coral science because of this work, and the groundbreaking husbandry technology and techniques being created that are revolutionizing the husbandry and welfare of corals in human care. These foundations have resulted from collaboration, dedication and the tenacity of the AZA community. They are the foundations of today's course setting to seek a hopeful destination of recovery for Florida's Coral Reef in the future.

Saving Slimy Rocks

Rescues/Broodstock Management/Genebanking: Flower Gardens Bank Sanctuary

Greg Whittaker, gwhittaker@moodygardens.org

Moody Gardens

Abstract not provided. Summary provided by the D&C Editor.

In August 2022 possible signs of Stony Coral Tissue Loss Disease (SCTLD) appeared on corals in the Texas Flower Garden Banks National Marine Sanctuary. This talk describes the initial quick assessment, treatment of diseased colonies, and collection of healthy specimens over four trips ending in March 2023. Disease progression was less intense in the Flower Gardens corals and may represent an outbreak of White Plague, rather than SCTLD. Moody Gardens has converted their FRTRP facility to a holding/display area for Flower Gardens corals. Florida corals were returned to holdings in that state.

Session 7: Land-based Nurseries and Sexual Propagation

Sponsor Presentation:
The Aquarium Vet

Development and Future Directions of Land Based Broadcast Spawning for Reef Restoration and Research

(Pre-recorded Presentation)

Dr J Craggs[1&2], jrkcraggs@gmail.com

V Thomas [2&3], vincent@aquarium-connections.co.uk

Prof M. Sweet [2&4], m.sweet@derby.ac.uk

[1] Horniman Museum and Gardens; [2] Coral Spawning Lab, coralspawninglab@gmail.org; [3] Aquarium Connections; and [4] University of Derby

Anthropogenic impacts such as climate change, pollution and disease are destroying coral reefs, resulting in devastating effects on the ecosystem services they provide, as well as the livelihoods of nearly a billion people who depend on them. With the intensity of these anthropogenic stressors predicted to increase, reef restoration approaches, aimed at increasing the resilience of corals, are crucial.

Given these mounting pressures, in 2012 we pioneered an innovative research program developing techniques to predictably induce broadcast coral spawning events in land-based bespoke systems. We designed these systems to accurately replicate the environmental cues (seasonal temperature, photoperiod, lunar cycles) that trigger natural spawning events. To date we have spawned over 40 species, with resulting research focused in three areas: 1) understanding reproduction at a fundamental level, 2) developing methods to enhance post-settlement survival and, 3) developed phase-shifted spawning at various temporal scales to produce multiple reproductive events within a single year, therefore increasing access of gamete material for experimentation.

Following eight years of R&D we founded the Coral Spawning Lab (www.coralspawninglab.org) to supply systems to enhance the capability and number of institutions that can work with land-based broadcast coral spawning for research and reef restoration. Since its inception in 2019 we have supplied >60 spawning and embryo rearing/settlement systems to 15 locations in 10 countries. Our innovation continues, with new upscaling grow-out systems to enhance post-settlement survival and growth so that the production pipeline of corals for restoration can be increased. This talk will cover the decades' work and provide insights into the future of land-based coral spawning.

Mote Marine Laboratory and Aquarium's Strategy to Preserve and Restore Florida's Coral Reefs

Erinn Muller, emuller@mote.org
Mote Marine Laboratory

Abstract not provided. Summary provided by the D&C Editor.

Describes the extensive network of coral nursery locations. This includes 3 land-based operations and 4 in-water nurseries that focus on Acropora spp. 25% of space is dedicated to assisted gene flow. Genets are being tested for tolerance of environmental extremes and production is adjusted accordingly. Currently installing 50,000 outplants per year and that capacity is projected to double in 2 years. Eastern Dry Rocks off Key Largo is a particularly successful outplant site

Spawning and Rearing Rescued Corals at The Florida Aquarium

Keri L. O'Neil, koneil@flaquarium.org, Rachel Morgan, Emily Williams, Jenny Lee, Jessica Sandelli, Paula Holmes, and Samantha Tanguay
The Florida Aquarium Center for Conservation, Apollo Beach, FL

As coral reefs continue to decline across the globe, an increased interest in ex situ gene banking and propagation has developed. In Florida, an ongoing disease outbreak has greatly reduced population densities of disease susceptible species, potentially limiting sexual reproduction and natural recovery. Large distances between surviving colonies also make in situ spawn collection from diverse parents difficult. In response to the ongoing outbreak, a variety of genetic rescue projects have been implemented to bring disease susceptible species into ex situ culture and preserve living genetic diversity. At The Florida Aquarium, corals being held in a long-term ex situ genetic bank were maintained using artificial lighting and temperature cues programmed to mimic natural cycles in Key Largo, FL. Some individuals were also held in greenhouses exposed to natural lighting with less precisely controlled temperature cycles. Synchronized broadcast spawning events were documented in aquaria over four annual spawning cycles from 2019 - 2022. Repeatable and predictable ex situ spawning events such as these are an essential tool for managed breeding and assisted fertilization in species suffering from severe population declines. These predictable and well-synchronized ex situ spawning events show that long-term living genetic banks can be used to preserve coral genotypic diversity in the face of severe wild declines and small ex situ populations can be used to produce thousands of offspring annually for future restoration activities.

Developing a Propagation Strategy for Restoring Florida's Coral Reef Using Rescued Corals from Stony Coral Tissue Loss Disease

Morgan Eason[1*], Tanya Ramseyer[2], Stephanie A. Schopmeyer[2], Rob Ruzicka[2], Beth Firchau[3], Jennifer Moore[4], Lisa Gregg[5], Keri O'Neil[6], Maurizio Martinelli[1]
[1] Florida Sea Grant, Morgan.Eason@ufl.edu, mmartinelli1@ufl.edu
[2] Florida Fish and Wildlife Research Institute, Tanya.Ramseyer@MyFWC.com, Stephanie.Schopmeyer@MyFWC.com, Richard.Ruzicka@MyFWC.com
[3] Association of Zoos and Aquariums, bfirchau@AZA.org
[4] NOAA Fisheries Southeast Regional Office, jennifer.moore@noaa.gov
[5] Florida Fish and Wildlife Conservation Commission Division of Marine Fisheries Management, Lisa.Gregg@MyFWC.com
[6] Florida Aquarium Center for Conservation, koneil@flaquarium.org
*Primary Author: FWC Fish and Wildlife Research Institute, St. Petersburg, FL
Morgan.Eason@ufl.edu, (352) 810- 6125

Florida's Coral Reef (FCR) is experiencing an unprecedented disease outbreak described as stony coral tissue loss disease (SCTLD). First reported near Miami in 2014, SCTLD has since spread throughout the entire reef tract including the Dry Tortugas resulting in the mortality of millions of colonies from >20 coral species, including primary reef builders and species listed under the Endangered Species Act. A multi-agency Coral Rescue Team (CRT) was developed to 1) design and implement a reef tract-wide coral collection plan for SCTLD-susceptible species, 2) preserve genetic diversity of Rescue corals in land-based holding, and 3) plan for future propagation, restoration, and reintroduction of Rescue corals to the wild. Since 2018, the CRT has collected over 2500 corals across 20 different species which are being held at 25 long-term housing facilities, labs, and organizations across 13 US states including many Association of Zoos and Aquariums (AZA) accredited facilities for care. Propagation of these corals is now the main focus in these land-based facilities to create sexual offspring which will eventually be outplanted in hopes of restoring the coral populations of FCR. The "Propagation Strategy" was established to take the Rescue Project from holding broodstock through the steps of propagation, including spawning, assisted fertilization, settlement, and growout in preparation for outplanting and restoration. The Propagation Strategy encompasses four primary components, (1) the genetic management and breeding plan, (2) in-water infrastructure plan, (3) land-based infrastructure plan, and (4) transfer plan. Initial planning efforts are focused on the land-based infrastructure plan with the goal of increasing capacity at current facilities and bringing new facilities into the program and preparing them for propagation. The genetic management and breeding plan will determine which corals are spawned and outplanted to create the highest potential genetic diversity, while the in-water infrastructure plan will determine the need for in-water nurseries to hold and acclimate corals to prepare them for introduction to the wild. Current wild populations, genetic needs for restoration, and production abilities determine which corals must be prioritized, the location of outplanting, and the number of corals to be outplanted. The development of the "Propagation Strategy" is essential for scaling up activities and maximizing current and potential resources necessary for restoring corals lost to SCTLD.

Sponsor Presentation:
Vitalis Aquatic Nutrition

Session 8: Conservation 1

Sponsor Presentation:
Aquatic Equipment and Design
with
Minorities In Aquarium and Zoo Science (MIAZS)

Stegastoma tigrinum **Augmentation and Recovery (StAR) Project**

Leah Neal[1], lneal@georgiaaquarium.org
Tim Carpenter[2], t.carpenter@seattleaquarium.org
Lise Watson[3], lwatson@sheddaquarium.org
[1] Georgia Aquarium, [2] Seattle Aquarium, [3] Shedd Aquarium

Stegastoma tigrinum (Zebra shark) populations have undergone dramatic declines over the past 30 years, due to habitat degradation and targeted fishing for their fins throughout their range in the Indo-West Pacific. In 2016, they were classified as Endangered by the IUCN Red List. Paradoxically, zebra sharks (also known as e.g., Indo-Pacific leopard sharks) thrive and reproduce readily in public aquariums. The Raja Ampat archipelago contains an extensive network of well-enforced marine protected areas (MPAs) and a shark and ray sanctuary, established in 2012. However, zebra sharks remain functionally extinct in the region, in contrast to other elasmobranchs that are showing signs of recovery. This world-first conservation initiative leverages the expertise of the aquarium sector, conservation NGOs, and local communities to reinforce zebra shark populations throughout their historical range within Raja Ampat, Indonesia. Egg cases produced by aquariums have been shipped to two hatcheries in Raja Ampat. Indonesian husbandry staff, trained by aquarium staff, are rearing zebra sharks in tanks and sea pens until they reach 70 cm (~1 kg). Sharks have been tagged and released within designated MPAs and monitored for post-release survival and behavior. This project seeks to re-establish a self-sustaining, genetically diverse breeding population of zebra sharks in the Raja Ampat Archipelago, Indonesia. The husbandry working group worked to establish training manuals, design and commission life support systems, and train Indonesian biologists to be aquarists.

SAFE Sharks and Rays in Action!

Beth Firchau[1], bfirchau@AZA.org
Kelli Cadenas[2], kelli.cadenas@merlinentertianments.biz
[1] AZA, [2] SEA LIFE Orlando

AZA Zoos and Aquariums can make a significant contribution to the recovery of global ocean health through the SAFE: (Saving Animals From Extinction) Sharks & Rays program. By coordinating action across zoos, aquariums and a global network of partners, we can influence positive change for sharks and rays. Through exploration of the program's online action hub, details of the program's resources and project initiatives to include but not be limited to details of the shark handling project, blood database project, international elasmobranch census, and sustainable feeds project, will be shared. The presentation will culminate in how to get involved and make the world's oceans safe for sharks and rays.

Three New Nautiluses! Conservation Planning for the Future

Gregory J. Barord[1,2]
[1] Central Campus, Department of Marine Sciences, Gregory.barord@dmschools.org
[2] Save the Nautilus, Gregory.barord@dmschools.org

Three new species of nautiluses were recently described that had been lumped under the catch all species, *Nautilus pompilius*. These new species are distinct from other populations of nautiluses based upon their morphological and phylogenetic data. The new species designations are *Nautilus vitiensis* (Fiji), *Nautilus vanuatuensis* (Vanuatu), and *Nautilus samoaensis* (American Samoa). These new descriptions of nautiluses provide insight into the overall evolutionary relationship between extinct and extant nautiluses. However, what do these new species descriptions mean to current and future conservation efforts of nautiluses? Do they hinder or support previous management plans? Is there a role for zoos and aquariums to promote species conservation efforts for organisms not in their current collection? These questions are all important to address, not only for nautiluses, but for other species as well. As technology and our understanding of the natural world improves, are we setup to answer these questions, quickly and efficiently?

Practical *Pycnopodia* Propagation Pointers

Ashley Kidd[1], ashley@sunflowerstarlab.org
Ben Morrow[2], ben.morrow@omahazoo.com
Jen Burney[3], jburney@aop.org
Fiona Curliss[4], fcurliss@uw.edu
Kylie Lev[5], klev@calacademy.org
[1] Sunflower Star Laboratory, [2] Omaha's Henry Doorly Zoo, [3] Aquarium of the Pacific, [4] Friday Harbor Laboratory, [5] California Academy of Sciences,

The sunflower star, *Pycnopodia helianthoides,* is a keystone predator of sub-tidal kelp and rocky reef ecosystems from Alaska, USA to Baja, Mexico. Due to the sea star wasting disease that decimated the population throughout their entire range, they are listed as critically endangered by the IUCN and are under review for federal protection under the ESA (USA) and COSEWIC (Canada). The sunflower sea star roadmap to recovery, published in November 2022, outlines seven key objectives to restore the species. Using this roadmap, public aquariums & zoos, academic labs and conservation nonprofits have partnered around sunflower sea star recovery through expanding echinoderm aquaculture, spawning sunflower sea stars at aquarium facilities, and diversifying sunflower sea star larval rearing efforts. Here we summarize the roadblocks and breakthroughs from this initial collaborative, concerted effort.

RAW Business Meeting

- Paul Clarkson (Monterey Bay Aquarium) has agreed to chair the RAW Advisory Committee.
- The National Aquarium (Baltimore, MD) was chosen as the host venue for RAW 2025.
- Chris Spaulding (Point Defiance Zoo and Aquarium, Tacoma, WA) discussed plans for RAW 2024 (aka Tacom-RAW). May 5-10 are the expected dates.

Session 9: Conservation 2

Sponsor Presentation:
Gulf Specimen Marine Laboratories

Training Giant Manta Rays, *Mobula birostris,* for Collaborative Research and Conservation Efforts

Alyse Stitt, astitt@georgiaaquarium.org
Candice Latini, clatini@georgiaaquarium.org
Georgia Aquarium

Over the past decade, the Ocean Voyager team has advanced the training of our population of Giant Manta Rays, *Mobula birostris.* This presentation addresses the use of training techniques to combine initiatives between the Zoological and Research and Conservation departments at Georgia Aquarium.

By taking advantage of a positive reinforcing scenario with divers, the team was able to introduce tactile to the ventrum of our resident male individual, "Blue" to stimulate calm behavior while divers successfully deployed a satellite tagging device via active suction. These novel tagging techniques will be further developed for use in the field on their counter parts in the ocean. Future plans include further advancement of this technique to encourage the rays to participate in their own healthcare, including blood collection.

Eye in the Sky May Reveal More About the Elusive Ornate Eagle Ray

Lyle Squire, Lyle.jnr@cairnsmarine.com
Cairns Marine

Listed as Endangered in most of its range, the ornate eagle ray, *Aetomylaeus vespertilio*, is an enigma. Few people have had the fortune of viewing this species in the wild, even though they grow to the size of a manta ray.

Little is known of their natural history and, outside of Australia, their populations are in steep decline. The primary pressure for ornate eagle rays is overfishing; they are kept as by-catch for meat and cartilage; caught by unmanaged demersal fishing which prevails in many countries.

New evidence suggest that this incredible species is not rare in Australia, where fishery management is strong. However, they live in the turbid, low visibility waters near shore, on the reef shelf while young and will dwell at depths of up to 100 metres as adults, so they are rarely seen.

Use of drones is being used for research and assessments of several aquatic species but there is a lack of use of this technology in remote, difficult to survey locations. Additionally, little to no work is being done with *A. vespertilio* in situ.

Experience with this species ex situ is also limited. To date, only a few specimens have ever been collected and housed long term, with only one ever being displayed in a public aquarium.

Public aquaria could be key to expanding understanding of ornate eagle rays, through display, husbandry and potential conservation and breeding programs; use of drones is being implemented to enable sustainable collection of this incredible species in Australia.

Sea-Ing Is Believing: Exploring Seadragon Reproductive Events with Data Visualization Tools

Nancy Kim Pham[1], Nancy.PhamHo@sezarc.com
Leslee Matsushige[2], LMatsushige@ucsd.edu
Paula Carlson[3], Paula@dwazoo.com
Linda Penfold [1], Linda.Penfold@sezarc.com
[1] South-East Zoo Alliance for Reproduction & Conservation, [2] Birch Aquarium at Scripps Institution of Oceanography, [3] Dallas World Aquarium,

The common seadragon (*Phyllopteryx taeniolatus*), also popularly known as the weedy seadragon (WSD), has a long but sporadic history of breeding events in aquaria. One of the earliest documented successful hatchings of WSDs was recorded 30 years ago. Since then, at least twelve institutions have successfully brooded and hatched WSDs. These successes are archived across various platforms, such as peer-reviewed articles, conference presentations, husbandry manuals, public forums, and personal communications records. To increase our industry knowledge, retention, and the transfer of information about reproductive successes in WSDs, we have compiled these historical records into a platform designed to handle complex datasets and generate interactive visual aids.

This standardized database has allowed us to take a closer look into parameters that are anecdotally believed to be important but have yet to be systematically examined. These parameters include temperature, photoperiod, and lunar cycles, as well as physical habitat characteristics, and social group composition that tie back to the dates of the successful egg transfer event. To establish how this database can be updated as new information is obtained, we have piloted it with recent egg transfer events to identify pinch points in data mining and data entry. The goal of this first phase is to strengthen the current database we have collaboratively established and to recruit stakeholders to join, contribute, and aid in phase two. The aim of phase two will focus on conducting hypothesis driven research to further our understanding of the effects of environmental and habitat conditions in WSD reproduction.

Raising Rip: From Conception to a Year of Age for a Sand Tiger Shark (*Carcharius taurus*).

Stacia M. White[1], Robert George[1], J. Wyffels [1,2], S. Anderson[1], C. Buckner[2], F. Latson[3], V. LaPage[4], T. Handsel[1], F. Bulman[2], J. Durrett[2]
[1] Ripley's Aquarium of Myrtle Beach, swhite@ripleys.com
[2] Ripley's Aquarium of the Smokies,
[3] Ripley's Aquarium Marine Science Center, Cheektowaga, NY, [4] Ripley's Aquarium of Canada

Sand tiger sharks (STS), *Carcharias taurus,* are a popular and one of the more common large sharks displayed in public aquariums. They often live for 10-15 years in managed care situations, with survival times reported well over 20 years. During a relatively long life on display female STSs go through many reproductive cycles but rarely become pregnant. Although there have been several successful pregnancies for sharks maintained in flow through systems around the world, there are no reports of a successful conception and live birth having occurred in the U.S. in either an open or closed system.

Starting in 2015 Ripley's Aquariums initiated an ongoing effort to induce pregnancy in its STS collection through the use of artificial insemination (AI). Initial AI procedures were completed with cycling females maintained at Ripley's Aquarium of the Smokies in Gatlinburg, TN, as well as a holding facility in Buffalo, NY. During 2020, the AI research expanded to include STSs maintained in a newly constructed 214,000-gallon tank in Myrtle Beach, SC, to support STS reproduction. Semen was collected from males in the system or from SC coastal catch and release *in situ* males. Inseminated females were passively monitored for changes in size and shape. Ten months post-insemination, the females were examined using ultrasonography to determine pregnancy status. A STS inseminated on May 5, 2021 was determined to be gravid with one young developing in the right uterus. On March 1, 2022, the gravid female was sequestered in a nursery area in preparation for parturition. Reports in the literature estimate a gestation period of 280-290 days for STSs. When the gestation period reached 303 days, the female was evaluated ultrasonographically and the young was confirmed live and determined to be pre-term based on length and gut contents. The female and young were re-evaluated after approximately 3 weeks and the neonate was manually delivered on March 24, 2022. The female was immediately returned to the main population and the neonate remained in the nursery area. The total gestation time was 322 days.

After a brief period alone in the nursery pen the neonate, nicknamed "Rip", was placed in a 14,000-gallon holding tank with a variety of small sharks and rays. In addition to morphometrics obtained at birth, Rip was weighed and measured monthly and his diet adjusted based on weight gain and loss. The nutritional goal was to balance growth rate with body condition. At birth Rip was 93 cm total length and 7.9 kg, at 8 months he was 139 cm total length and 17.2 kg.

Acknowledgements: The authors wish to thank the husbandry staff of Ripley's Aquariums for their efforts and dedication on behalf of this project and elasmobranch conservation.

Thursday, May 11th

Session 10: Morning Buzz (shorter presentations)

Sponsor Presentation:
Asahi America

Buffing Your Way to a Better pH

Charlene M. Burns, Charlene.M.Burns@Disney.com
Nick Andrew, Nickolas.J.Andrew@Disney.com
Karen J. Echeverria, Karen.J.Echeverria@Disney.com
Disney's Animals, Science & Environment

Tricaine methanesulfonate (MS-222) is the most widely used anesthetic for fish and the only one FDA approved. While Tricaine is highly desirable due to its solubility in water, it does create an acidic aqueous setting in both fresh and saltwater environments. For this reason, a buffer is recommended to increase the pH to be consistent with an animal's environment. Due to availability, aquariums have traditionally used sodium bicarbonate (baking soda) to aid in the correction of water pH. But is there a better choice?

Sodium bicarbonate has been the preferred choice for buffering tricaine since its development. This buffer is used at a 2:1 ratio with tricaine to increase the pH of the anesthetic water but it often has its limitations. Due to its chemical formulation, bicarbonate can only buffer to a certain point and may not reach the original environmental pH.

Utilizing sodium carbonate instead of bicarbonate can help eliminate these adverse reactions. Sodium carbonate, a stronger base than bicarbonate, can more adequately absorb the acidity generated by the tricaine solution. The further you get from the original pH the harder it is physiologically for the animal to manage homeostasis and it's a stressor. In addition, sodium carbonate has less overall carbon dioxide contribution to the anesthetic environment, which contributes to blood gas and respiratory stability of the patient. Due to these factors, sodium carbonate is a recommended alternative to sodium bicarbonate for tricaine anesthesia buffering.

ACSC Larval Programs Production and Distribution Program

Barbara Bailey[1], bbailey@neaq.org
Kylie Lev[2], klev@calacademy.org
Rachel Zak[3], rzak@sheddaquarium.org
[1] New England Aquarium, [2]California Academy of Sciences, Steinhart Aquarium,
[3]Shedd Aquarium,

Developed through the AZA's Aquatic Collections Sustainability Committee (ACSC), the Larval Programs Production and Distribution program aims to support a collective effort across AZA Aquariums for sustainable and ethical collections. This program partners AZA institutions consistently rearing tropical marine fish with other AZA members to share costs and make sustainable aquacultured fish more accessible. The 2022 pilot program focused on production of five species distributed across 22 institutions. Production institutions have a designated species coordinator who are responsible for raising and shipping fish to participating institutions. These coordinators collect valuable rearing and shipping data that help develop best practices for this program moving forward.

Each year as this cooperative program continues to grow through membership interest and contributions, the ACSC sustainability fund also grows, supporting ACSC wide initiatives including workshops and training, welfare and longevity studies, R&D on new species and existing species, starting and expanding larval programs at member institutions, and more. By producing fish at AZA facilities, there is greater access to aquacultured fish that are important to our members for sustainable exhibit collections, with the added benefit of conditioning, biosecurity, known provenance, and potential welfare concerns related to handling and quarantine.

Case Studies in Marine Animals Handling, with an Emphasis on Specimens Under Human Care

Clem Kouijzer, clemaureen@gmail.com
1-2-1 Animal Handling Products Ltd, United Kingdom

Handling of animals under human care requires specialized equipment and a professional attitude. Some species living in zoological facilities around the world may pose life threatening abilities to their human care takers, and the methods used to handle them must take into consideration their life history, biology and behavior, thus ensuring neither party - animal and/or human - are harmed in any way. Handling is a necessity, during veterinarian procedures, during collection from the wild, during moves between exhibits, or even during inter-institutional transports, such as breeding loans.

I present to you a case study of large, marine animal handling, with an emphasis on reducing collection and movement induced stress with the use of industry led, practical equipment solutions that have animal welfare at their core and can supplement positive reinforcement behavioral training protocols. In addition, a brief introduction to concepts aimed at enhancing visitor-exhibit interaction with reference to recognized benefits.

Jelly Madness: The Project that Turned us into Gelatinous Blobs

Ben Houghton, BRHoughton@wondersofwildlife.org
Wonders of Wildlife

Over the past year Wonders of Wildlife has been working towards installing one of the largest Jellyfish displays in North America. This talk will cover the husbandry side of the project. Highlighting the growing pains of building a giant exhibit, creating a jelly culture lab to sustain the collection needed for the display, along with a live food room capable of supporting our entire collection, all tucked into our existing footprint. All the while dealing with the ins and outs of ordering culture equipment from the other side of the world, collection plan changes mid-project, undefined timelines, and building a new team to maintain this endeavor.

Veterinarian Hands Across the Atlantic Ocean

João Correia[1], Rui Guedes[1], Gonçalo Graça[1], André Neto[2], Juliana Fomagio[2]
[1] Flying Sharks, info@flyingsharks.eu, guedes@flyingsharks.eu, goncalo@flyingsharks.eu
[2] Oceanic Aquarium, andre@oceanicaquarium.com.br, veterinaria@oceanicaquarium.com.br

In December 2020, two 2.0 m tiger sharks, *Carcharias taurus*, flew from Dynasty Marine (New York) to São Paulo and were then driven for 20 hours to the Oceanic Aquarium in Balneário Camboriú, southern Brazil. Approximately seven months later, the female shark developed a strange-looking lesion on her caudal fin, with continuous progression.

Two members of the Flying Sharks team then flew to Brazil to help contain the animal, using a 'Shark Bag'. A detailed veterinary examination was then performed, focusing on the lesion, blood and also an ultrasound of the coelomic cavity. This examination was repeated with the second animal, as prophylaxis.

Detailed results of water quality and environmental variables were investigated before the injury occurred to trace its origin. With all the analyses and exams in hand, it was found that it was a traumatic lesion evolving into dermatitis with secondary bacterial infection.

The team then began mapping the sharks' swimming and noticed that the female's caudal fin was in constant contact with some of the scenographic structures of the tank. The solution was to modify the shark's swimming lane with structures that prevented the collision with the wall and treat the injury with injectable medication for 4 weeks.

Session 11: Sustainable Collections | Expanded Session

Sponsor Presentation:
Cairns Marine

Where Do Aquariums Get Their Fish?

Christopher Ben Daughtry[1,2], Ben@Aquariumencounters.net
Frank A Young[2], Frank@Dynastymarine.net
Carol "KEE" Bligh[1], Kee@floridakeysaquariumencounters.com
[1] Florida Keys Aquarium Encounters, Marathon FL, [2] Dynasty Marine Associates Inc.

Aquariums see hundreds of millions of visitors each year. Most Aquariums would put educating those guests as one of their top priorities and is often even written into those facilities' mission statement, but what about educating those guests to being a responsible fish hobbyist?

There is little doubt that at the end of a day's visit at an aquarium that at least some of those guests leave with the notion of being able to keep their very own home aquarium. In some ways, public aquaria help to drive the pet trade. Many professional aquarists are home hobbyist. Most aquariums spend resources informing guest of larger issues such as global warming, overfishing, and coral reef bleaching. Some direct guests to sustainably sourced seafood to eat. Yet, there is often very little information on where and how these aquariums source their collection of animals; while it is typically a combination of institutional trading, propagated, and wild caught; many times, there is little to nothing to inform guest on how they can sustainably source their animals from the pet trade.

This presentation will look how at messaging at Aquarium Encounters is used to educate guests on "where we get our fish". As well as different ideas and approaches to make this information more available in public aquaria, in hopes to help make the pet trade a more ethical and sustainable industry.

Why Aquariums Need to Educate Visitors on How They Source Their Collection and Why It's Important to Educate the Public on Making Ethical Decisions for the Home Hobby

Frank A Young[1], Frank@Dynastymarine.net
Christopher Ben Daughtry[1,2], Ben@Aquariumencounters.net
[1] Dynasty Marine Associates Inc. Marathon FL
[2] Florida Keys Aquarium Encounters, Marathon, FL

The general public is naive to how public aquariums source their collections; and with lack of education, they may often come to an inaccurate conclusion. As an industry, we need to be proud of our collection management plans that combine ethically and managed wild caught and aquacultured animals and present that information to our guests so they can be informed of the efforts being taken. Proactive promotion of this information to the guests through signage and guest services will foster this awareness and create a sense of understanding and appreciation.

A positive experience at a public aquarium is a typical starting point for a home hobbyist. With that in mind, it is a fundamental that we inform our guests on how to be responsible hobbyists so they follow best practices when sourcing animals for their home aquariums.

People are impressionable and with a lack of proper guidance, can be led astray from misguided information which seems to be more and more prevalent these days.

Consolidated Supplier Reference Group

Nate Jaros, njaros@lbaop.org
https://supplierreference.com/login

Abstract not provided. Summary provided by the D&C Editor.

This website compiles all vendor information needed to complete acquisition paperwork. Participating vendors have supplied their current permits, licenses and profiles. This eliminates the repetitive back-and-forth previously used to obtain these materials, saving time for both vendors and public aquarium customers.

Aquatic Collections Sustainability Committee Ethical Acquisition Action Plan

Chris Coco[1], ccoco@georgiaaquarium.org
Andrew Pulver[2], apulver@sheddaquarium.org
[1] Georgia Aquarium, Inc., Atlanta, GA, [2] John G. Shedd Aquarium, Chicago, IL

The Association of Zoos & Aquariums (AZA) Ethical Acquisition Task Force has created a new template to achieve a broadly adopted model for AZA aquariums to identify and support suppliers who acquire animals ethically and sustainably, with initial emphasis upon marine ornamental fishes. The template was shared by the Task Force at AZA's Directors Policy Conference in January, 2023. Broad support exists among institutional CEOs/Directors that a consistently transparent and holistic impact validation system be created for acquisition of marine fishes as a top priority for development by the Aquatic Collections Sustainability Committee (ACSC). The ACSC Ethical Acquisitions Working Group, in close collaboration with appropriate

Taxon Advisory Groups, live specimen suppliers, and a variety of colleagues will create a framework of ethical standards to be evaluated. These standards include fishery/environmental impact, animal welfare/handling, chain of custody documentation, and human livelihood impacts. Ultimately, the mechanism created to validate these standards will be operationalized by a third party, allowing AZA members the tool to reach new AZA accreditation standards for collection planning. The Ethical Acquisitions Working Group is comprised of AZA institutional representatives, AZA staff, and colleagues from related professions.

Animal Wellbeing Considerations for Institutional Collection Planning

Allen McDowell, allen.mcdowell@ncaquariums.com
North Carolina Aquarium on Roanoke Island

An appropriately developed Institutional Collection Plan (ICP) is one of the most critical components to ensuring all animals at a zoological facility are experiencing good animal wellbeing. As a result, it is important to consider practices and philosophies that will incorporate animal wellbeing concepts at each step in the ICP process.

ICPs are intended to guide facilities with the acquisition of their animal collection to support their mission and education goals. ICPs may include many different elements such as the animals' status in the wild, involvement of the species in ongoing conservation initiatives, possession of the appropriate knowledge and resources by staff to provide optimal care for the animals, guidance on appropriate acquisition methods, etc. When defining these elements, the greater extent that wellbeing considerations are being incorporated, the greater potential that the entire animal collection will experience good wellbeing.

The strategies of incorporating animal wellbeing considerations into an ICP may vary dependent on the institution but asking the right questions at each step in the process can have a significant overall effect. Some of these questions are related to how we can reduce acute and chronic stress from initial acquisition all the way through the rest of its life. Other questions deal with how we can optimize the care we are providing to ensure the animals live long and healthy lives. To be effective, facilities need to be honest with themselves and ensure that each incorporated consideration is being fully applied to every animal that is acquired.

Building an Egg Identification Catalog: Life Doesn't Have to be Like a Box of Chocolates

Kylie Lev[1], klev@calacademy.org
Barbara Bailey[2], bbailey@neaq.org
Andrew Rhyne[3], arhyne@rwu.edu
Paul Anderson[4], paul@aquariumfisheries.com
[1] California Academy of Sciences, Steinhart Aquarium, [2] New England Aquarium, [3]Roger Williams University, [4]University of Connecticut
https://www.larvalcultureproject.org/

Collecting fertilized eggs from broadcast spawners in complex marine habitats, will often produce a large volume of eggs from a variety of species. When reared together, there are often significant challenges with competition and outcompeting that impact survivorship. Being able to accurately distinguish eggs allows facilities to strategically select and prioritize fish species on which to invest resources.

Originally developed in 2010 by Roger Williams University and the New England Aquarium, the use of DNA barcoding and photo-documentation was used to create an Open-sources Marine Fish Egg Catalog. In 2021, through an AZA Conservation grant fund, this work expanded to include eight additional collaborators. There are nine AZA institutions currently collecting, photo-documenting, and submitting eggs for DNA barcoding from marine fishes spawning in their habitats. The DNA barcoding produces species identification that can be connected with individual egg photos which allowing egg morphometric data to be collected and analyzed. This information is then published on the Larval Culture Project website making it available to any industry professional. This presentation will review the tools and techniques used to build the egg catalog and highlight the practical application of this open-sourced resource.

Session 12: Life Support/Knowledge Retention

Sponsor Presentation:
U. S. Mysids, Inc.

Why We've Aged Rapidly: A Review of The Start-Up of Monterey Bay Aquarium's New Gallery, "Into the Deep: Exploring Our Undiscovered Ocean"

Megan H. Olhasso, molhasso@mbayaq.org
Monterey Bay Aquarium

Providing a rare look at animals that thrive in the least explored part of our planet, the new "Into the Deep: Exploring Our Undiscovered Ocean" gallery at Monterey Bay Aquarium offers a unique deep-sea experience to visitors. How did it go? A review of the enormous collaborative effort it took to get from concept to fruition including species research, development and deliverables, innovative low oxygen life support systems, deep sea collection, and guest facing conservation & ecosystem messaging. How is it going? A look back at our first full year open to the public: what is going as planned, unexpected challenges and public perception. What's next? Our early thoughts on continuous gallery innovation, evolution, and ongoing success.

Mobile Holding Station: A New Paradigm in Fish Transport

João Correia, Rui Guedes, Gonçalo Graça, Ivan Beltran, David Silva, Pedro Marques, João Reis, Telmo Morato
Flying Sharks, info@flyingsharks.eu, guedes@flyingsharks.eu, goncalo@flyingsharks.eu, ivan@flyingsharks.eu, david@flyingsharks.eu, zepedro@flyingsharks.eu, reis@flyingsharks.eu, telmo@flyingsharks.eu

It's largely accepted that moving live fish is no easy task, given the constant deterioration in water quality, namely pH, oxygen, and ammonia. In 2020 we received some funding, which allowed us to revolutionize this concept, by shattering time constraints in transport. This involves state-of-the-art filtration, much like the one used in a standard holding station, which is why the concept was named 'Mobile Station'.

Technical innovations include, but are not limited to: 1) differentiated filtration modes, namely height of intake from the bottom, between sedentary elasmobranchs, ram ventilating elasmobranchs, 'motionless' teleosts, and schooling teleosts; 2) optimized water circulation for turnovers under one hour; 3) versatility of intake plumbing, allowing for water to be provided by numerous sources; 3) same for electrical current, allowing for the system to be powered by 110 V, 220 V, 380 V and other options; 4) differentiated lighting, allowing for a regular daily cycle and/or dimmed/red lighting for packing and unpacking; 5) automatic probes measuring water parameters in real-time, and relaying them to the team via GSM; 6) automatic chemical parameters correction; 7) router for generating own GSM network; 8) double - often triple - redundancy on all electric systems.

These details above are but a few of the multiple aspects that will be shared and discussed.

The Story of Creating a Professional Development Program

Theresa Nietfeld, tnietfeld@cabq.gov
Jason Olona, jasonolona@cabq.gov
ABQ BioPark,

Ongoing professional development and career path opportunities are critical to the retention of professional staff, thus institutional knowledge. Attempting to create an industry wide comprehensive program is challenging due to the uniqueness of each facility. Generating a curriculum that addresses these needs seems daunting, but in reality, is the easy part. Gaining institutional support, financial support, and having the staff to facilitate the program is where most facilities run into roadblocks.

The ABQ BioPark has recently managed to work through the many challenges that can stop the creation of a professional development program. The ABQ BioPark Animal Care Excellence Training and Certification Program was designed to provide a clear path for Aquarists and Zookeepers to further develop their knowledge and skills in all areas of animal care and wellness, guest engagement, and leadership.

Employees follow a curriculum of online and in-person trainings about a range of topics including animal wellbeing, nutrition, animal training and enrichment, species specific animal husbandry techniques, regulations and standards, communication, leadership, and conservation. A combination of skills demonstration, training certification, written exams, and time spent in a position is used to standardize advancement requirements. The program allows for four distinct animal care staff levels, with each level receiving increased responsibilities and higher pay.

This presentation is meant to describe our experience in creating a professional development program for our animal care staff, including the successes and the challenges faced.

My Little Paly: Friendship is Toxic

Adam Atlas, AAtlas@LBAOP.org
Aquarium of the Pacific

The genus *Palythoa*, *Protopalythoa*, and certain species of *Zoanthus* possess an extremely potent neurotoxin called palytoxin (PTX). PTX functions by preventing the pumping of sodium and potassium ions across the cell membrane, resulting in cell death. There is no direct cure that currently exists for PTX poisoning (palytoxicosis).

In aquariums, palytoxicosis most commonly occurs when aquarists attempt to improperly dispose of *Palythoas*. There are often multiple reasons for the removal of *Palythoa* from a marine system, such as keep the highly competitive *Palythoa* colonies under control and freeing up space for more desirable coral colonies. However, removing and disposing of *Palythoas* must be done with extreme care, to prevent PTX poisoning of both an individual and the system.

At the *Aquarium of the Pacific*, a standard operating procedure that allows the partial or full removal of *Palythoa* colonies in an exhibit from the surface or on scuba has been developed. The methodology has proven highly effective and safe for the aquarist staff and the other exhibit animals.

Trials in the Production of *Catostylus tagi* (Haeckel, 1869)

Hugo Batista, hbatista@oceanario.pt
Raúl Gouveia, rgouveia@oceanario.pt
João Lopes, jlopes@oceanario.pt
Oceanário de Lisboa, Esplanada D. Carlos I, 1990-005 Lisboa, Portugal

Over the years the understanding about jellyfish production has increased. Much of this knowledge has been enhanced due to research and information sharing in a collaborative work between public aquariums. However, there are still many species of jellyfish whose life cycle and production process are yet unknown. One of these species is *Catostylus tagi*, the only member of the family Catostylidae that is found in Europe.

Oceanário de Lisboa, in partnership with research institutions, carried out a scientific work on the reproduction of this species with specimens captured in the Tagus estuary (Portugal). It was observed that *Catostylus tagi* has the typical Rhizostomida metagenetic life cycle. Male and female gonads were identified and extracted. Gametes from both gonads were mixed and planulae were collected after 48h. Trials were conducted to evaluate the influence of different temperatures and salinity on planulae settlement survival and metamorphosis and, the influence of temperature and diet on asexual reproduction.

It was observed asexual reproduction of the scyphistoma only by podocysts. Two temperatures (15ºC and 20ºC) and a feeding regime (*Brachionus plicatilis*, Artemia nauplii an Unfed) were tested to evaluate the influence on asexual reproduction. No significant differences were found.

Strobilation occurred with better results with polyps kept at 20ºC fed with *Brachionus plicatilis* and Artemia *nauplii*. Transition from ephyra to fully developed juveniles occurred in 5 months.

More work must be conducted to improve the production of this species, but these first trials show that it´s possible to obtain animals by asexual reproduction in a controlled environment.

Session 13: Husbandry Techniques 2

Sponsor Presentation:
Tracks

Stripes To Spots: A Year with Stegostoma Pups

Jelani Reynolds[1], Jelani.Reynolds@seaworld.com
Libby Scudella[1], Elizabeth.Scudella@seaworld.com
Julie Young[3], Julie.Moore@seaworld.com
Celena Diaz[2], Celena.Diaz@seaworld.com
[1] Seaworld Orlando

Seaworld Orlando introduced a male Stegostoma into the main tank of the Manta Aquarium in September 2021. The first viable eggs were observed in October 2021. 61 eggs were laid in a 5-month period. After an average of 140 days 24 eggs hatched which gave us a hatch rate of 39% overall, 77% of "wigglers". Once eggs began hatching, we split the population between two systems in two different areas of the park. This gave us the opportunity to use two different methods to see which was more successful. There were also major differences in habitat: nitrates, sterilization, and population density. We were able to compare behavioral observations and dietary preferences between the two populations. A subset of the population was chosen for a monthly bloodwork study up to one year of age. This helped us establish baselines for different ages and individuals. Having a large population allowed us to treat each shark as an individual and adjust their care based on their needs. It also gave us an opportunity to discover potential developmental milestones as the pups grew.

Contributing the knowledge gained to the industry can not only increase desirable outcomes when raising Stegostoma neonates and juveniles but can also contribute to the welfare of juveniles of other shark species.

A Baby Boom

Caroline Cox, Caroline@aquariumattheboardwalk.com
Aquarium at the Boardwalk, Branson, MO

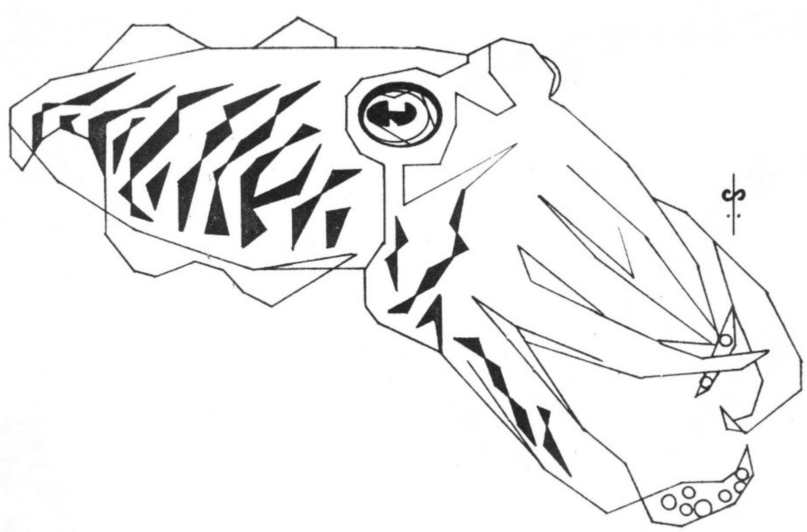
Yellow Stingrays (*Urobatis jamaicensis*), Blue-Spotted Ribbontail Rays (*Taeniura Lymma*), and Blue-Spotted Maskrays (*Neotrygon kuhlii*) are popular animals in zoos and aquariums due to their small size and docile nature, making them high demand species. Since

March 2021, the Aquarium at the Boardwalk in Branson, MO (est. November 2020) has had much success in the reproduction and rearing of these species, with 44 pups finding new homes in facilities across 13 different states. While there have been many exciting and joyous moments to raising so many pups, there have also been many challenging ones too. Our dedication to the survival of these juvenile rays has led to their success from the moment they are born to when they go to their new homes. I am excited to share about our breeding program and hands-on care through the first months of life and to discuss their first 72 hours, diets and assist feedings, observed trends in weight gains and losses, and unique circumstances behind mortalities.

How to Grow Your Sea Dragons: Breeding Weedy Sea Dragons

Mallory V. Haskell, Mhaskell@greaterclevelandaquarium.com
Greater Cleveland Aquarium

The Greater Cleveland Aquarium has celebrated a milestone that few aquariums around the world have achieved, breeding and raising Weedy Sea Dragons, *Phyllopteryx taeniolatus*. On January 23, 2020 we had a successful egg transfer, in our collection of 1.2.0 Weedy Sea Dragons. The egg development was a slower process than expected with only a few hatchlings that survived less than one week. This prompted a lot of literature research and adjustments to the light and temperature cycles in hopes of getting a second chance. On September 5, 2021 we had a second successful egg transfer. We began preparation of the holding tank and live food options for once the fry were born. Seventeen of the hatchlings have successfully grown past the one-year mark. To date we have been able to surplus eight individuals to other Aquariums, and we have nine 1+year old hatchlings on exhibit with the three adults at the Greater Cleveland Aquarium. We are dedicated to sharing the lessons learned and successes to the industry, and presenting at RAW to peers will further the progress of husbandry practices for this species.



USING ZIMS DATA TO DEVELOP AVERAGE LONGEVITY VALUES

Jay Hemdal, jay.hemdal@toledozoo.org

The Toledo Zoo and Aquarium

Two demographic measures; life expectancy and lifespan equality, can provide an indicator for welfare. Life expectancy is the average lifespan of animals in a population, while lifespan equality measures the number of deaths due to "old age" relative to life expectancy. In other words, animals experiencing better welfare will demonstrate an above-average life expectancy, as well as having more of the mortalities grouped towards the end of the expected lifespan. Ironically, it is sometimes stated that "dead animals have no welfare consideration". However, an early death is certainly an indication of poor welfare.

Aquarists are often interested in record longevity of their animals – who have kept an individual of a given species longer than anyone else? While these records are fascinating, average life expectancy is a more important measure of good husbandry. The question is then, how can this information be acquired? Facilities using the Zoological Information Management Systems (ZIMS) developed by Species 360 have access to global animal records that can be exported and evaluated in Microsoft Excel. It turns out that the process for doing this is not straightforward and needs to be done with careful consideration.

The following steps can be used to extract usable longevity data from ZIMS:

Run an Animal Simple Search in the Animals Module. Search ZIMS for "all animals, global, include dead animals" and filter by the taxa of interest. This could be a species, or even a genus or family. It helps to use the Animal Advanced search function and choose individual accessions (group counts cannot contribute to this data). It is also beneficial to select the global status of "dead animals", as those still living also cannot be used to calculate longevity. Then, run the report and export it to an XLS file. In Excel, first delete any unneeded columns. Then, delete all listings that are obvious errors, such as acquisition dates from hundreds of years ago or entries with missing dates. At the same time, delete any "merges" or "lost to follow up" entries.

The DOB/Established column gives a date, but it is not formatted correctly for analysis. The following steps will convert that information into actual dates that can then be managed.

Select the data in the column.
Go to the Data tab and choose "Text to Columns".
On the first screen, leave the radio button on "delimited" and click Next.
Unselect any delimiter boxes (everything blank) and click Next.
Under column data format choose Date.
Click Finish.

The death date "Status" is a text string, not an actual date and also has to be handled carefully:
Strip out the "Dead as of" text using search and replace
Convert the remaining text using the function =DATEVALUE()

Apply =YEARFRAC() to the DOB/Established and Status columns to create a "Years Alive" column. Format that column as a number with two decimal places. At this point you can average the values in that column and use that as a calculated average longevity for that species.

The data may need additional "clean up", depending on your application:

- If any rows for animals that lived less than 0.2 years are deleted, the result will give a modified "post quarantine" longevity average.
- Using this method, any cohorts that are still alive will not be included. That means that animals with later DOB/Established dates will be skewed to showing only those that died prematurely. One workaround is to delete these later acquisitions if they are within the expected longevity average time window.
- This method can only track individual accessions. Very often, larger aquariums, and those breeding smaller aquarium species use group accessions. The facilities who tend to use individual accessions are small aquariums, or zoos that have an aquarium in an education classroom, etc. It is possible that those facilities do not have the same level of husbandry knowledge and veterinary support as the larger aquariums have. There is then the possibility that the average longevity value calculated here may be shorter than is seen in the collections at larger facilities.
- The DOB/Established date is used differently by institutions; it can be an estimated date of that animal's birth, or it can be the date that the facility received that animal into their collection. There is no real fix for this, other than to understand that most animals are acquired as juveniles, so that the different values may still be used to create estimates.
- Some animals will be shown to have a DOB/Established date of "January 1" of a given year. Nobody ships animals on New Year's Day. This is an artifact created by some registrars when they do not know the actual date of acquisition. This could invalidate those entries. A potential workaround would be to convert those dates to July 1 of that year. In that case, the actual date would be at most, six months off, versus as much as 12 months off using January 1.
- The cause of death won't be known for the extracted data. Some deaths may be from predation, others may be from euthanasia. However, both events lower average longevity and need to be accounted for.

For an example, this method was applied to the Banggai cardinalfish, *Pterapogon kauderni* listed in ZIMS.

Usable data included 100 individual Banggai cardinalfish that were acquired from 1996 to 2021.
The longevity ranged from 2 days to 8.9 years
Average lifespan was 1.8 years, with a median of 1.2 years.
If quarantine losses were removed, average lifespan was 2.4 years with a median of 2.1 years
25% of the fish died less than one month after acquisition
50% died less than a year after acquisition.
10% lived longer than five years.

Even if you choose not to run a full data extraction for a given taxonomy, simply looking at the raw data may offer some insight as to what an acceptable life expectancy might be for a taxa. Running an Animal Search for Emperor angelfish, *Pomacanthus imperator*, shows a maximum longevity of about 25 years, with a number of fish that lived over ten years. Aquarium staff, seeing this, would be able to conclude if their Emperor angelfish were surviving longer than about ten years, they are likely offering those fish good welfare.

Reference:

Hemdal, J. 2009. **Mortality Rates of Fishes in Captivity**.
https://reefs.com/magazine/aquarium-fish-mortality-rates-of-fishes-in-captivity/

Trio Of Mahi Mahi, Bruce Koike

CAPTIVE LONGEVITY OF A BUTTERFLYFISH *Heniochus acuminatus* AT THE TULSA ZOO

Stephen Walker, retired sdwalker17@gmail.com

5438 E. 24th Pl, Tulsa, OK 74114

On March 4, 1983, the Tulsa Zoo received 4 adult butterflyfish *Heniochus acuminatus* from Salt & Sea Enterprises for $6.50 each. At the time, I was Curator of Aquariums, and the four fish were placed on display into a 120-gallon aquarium in the "Secrets of Survival" area at the Zoo. Over the years, we lost three of these butterflyfish, but specimen #5821 (Figure 1) survived the others. As of this writing in December of 2023, this specimen is still alive and swimming and has been in the Tulsa Zoo's collection for over 40 and ¾ years!

During this fish's *tenure*, it has been moved within the Zoo at least 21 times either to a display or an off-exhibit enclosure and has had an innumerable parade co-inhabitants. The Zoo's Specimen Report shows no instances of this fish ever needing treatment for pathogens or injury.

Heniochus acuminatus goes by many common names including pennant coralfish, black and white *Heniochus*, longfin bannerfish, phantom bannerfish, and common bannerfish. Old-timers may even remember that this fish was once called "The poor man's Moorish idol."

Admittedly, I don't know how significant this 40 plus year of captivity actually is. I suspect there are other institutions housing *Heniochus* with similar longevity, but they may be held in large enclosures with many conspecifics where identifying a single individual would be impossible. On the other hand, the history of this specimen in captivity is clearly established.

The many members of the Tulsa Zoo staff who have cared for this fish over the intervening years should be congratulated and acknowledged for the achievement of this fish's longevity, and here's hoping for many more years for this intrepid butterflyfish.

Figure 1. *Heniochus acuminatus*, specimen #5821 from the animal collection of the Tulsa Zoo, Tulsa Oklahoma

ABSTRACTS from AALSO 2023
March 25-29.
Milwaukee County Zoo, Milwaukee, WI, USA.

Chattanooga Water Outage on Friday the 13th

Sean Hill
Tennessee Aquarium 1 Broad Street, Chattanooga, TN 37402 USA

On Thursday, August 12, 2019, at 11:00 pm, the Tennessee American Water company experienced a catastrophic water main break which shut down water service for the entire downtown Chattanooga area including the Tennessee Aquarium. The Aquarium campus includes three buildings with cooling towers and chillers: River Journey, Ocean Journey, and IMAX Theater. The operator on shift began getting alarms on cooling towers and chillers around 2:00 am on Friday, August 13. He notified the director of facilities and senior operator. At that point it was discovered the building's domestic water was without pressure and the water company was contacted. With no timeline available as to when necessary, repairs may be completed, the Aquarium assembled a multi departmental team to work through the issues the outage would cause. Operators begin going evaluating systems to discontinue use of non-essential heat generating equipment while husbandry staff began assessing all exhibits. Without cooling capacity for the exhibits or public spaces and no access to municipal water, the Aquarium would remain closed for business on Friday. Focusing on the buildings containing animals, River Journey and Ocean Journey, the response team needed to figure out how to get water to the cooling towers to regain temperature control of the cool water exhibits. With the help of a contractor and the Chattanooga Fire Department, a tanker and water trucks were secured. The fire tanker was able to source water directly from the nearby Tennessee River while the contractor filled his trucks from a local rock quarry. The team was then able to get water to the River Journey and Ocean Journey cooling towers restoring temperature control to all necessary exhibits and building spaces. The operator on shift Saturday morning noticed municipal water pressure return around 7:00 am. The response team was able to restore system control and open the Aquarium for normal business hours at 10:00 am Saturday. Through this outage many strengths and weaknesses were identified, and new protocols were put in place in the event water service is disrupted in the future.

Problem Solving Disinfection for Extreme Bio-load Variability at the World's Largest Marine Mammal Hospital

Micah Buster
2000 Bunker Rd, Sausalito, CA 94965, USA

The Marine Mammal Center is the world's largest marine mammal hospital that has seen more than 24,000 patients since it was founded in 1975. The extreme seasonal patient load variability presents unique challenges for the Center's ozone-based disinfection. An ozone balancing spreadsheet was created, which highlighted some of the gas balancing issues inherent in the system. Additional ozone generation equipment was installed to address some of these issues, which resulted in significant improvement to the functionality and flexibility of the system. Future

modifications, including proportional ozone flow control valves and modified PID control, are being considered as possible solutions to further improve the system's flexibility. The design considerations discussed may be useful to other organizations experiencing similar challenges with variable bio-loads.

NOB's: The SOB's of New Tank Syndrome

Hannah Mewhirter
Seattle Aquarium, 1483 Alaskan Way, Seattle, WA 9810, USA

The first of Seattle Aquarium's multiphase expansion includes the Ocean Pavilion, exclusively featuring tropical species. After 46 years of temperate, flow-through systems featuring local species, this expansion represents a paradigm shift towards closed, recirculating systems. Preparing for a 2024 opening necessitated an offsite facility with 22 individual systems ranging from 110-31,000 gallons. Dosing began in June, but cycling was not confirmed until September, taking roughly four months to cycle 1 mg/L NH3 in 24 hours. Challenges included over-ozonated makeup water, initial low-level dosing, and adjustment to suitable testing methodologies, but the most persistent challenge was a prolonged nitrite peak wherein nitrite-oxidizing bacteria (NOB) were slow to populate. Alkalinity and temperature were increased to coax NOBs in time for scheduled animal acquisitions. Either parameter may have helped, but ultimately it was time that conquered the initial nitrite peak. NOB issues persist and remain largely outcompeted by ammonia-oxidizing bacteria in cycled systems.

The Alaska SeaLife Center: Refurbishing an Aging Flow through Life Support System and Associated Infrastructure in a Post-Pandemic World

Juan Sabalones
Alaska SeaLife Center, 301 Railway Ave, Seward, AK 99664, USA

On March 24, 1989, the supertanker Exxon Valdez spilled 11 million gallons of oil into Prince William Sound, damaging nearly 1,500 miles of Alaska's pristine shoreline and causing widespread harm to the region's wildlife, economy, and ecosystems. Thousands of marine birds, mammals, and fish were destroyed in the days and weeks following the spill. The disaster drew attention to the need for improved facilities to treat injured wildlife, as well as to enhance Alaska's collective research infrastructure. On May 2nd, 1998, The Alaska SeaLife Center (ASLC) opened its doors as a result of this need. Having been constructed between 1995 and 1998, the bulk of the Life Support System and associated infrastructure is twenty-four to twenty-seven years old and beginning to show it. Issues we will focus on concern but are not limited to, attracting, training and retaining competent staff, the deterioration of primary electric services, the fouling of seawater intake lines that go as deep as 250 feet, and the design of the seawater lines cooling the heat pump heat exchangers. This presentation will focus on several aspects of how we are dealing with such issues especially in light of complications brought on by the recent pandemic. While our unique location contributes to the issues, we believe many other institutions face similar issues.

Water Quality and its Impact on Fish Health and Welfare

Dr. Rob Jones
PO Box 2327, Moorabbin Victoria, Australia 3189

Understanding welfare considerations for all species displayed in aquariums and zoos is a high priority. As an industry we must be able to provide evidence that we are caring for our animals in the best way possible. This will become increasingly important in the years ahead as we face our detractors that would close down aquariums and zoos. In the past ten to fifteen years, the Five Domains of animal welfare have completely replaced the Five Freedoms and become the standard for examining animal welfare globally. Studying and understanding the Five Domains of nutrition, environment, health, behaviour and how these first four Domains lead to the fifth Domain (the mental domain) can provide valuable insight into the welfare of the animals in our care. Water Quality is the main parameter in the Environment Domain and is thus critical to ensuring the good health and providing excellent welfare for the fish and other aquatic animals under our care.

Modeling Lanthanum Chloride Reaction Rates for Practical Applications in LSS

Barrett L. Christie
The Maritime Aquarium at Norwalk, 10 N Water St, Norwalk, CT 06854, USA

Lanthanum chloride ($LaCl_3$) dosing to remove orthophosphate (PO_4^{3-}) from water is a common practice in both aquaria and zoos. Limitation of phosphorus in outdoor pools with heavy nutrient loading has some benefits in algal control, and the resulting flocculation also may benefit turbidity by removal of particulates in solution. The most common downside of regular lanthanum chloride dosing is fouling of mechanical filters, piping, and valves with insoluble lanthanum phosphate (LaO_4P). Several strategies such as the use of sacrificial sand filters are commonly used to mitigate this, but a common question that operators have is how to calculate the length of pipe run that is needed between point of lanthanum injection and mechanical filtration. This talk will examine the rate of reaction as a function of PO_4^{3-} concentration and temperature, and show examples of how these data can be used to model optimal dosing as a function of pipe diameter, water flow, and flow velocity while avoiding breakthrough of fouling precipitates after the filter.

Weighing the Scales on the Skalar: The Pros and Cons to Continuous Flow Analyzers in an Aquarium Setting

Veronica Thompson
Florida Aquarium, 701 Channelside Dr, Tampa, FL 33602, USA

The Florida Aquarium recently completed construction on a new water quality laboratory. This has allowed for introduction of new analytical tools to be used in an ongoing effort to improve the water quality data provided to the aquarium's biological operations teams. The first such tool is the Skalar San++, a continuous flow analyzer that is being evaluated as a primary tool for ammonia, nitrite, nitrate and phosphate analyses. The Skalar, while in early stages of operation, is showing promising signs of reducing the Aquarium's dependency on single-use reagent packets. The data gathered by the Skalar to date has showed accuracy and precision at the low detection levels required by aquarium biologists. In addition to increasing the sustainability of, and the quality of data output by the water quality laboratory, the operation costs associated with the Skalar appear to be less than those associated with the use of single-use reagent packets.

How Beer has Shaped Water Chemistry Analysis in Aquariums

Barrett L. Christie
The Maritime Aquarium at Norwalk, 10 N Water St, Norwalk, CT 06854, USA

For over five millennia humans have practiced the art of zymurgy, the controlled fermentation of sugars to ethanol in beverage production. By comparison, we have only understood concepts such as the nitrogen cycle, enzymes, or pH for about a century. The end products of zymurgy are undoubtedly familiar to many of us, but the scientific advances that have evolved from brewing are probably less well known. A number of instruments we all use daily were originally developed for the brewer, and there are numerous chemical reactions in water we understand mainly because of their importance in fermentation. We will review eighteen of the technological and analytical advances that shape the lab work performed in aquariums every day, ranging from testing equipment to chemical processes used in analysis of water. The synergy between brewing and water chemistry has a long and fascinating history, let's hoist a pint to science and explore these connections.

Plastic Welding of a Protein Skimmer (PSK)

Brett Funk, Carlos Cruz Reyes
Georgia Aquarium, 225 Baker St NW, Atlanta, GA 30313, USA

Protein Skimmers (PSK) are an important and expensive part of life support equipment. Over time, wear causes the vessels to leak. Unfortunately, the logistics of replacing a PSK is both time and money consuming. We decided to hire an outside company to come in and weld our PSKs. Although a better option than replacement, the same logistical problems occurred. This pushed us into welding in-house. Over the past year, we have welded dozens of protein skimmers. This has increased the life span of our PSKs and has helped with both time and monetary constraints.

Fun with PVC! How to be Green by Hill-William-ing our Best Friend

Nick Zarlinga
Cleveland Metroparks Zoo, 3900 Wildlife Way, Cleveland, OH 44109, USA

PVC is a material that we cannot live without and yet, so often, we waste a significant percentage of it in our daily projects. Unfortunately, it isn't recyclable so we are left to just dispose of it in landfills. However, what if we can get creative and utilize it for other purposes in our daily lives? In this presentation, we are going to learn how to Hill-William our leftover PVC to our benefit.

Clam Cannons as an LSS-Oriented Enrichment Tool for Sea Otters

John Drummond
Georgia Aquarium, 225 Baker St NW, Atlanta, GA 30313, USA

Part of the life support system for the Georgia Aquarium's sea otter exhibit is a simple side loop of pipes and valves used to feed, enrich, and entertain the animals all at once, in a coordinated effort between LSS and husbandry staff. Clams are loaded into a section of pipe that branches off of the sand filtered water returning to the exhibit, through open-ended ball valves. Each valve returns to a different part of the pool, and the operator watches the otters on a screen in order to better engage them. By diverting some of the filtration flow supplying an exhibit, many operators may be able to replicate this fun enrichment tool and adapt it to their needs.

**The Design and Build Process of Moon Bay;
A Jelly Touch Experience at The Florida Aquarium**

Libby Nickels
Zoo Tampa at Lowry Park, 1101 W Sligh Ave, Tampa, FL 33604, USA

In early 2018, The Florida Aquarium made the decision to renovate its main lobby exhibit and the surrounding area with the goal to provide an updated look to that portion of the lobby. In the past 10 years there had been various attempts to capture the guest's attention as the system had morphed through a number of different taxa from stingrays to horseshoe crabs but the system components (tank and LSS) always stayed the same with only minor modifications. The exhibit had always been a touch habitat and that component was to stay but the decision was made to completely start from scratch on the taxa and system components this time. By the middle of the 2018 it was decided to build a moon jelly touch habitat and thus began an intense year long journey to design, demo, and build this improvement using internal resources wherever possible in the process. On January 8th 2019 after business hours LSS techs and Aquarists completely removed the old tank, LSS, and façade. The next week construction began and 6 months later on World Oceans Day (June 8th) Moon Bay was successfully opened.

You Want to Put What Where?! Building One of North America's Largest Jellyfish Exhibits into a Fully Functioning Aquarium

Richard Prince
Wonders of Wildlife Museum & Aquarium, 500 W Sunshine St, Springfield, MO 65807, USA

Wonders of Wildlife in Springfield, Missouri is one of the largest Aquariums in the US. It is now home to one of the largest Jellyfish displays in North America. Over the last year, we have modified a movie theater in our Shipwreck exhibit into this new gallery. The only problem is that the floor was not engineered to hold the weight of this exhibit and there is a densely packed mechanical room below the theater that houses the filtration for our Stingray Touch Tank and Shipwreck Cylinder as well as a large saltwater recovery system and several ozone contacts. This presentation will show how we were able to engineer our way around this obstacle by building a steel aquarium stand around the sand filters and modify several large aquarium systems in order to accommodate this new exhibit.

Between a Rock and a Hard Place:
Impacts on Water Quality during Rockwork Replacement

Karen Tuttle[1]; Christopher Wojcik[2]
[1]Monterey Bay Aquarium, 886 Cannery Row, Monterey CA, 93940, USA
[2]Animal Exhibits and Design, PO BOX 145, Bay Head, NJ 08742, USA

After almost 40 years of constant wave action and aging infrastructure, the original rockwork in the Kelp Forest Exhibit at the Monterey Bay had started degrading. The 335,000-gallon exhibit is the first stop for seawater through the Aquarium's open system, flowing through a number of other exhibits and eventually back into the Monterey Bay. The Kelp Forest exhibit is home to live giant kelp, sardines, giant sea bass, leopard sharks and a host of other fishes. Without a way to isolate the flow of water, repairs had to occur without removing water or animals from the exhibit. Temporary patches kept the exhibit functioning while we researched the best way to repair it. Animal Exhibits and Design worked closely with Aquarium staff to develop methods to remove over 100,000 pounds of rockwork and replace it with over 60,000 pounds of lighter interlocking rockwork panels and framing. Water quality was monitored throughout the process, including parameters like pH, Total Gas Pressure, Turbidity, and Trace Elements.

Giant Featherback, Bruce Koike

IN MEMORIAM: CHARLES J. YANCEY (1959-2023)

Barrett L. Christie

This past year a long-time member of the aquarium community passed away. Charles Yancey was an aquarist for over 40 years. A native of Washington DC, he spent most of his career at the Dallas Aquarium at Fair Park (since the late 1980's) but got his start under the tutelage of Craig Phillips at the National Aquarium in Washington, the '*REAL'* National Aquarium, as he was always quick to emphasize.

Charles specialized in working with freshwater fishes and horticulture, and spent most of his career dedicated to keeping beautifully planted tanks. He was a tinkerer and a perfectionist in exhibitry and would spend countless hours tending to the smallest details, or propagating plants from cuttings. It was not unusual for him to become so lost in his work that he had scarcely realized it was dark and he was the last one in the aquarium. He loved barbecuing, gardening, and the Dallas Cowboys, and when not working was likely to be found hanging out in punk rock or rockabilly bars, surrounded by the most eclectic people he could find.

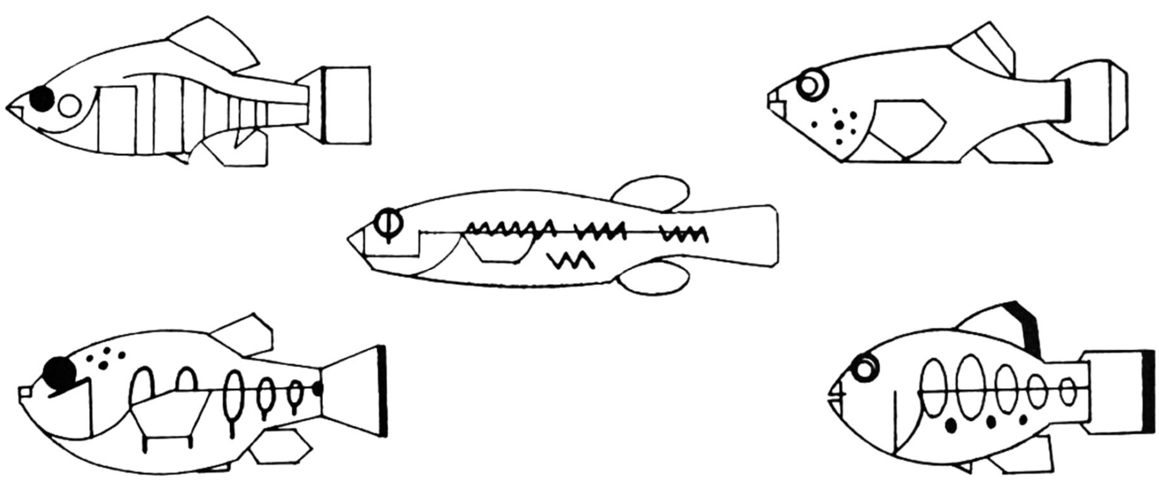
Charles was also involved with the Desert Fishes Project of the Freshwater Fishes TAG from its inception, traveling to México with colleagues in the early 1990's to collect ultra-rare desert pupfishes as founder stock for *ex situ* breeding. Some of these collections represent the last time these species were ever seen in the wild, and Charles devoted decades to breeding and distributing these fishes to other aquaria, universities, and to dedicated hobbyists. For nearly three decades starting in 1991 he bred thousands of these fishes, some through as many as 29 consecutive generations. He had a special knack for breeding pupfish, and several species owe their continued existence to his work. Without the curiosity and passion Charles had for these fishes, it is not exaggeration to say that a few more of these unique creatures would have gone extinct in the 1990's.

An iconoclast is traditionally defined as a maverick, one who does not conform to generally accepted standards or customs. As a truly unique individual, one who marched to the beat of his own drum, Charles was an iconoclast, arguably more so than any other person in our industry. Anyone who has the privilege of working with him likely has no short supply of stories to tell, all of which are hilarious, and most of which are completely inappropriate to be told in polite company. These outrageous stories will always be treasured by those who knew him.

His respect was never simply given, it had to be *earned*. But once you had gained his respect, he was a devoted friend and colleague who taught so much to both the young aquarists he trained, as well as the supervisors who dared to try and manage such a force of nature.

Charles was a mentor to many aquarists, a teacher, and a friend, and we are all better for having known him and having had him as part of our aquarium community. Rest in peace Charles, your friends, and the pupfish, will never forget you.

"*Everybody's got a little light, under the sun*"
-George Clinton



Desert pupfishes, drawn by Craig Phillips (1922-2009), conserving pupfishes was a large part of Charles' work and Craig hired a young Charles at the National Aquarium as an aquarist at the outset of his career.

MODELING LANTHANUM CHLORIDE REACTION RATES AND PRECIPITATE FORMATION FOR APPLICATIONS IN LSS PARTICLE CAPTURE

Barrett L. Christie, Director of Animal Care, bchristie@scaquarium.org

South Carolina Aquarium, 100 Aquarium Wharf, Charleston, SC 29401

Introduction

Lanthanum chloride ($LaCl_3$) is a flocculant that originated in the pool industry that has been widely used in aquaria and zoos to reduce concentrations of orthophosphate (PO_4^{3-}). Lanthanum has been safely used in a variety of systems, including reef systems housing delicate invertebrates, tanks with fishes, and marine mammal tanks (Young and Thoney, 2014). Lanthanum chloride reacts with orthophosphate and water to form insoluble lanthanum phosphate and chloride ions. In seawater or alkaline freshwater, it also reacts with sodium bicarbonate to form lanthanum carbonate, sodium chloride, and hydrochloric acid. These reactions occur simultaneously in alkaline waters as governed by the following equations:

$$2LaCl_3 + 2PO_4^{3-} \rightarrow 2LaPO_4 + 6Cl^-$$
$$2LaCl_3 + 3NaHCO_3 \rightarrow La_2(CO_3)_3 + 3NaCl + 3HCl$$

Particle capture of the precipitates formed when dosing lanthanum is essential, as these solids will increase turbidity and foul piping and equipment (AALSO, 2020); some studies also show detrimental effects to animals from exposure to high concentrations of lanthanum ions (Hua et al., 2017). Zhang and Wong (2012) concluded that application and filtration of lanthanum needs to be carefully controlled and is best conducted in a side loop of the LSS Young and Thoney (2014). However, in any application, sufficient reaction time is critical to allow precipitation for effective removal by filtration (US Dept. Interior, 1970).

Methods

To simulate the particle capture of a rapid sand filter a 20µm filter (Whatman Grade 41) was used in conjunction with a filter flask and Büchner funnel. The filter flask was attached to a 2-gallon steel pressure tank and a rotary vane vacuum pump capable of pulling a vacuum of -37.3 mmHg (-20 inH_2O/-0.723 PSI). The vacuum was actuated by a solenoid valve controlled by a momentary switch (Fig.1). The assembled benchtop apparatus (Fig. 2) was capable of filtering 200ml of sample in <1sec, and a stir mixer kept the solution homogenized until passing through the filter. Solutions were prepared of 0.1M $LaCl_3$ and 0.1M $NaH_2PO_4 \bullet 7H_2O$ in deionized water, artificial seawater (30 g/L - Fritz RPM®), and deionized water made alkaline (3.5 meq/L) with sodium bicarbonate. 100ml of the lanthanum solution and 100ml of the orthophosphate solution were combined and allowed to mix for 1, 5, 15, 20, 30, 60, and 120 seconds before filtration to allow precipitation and crystallization of $LaPO_4$ at various time intervals. Ambient temperature (and temperature of solutions) was 21ºC (70ºF), 10 replicates each of seawater and alkaline freshwater were run with a 5 second reaction time, and 92 replicates with deionized water were run over the 7 time intervals described above. The original intent was to run 200 replicates at two different temperatures, however after 112 cycles the filter flask (which was not rated for high negative pressures) displayed signs of catastrophic inward fragmentary auto-disassembly (i.e. it imploded in spectacular fashion).

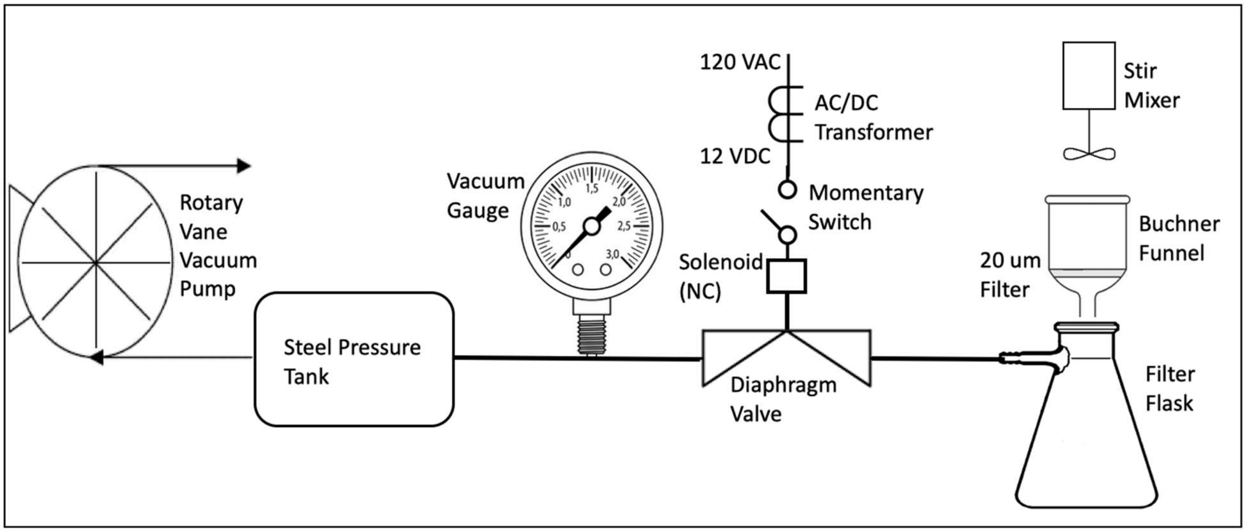


Figure 1. Schematic diagram of benchtop filtration apparatus used to mix lanthanum and phosphate solutions and allow for rapid filtration to 20µm to simulate the particle capture abilities of a high-rate sand filter with standard media.

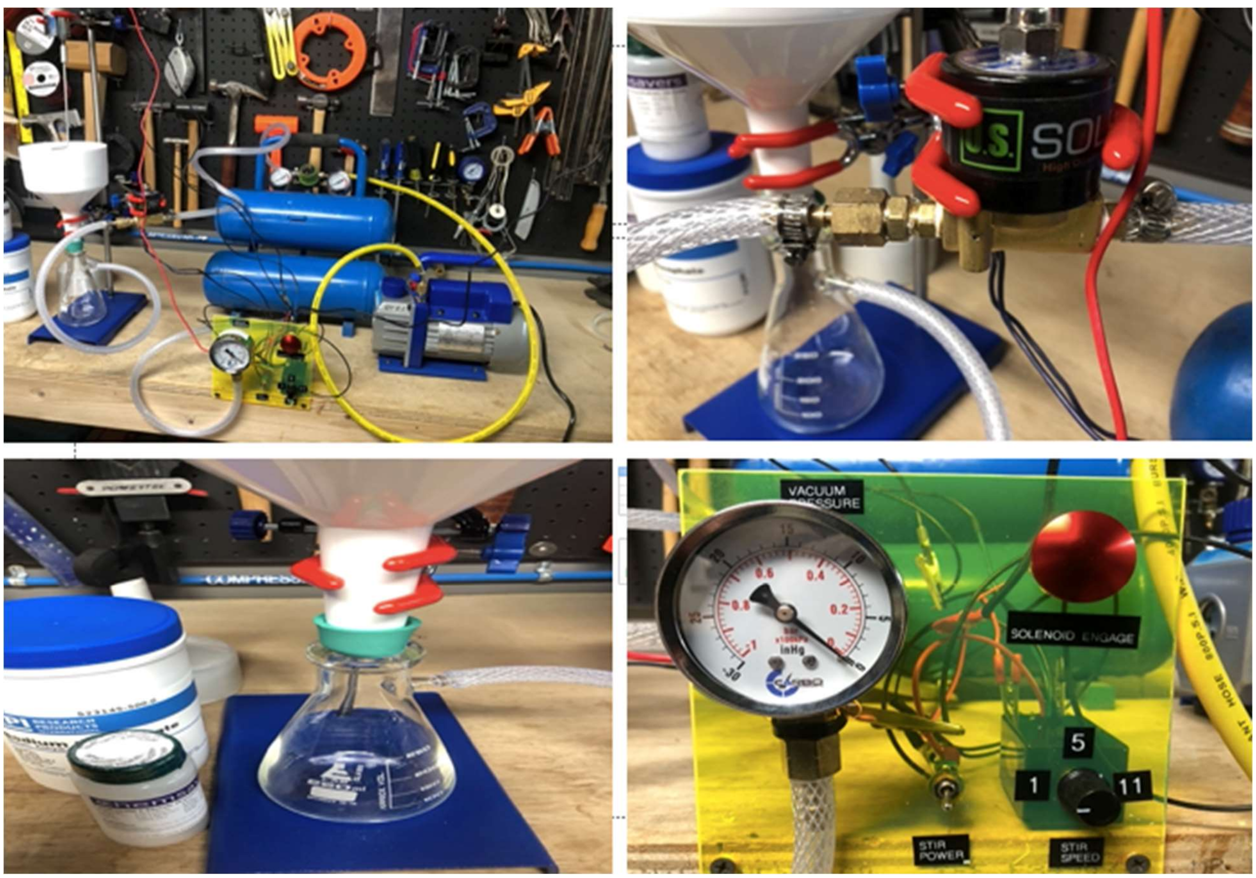


Figure 2. Benchtop apparatus, (top left) full rig with vacuum pump, tank, and filter flask (top right) 12VDC solenoid valve to allow instantaneous application of vacuum pressure, (bottom left) filter flask that receives solutions and any $LaPO_4$ precipitate breakthrough, (bottom right) control panel: vacuum gauge, momentary switch (big red button) to actuate solenoid, and speed control for stir mixer (note that it goes to eleven, its one faster than ten, isn't it? Most blokes, you know, will be stirring lanthanum at ten…where can you go from there?)

Results

A student's t-test was used to determine if there were differences between $LaPO_4$ recovered from solutions made with deionized water and seawater/alkaline freshwater. A significant difference ($\alpha < 0.05$) was not found to exist between deionized water samples and seawater (α = 0.14925) or between deionized water and alkaline freshwater (α = 0.17258) with 5 seconds of reaction time. As no statistically significant differences were found in the seawater and alkaline water datasets (n=20, combined), the data were incorporated into the larger dataset (n=92) for a total of 112 replicates. This was done to better predict the behavior of $LaPO_4$ precipitation as a function of time.

Mass recovered ranged from 19-128mg of a maximum 138mg across all samples run. For 1 second replicates change in mass ranged from 19-84mg, 27-45mg for 5 second replicates, 20-54mg for 15 seconds, 22-71mg for 20 seconds, 49-68mg for 30 seconds, 57-88mg for 60 seconds, and 91-128mg for 120 second replicates. It was found that increasing time for solutions to mix resulted in increasing recovery of $LaPO_4$, average mass recovered for each of the time intervals ranging from 34.6mg to 114.8mg (Fig. 3), corresponding to an average percent recovery of 25-83% (Table 1). These data show that the mean reaction time needed for 50% or more capture of $LaPO_4$ is 62 seconds, and that 120 seconds is required for >85% particle capture (Fig. 4).

The mean of all percent recovery data points (n=112) divided by reaction time gives a constant of 1.29912 seconds per percent solids retained which can be used to estimate particle capture as a function of time in practical applications. This corresponds to the slope of the trendline in Fig. 3, and allows extrapolation out to 100% recovery, as discussed below.

Table 1. Mean recovery of $LaPO_4$ with 20μm filter at various reaction times

Time (sec)	Start (mg)	End (mg)	Δ Mass (mg)	Mean Recovery (%)	Recovery Range (%)
1	571.3	606.0	34.6	25.1%	13.6 - 60.9%
5	571.1	606.5	35.4	25.6%	21.7 - 30.4%
15	571.6	616.1	44.5	32.3%	26.1 - 39.9%
20	571.3	617.6	46.3	33.5%	27.5 - 51.4%
30	571.4	627.9	56.5	40.9%	35.5 - 45.7%
60	571.4	635.7	64.3	46.6%	20.3 - 55.1%
120	571.2	686.0	114.8	83.2%	72.5 - 92.8%

Additionally, the negative pressure rating of a $12 generic filter flask purchased from Amazon was found to be somewhere between 0 and 112 one-second cycles at -37.3 mmHg, which can cumulatively be expressed as -5.57×10^5 Pascal-seconds. It was also found that Amazon will provide a refund for an imploded filter flask even when one is completely straightforward about the negligent way in which it was destroyed, provided one returns the lanthanum-covered glass shards to a Whole Foods.

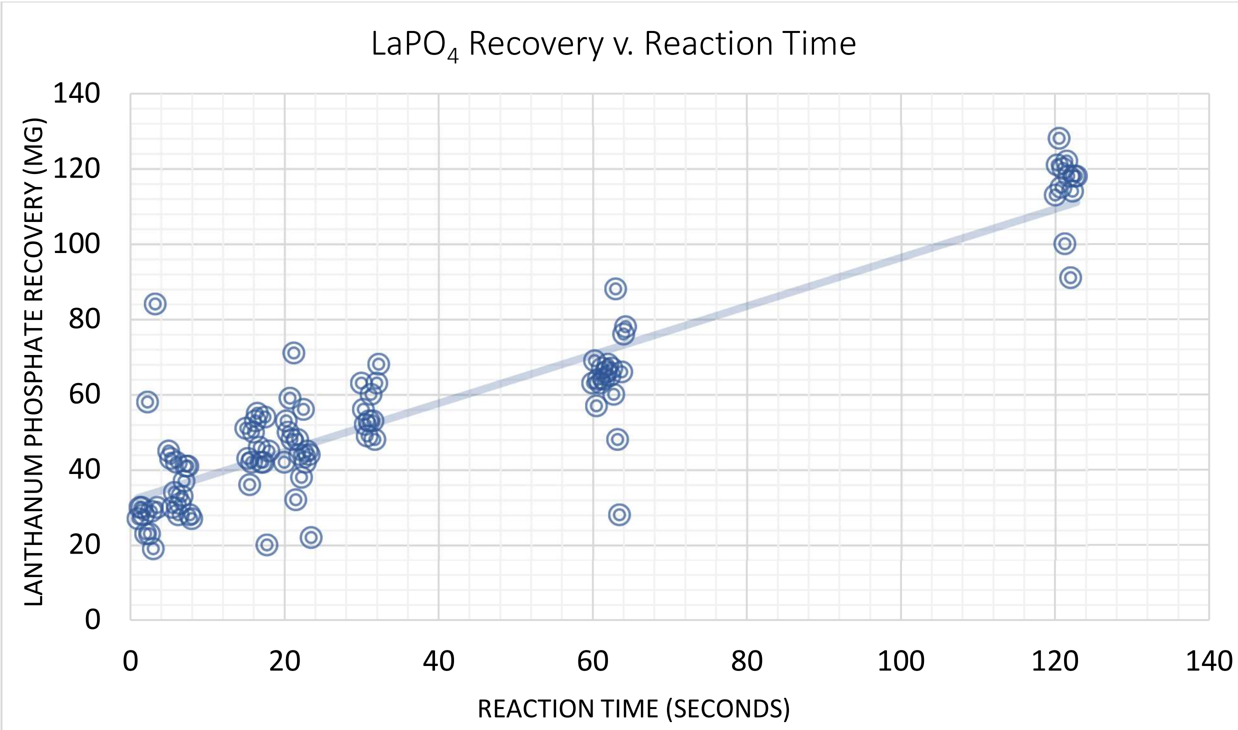


Figure 3. Scatter plot of change in mass from $LaPO_4$ precipitation of 20μm filter papers at various reaction times of 0.1M NaH_2PO_4 and 0.1M $LaCl_3$.

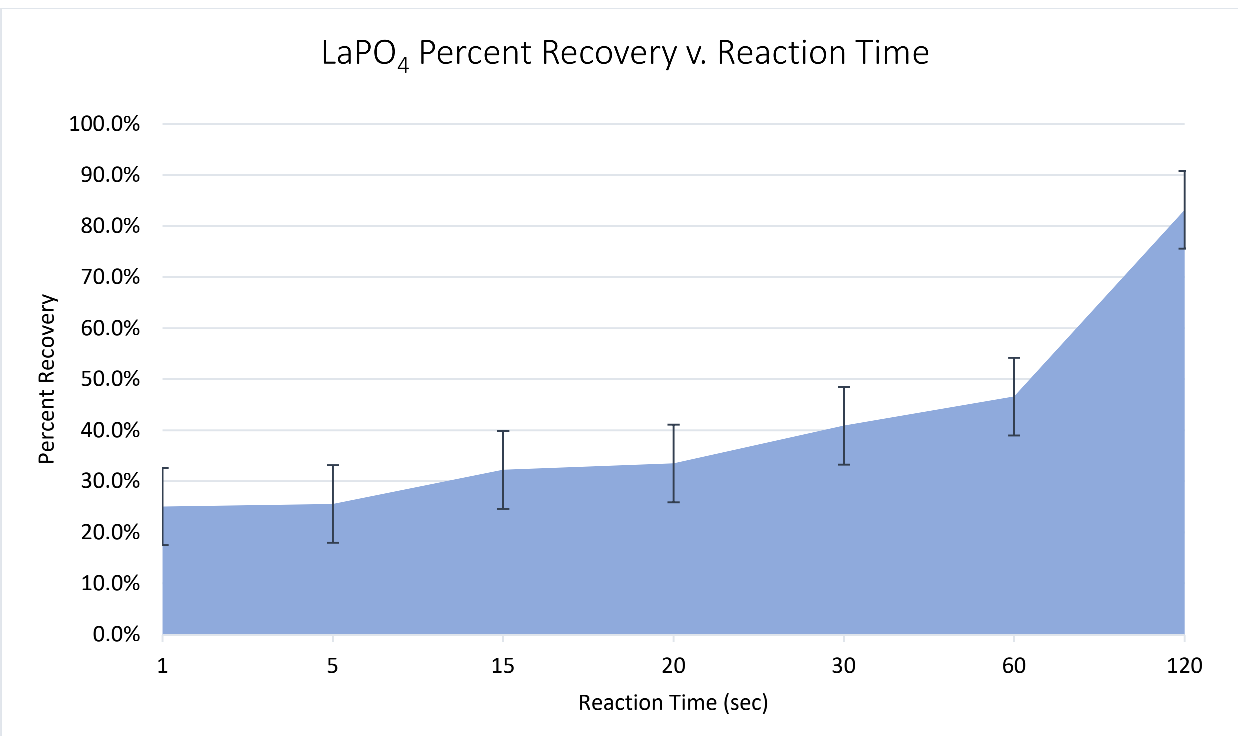


Figure 4. Percent recovery of LaPO4 from reaction of 0.1M $LaCl_3$ and 0.1M NaH_2PO_4 at various reaction times with a 20μm filter. Error bars show standard deviation. Note that recovery increases dramatically at120 seconds, indicating a far longer required time for lanthanum precipitation and crystallization than has commonly been assumed.

Discussion

While the reaction of lanthanum chloride and orthophosphate occurs nearly instantaneously, the data collected show that the time required for crystallization to a size that can be effectively removed by large scale LSS, namely high-rate sand filtration, is longer than has been previously assumed by many users of the flocculant. These data show that at 21ºC a contact time of over a minute is required for >50% precipitate capture, with 2 minutes being required for >80% capture. By combining these data with standard flow velocity formulas, a model for estimating length of pipe run as a function of particle capture percent desired, flow rate, and pipe diameter may be constructed. The equation for determining length of pipe required is as follows:

$$\text{Length of Pipe Needed} = \left(\frac{F\,(2.22801 \times 10^3)}{\left(\frac{D/12}{2}\right)^2 \left(\frac{3.14159}{1}\right)} \right) \left(C\,(1.29912) \right)$$

In the above equation F is equal to the water flow expressed in gallons per minute (GPM), D is the inside diameter (ID) of the pipe in inches, and C is the approximate lanthanum solids capture percentage (1-100) for a 20µm mechanical filter. Note that as this model is extrapolated from the benchtop data it predicts the behavior of $LaPO_4$ and $La_2(CaCO_3)_3$ precipitate capture at a single temperature (21ºC/70ºF) and there may be differences in warmer or cooler water. The same seconds per percent particle capture (1.29912) may also be inserted into a standard dwell time calculation to estimate the volume needed in a retention vessel (header tank or similar) to increase residence time for lanthanum crystallization prior to filtration as such:

$$V = \left(\frac{C\,(1.29912)}{60} \right) (F)$$

In this equation V is equal to volume in gallons, C is the approximate lanthanum solids capture percentage (1-100), and F is water flow (GPM). This equation can be used to size a retention vessel to increase contact time in lieu of a long pipe run prior to filtration. To use these equations to model flow dynamics in LSS in practical applications a spreadsheet calculator was created, available at this link, which also includes a calculator for dosing various lanthanum products as a function of tank volume, orthophosphate concentration, and desired removal: https://www.dropbox.com/s/pejp55kbew94w7x/LaCl3%20Calculator.xlsx?dl=0

Another major limiting factor is the mixing time of two solutions in PVC pipe. In a side-stream application significant pipe runs are required before solutions homogenize because of laminar flow. Thus, the pipe length required for lanthanum precipitation and crystallization must be added to the length required for mixing. The mixing coefficient can be estimated from literature where the mixing rates of saline solutions in pipe are described. Extrapolating the data from Sun et al. (2020) we can approximate the pipe run for mixing, roughly 10 feet for 2" ID PVC, 15 feet for 3" ID PVC, and up to 60 feet for 12" ID PVC (Fig. 5). This would add significantly to the required pipe run for a side-stream lanthanum system, though this can be mitigated with the use of a static mixer (Fig. 6) which can achieve >90% coefficient of mixing in less than 2 feet of pipe.

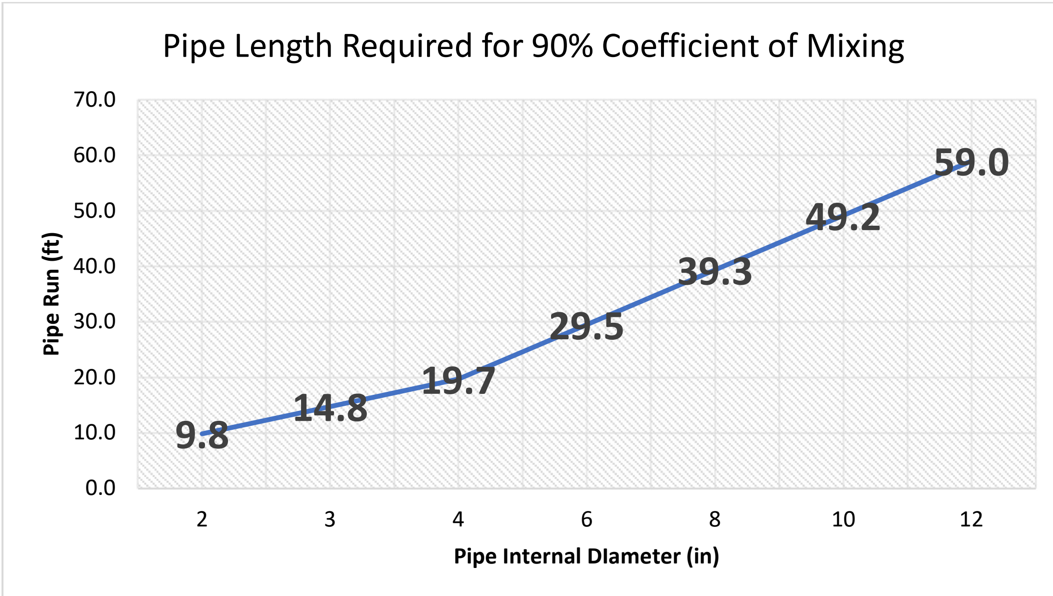


Figure 5. Pipe length required (ft) for mixing of two solutions with increasing diameter.

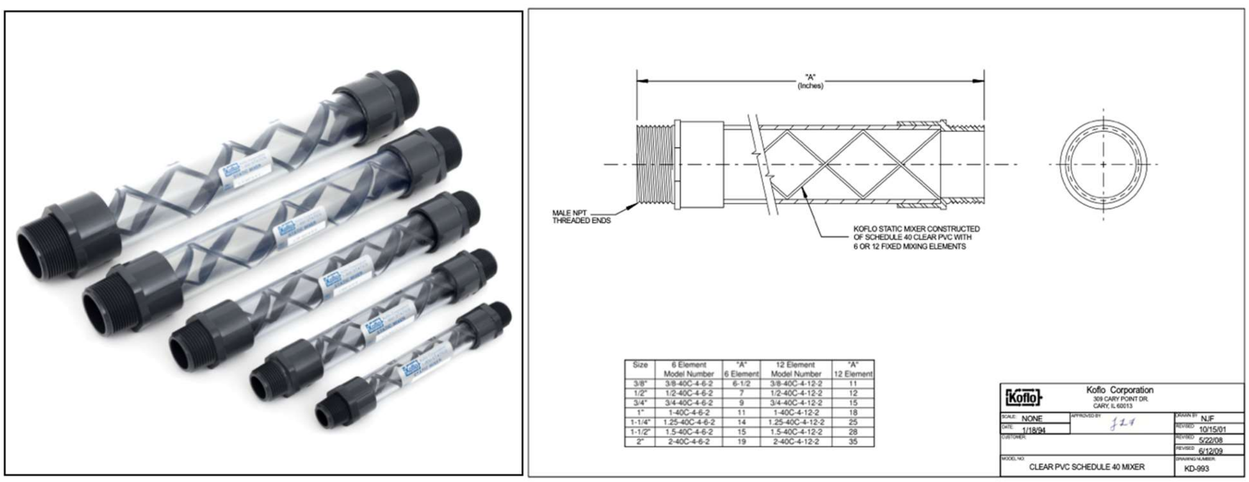


Figure 6. Static mixers which can substantially reduce pipe run required for mixing of two solutions. Such mixers are ideal for lanthanum injection into process flow to reduce pipe run required before filtration. Photo and specifications from Koflo Corporation & Ryan Herco Flow Solutions.

Assuming that mixing rate is not a contributing factor in pipe run required through the use of a static mixer or similar, as described above, the equations above can be used to create models predicting particle capture percentage as a function of a broad range of pipe sizes and flow rates. Four common pipe diameters were selected (2”, 3”, 4”, and 6”) were selected and appropriate flow rates for each pipe diameter were chosen (Hayward, 2023). These variables used in the above equation show that water velocity at standard flow rates is far too rapid to rely on precipitation and crystallization of $LaPO_4$ within PVC pipe as part of the LSS process flow. For example, with 2” PVC at 100 GPM a length of pipe over 140 feet would be required for just 25% particle capture, and over 550 feet would be required for 95% particle capture. For 3” PVC a length of 700-850 feet would be required for 95% particle capture, and values for other sizes yield similarly impractical pipe length estimates (Fig. 7).

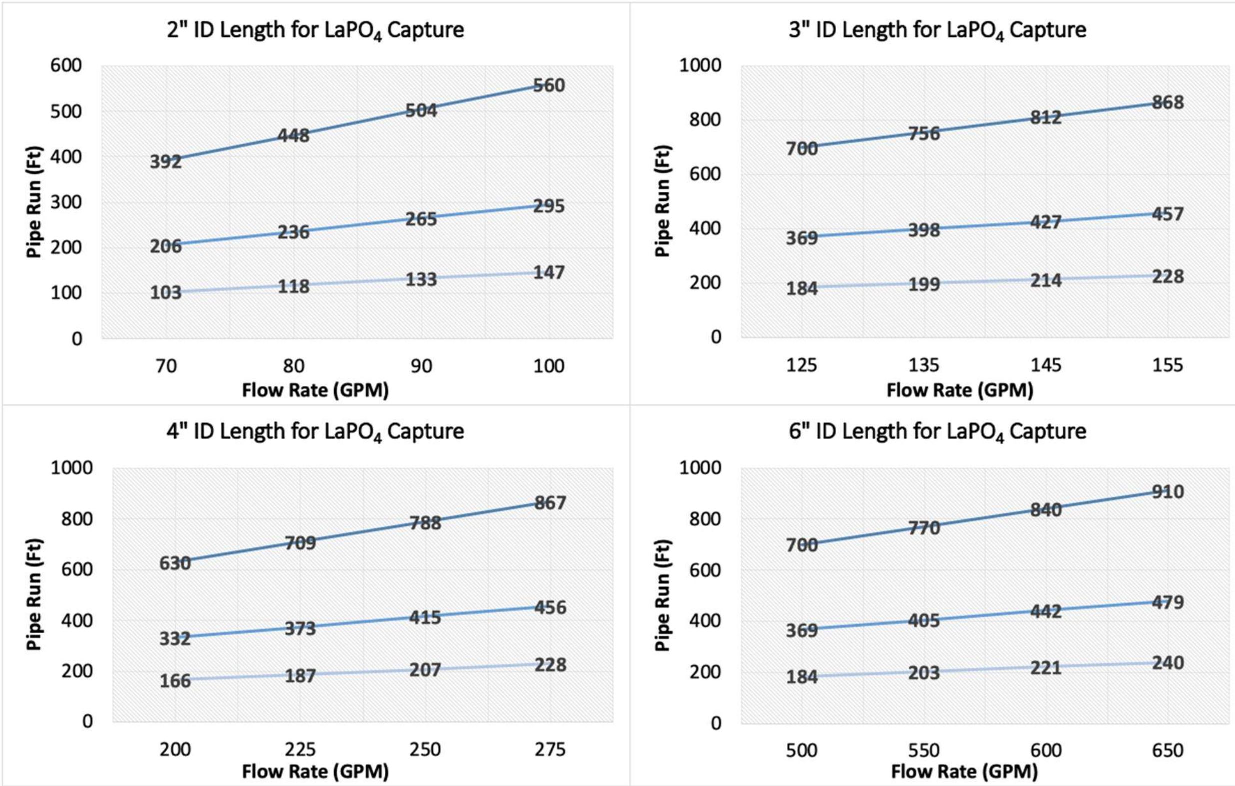


Figure 7. Pipe run required for four diameters of PVC pipe (2-6" ID) for lanthanum particle capture at various flow rates appropriate for each pipe size. Top line in each figure represent 95% particle sequestration, middle equals 50%, bottom line equals 25%.

However, when parameters in the model are adjusted, some potential solutions to efficient $LaPO_4$ precipitate capture become apparent. Area increases exponentially with increasing pipe diameter, and thus water velocity decreases exponentially with increasing pipe volume at a given flow rate. Therefore, the length of pipe required for precipitation of $LaPO_4$ decreases substantially as pipe diameter increases or flow rate decreases. While a pipe run of 560 feet in 2" PVC at 100 GPM flow would be required to allow 95% capture, if pipe diameter is increased to 6" and flow decreased to 10 GPM the length of pipe required for the same precipitation drops to less than 15 feet (Fig. 8).

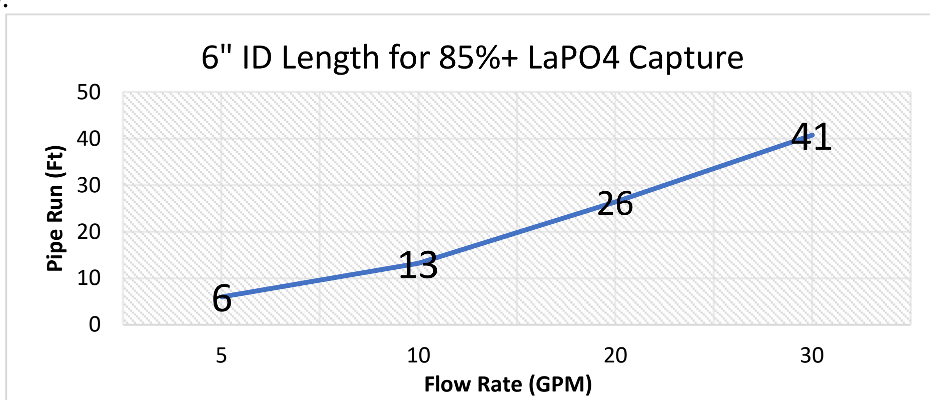


Figure 8. Pipe run required for 85% particle capture in 6" ID PVC at four low flow rates.

In a practical sense, manipulating the model as described above could be put into practice by creating a side-stream and running a low GPM water flow through a large trunk line, giving time for $LaPO_4$ to precipitate and crystallize to a size large enough for capture by a rapid sand filter. A standard side-stream filtration approach is reflected in the P&ID below (Fig. 4) where a 36" rapid sand filter is used to capture $LaPO_4$ precipitate as part of a larger LSS. In the hypothetical system below the lanthanum stock solution is injected into 2" PVC upstream of the sand filter with a conventional run of pipe. At 70-100 GPM the pipe run required for 95% particle capture would be 390-560 feet, and thus, in a conventional system well over 75% of the insoluble precipitate will break through the mechanical filtration and settle elsewhere in the system, potentially fouling piping, valves, or settling on acrylic panels or in the exhibit substrate.

If the hypothetical system in Fig. 9 is modified as described above to add a large diameter trunk line for precipitation of lanthanum solids, only 14 feet of 6" PVC would be required at a (slow) flow of 10 GPM in a side-stream (Fig. 10). This would decrease the velocity from 10.2 ft/s to 0.1ft/s and allow sufficient contact time in a short pipe run for precipitation and crystallization.

Alternatively, a retention vessel could be incorporated into LSS between the point of lanthanum injection and mechanical filtration. Sizing a large enough vessel will increase dwell time with increasing volume and allow more time for precipitation and crystallization of $LaPO_4$ without reducing the rate of process flow in the LSS. If the hypothetical LSS in Fig. 9 were modified to include a 200-gallon contact vessel (Fig. 11), then the time between the injection of lanthanum and mechanical filtration increases and as such 85-92% capture of lanthanum precipitates can be expected.

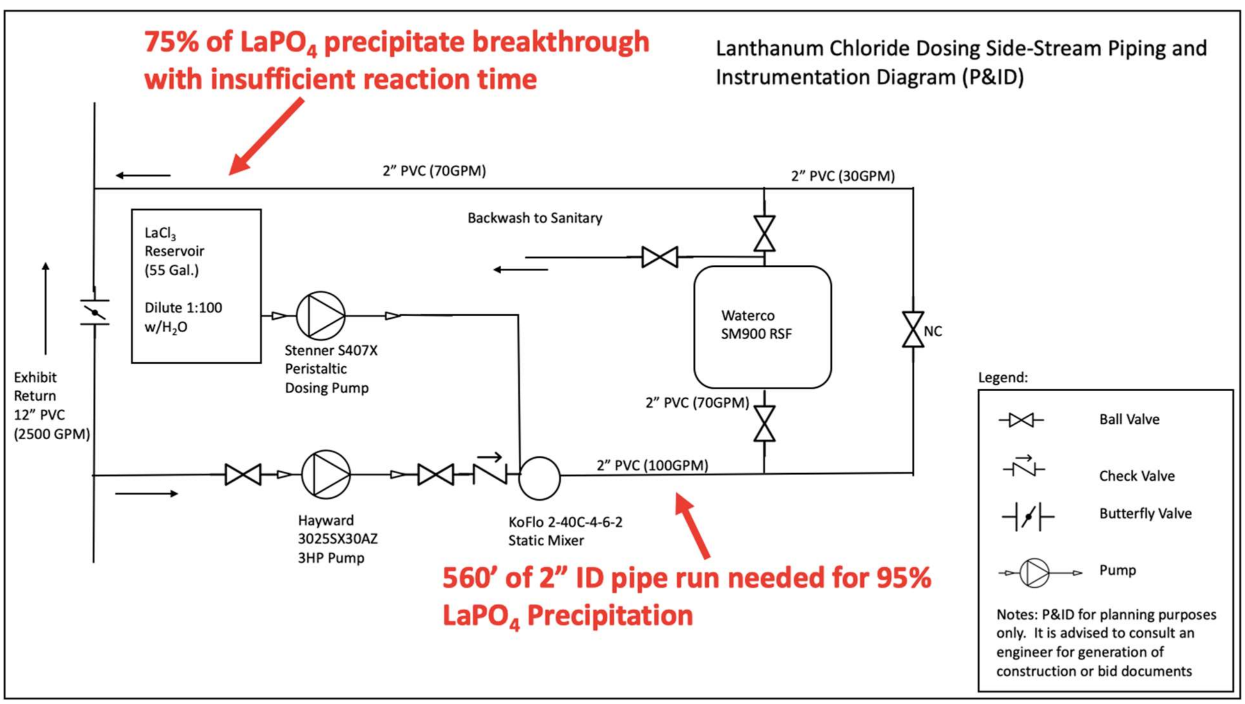


Figure 9. Diagram of a side-stream lanthanum filtration system. In this scenario, an impractical length of 2" PVC would be required for precipitation and crystallization of $LaPO_4$ and the majority of the precipitate would break through the filtration.

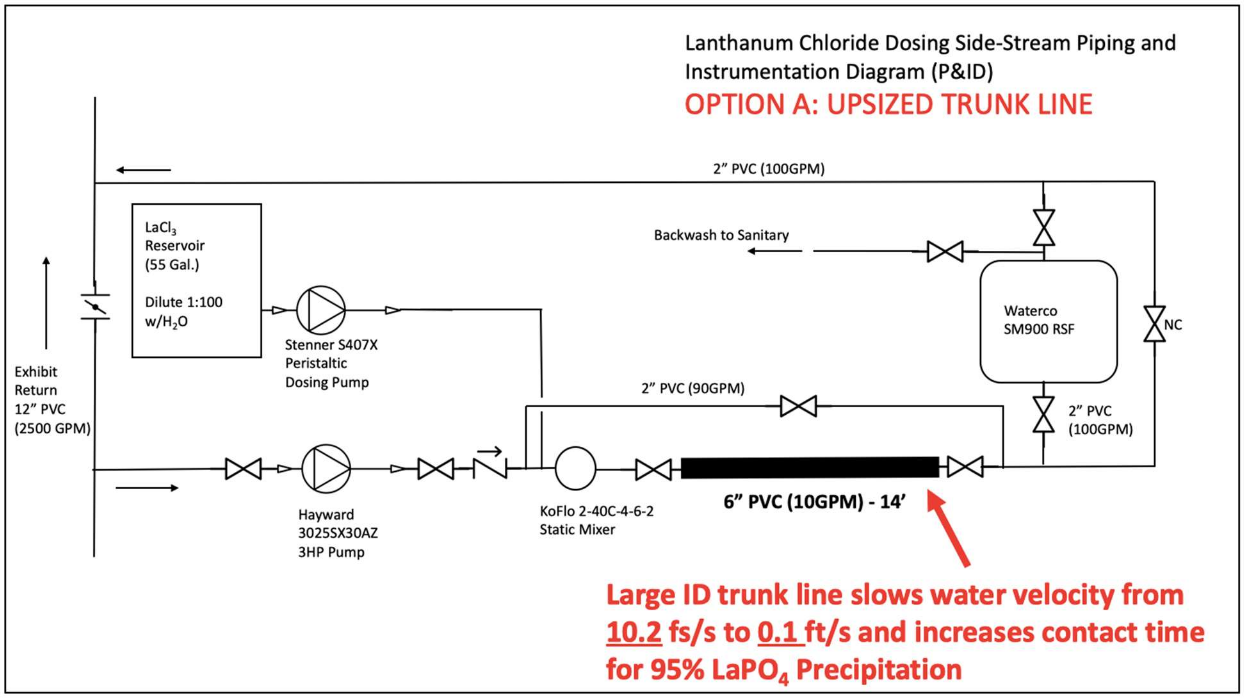


Figure 10. A side-stream lanthanum injection and filtration system with a large diameter trunk line (14 feet of 6" PVC) to provide a practical configuration to allow sufficient time for precipitation and crystallization of $LaPO_4$.

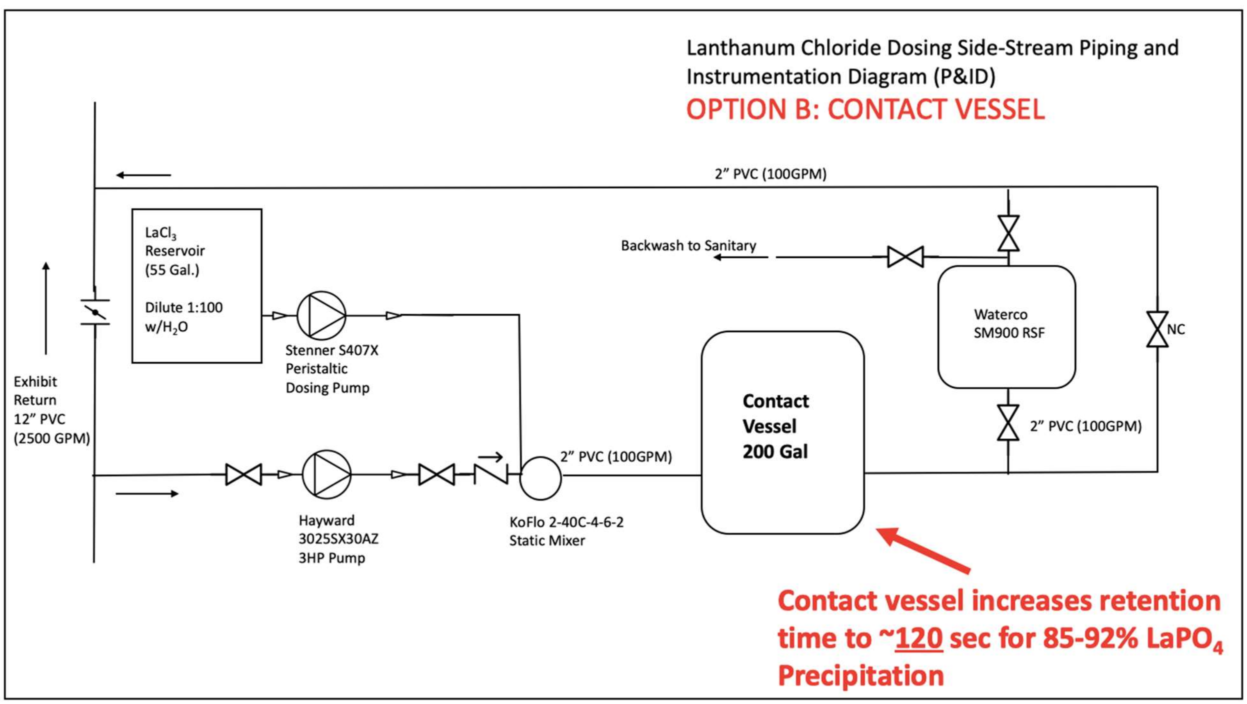


Figure 11. A side-stream lanthanum injection and filtration system with a 200-gallon retention vessel between lanthanum injection and filtration. This increased dwell time allows sufficient time for precipitation and crystallization of $LaPO_4$.

It should be noted that while the equations above based on the experimental data can be useful in predicting the dynamics of lanthanum precipitation and crystallization in LSS systems, the models do have limitations. Warmer temperatures may allow more rapid crystallization and reduce the amount of time required for effective particle capture, but to what degree these data cannot predict. Also, in smaller systems, filtration to a smaller pore size than traditional sand filtration allows (20μm) will decrease the time needed for crystallization of insoluble precipitates.

When starting lanthanum dosing, it is advisable to start slowly, and dose very conservatively at first, titrating up to a higher dosage. Adding a single large dose to a system will lead to excessive precipitation of insoluble $LaPO_4$ (and $La_2(CO_3)_3$ in seawater) and can be quite damaging to filters, piping, valves, acrylics, and irritating or toxic to live animals in high concentrations. Lanthanum flocculants are best added in dilute form very slowly, and deliberately removed via mechanical filtration. A sensible approach is to calculate the daily average rise in orthophosphate from multiple analyses, then size the daily lanthanum dose to this value, plus 10-15%, such that orthophosphate values are slowly reduced over time. Also note that in seawater, or alkaline freshwater, slightly more lanthanum will be required as it also reacts with carbonates, and this parallel reaction will drive down pH by consuming alkalinity (HCO_3^-) and producing hydrochloric acid (HCl). Additional information on dosing rates is included as an appendix.

These experimental data, and the models to predict pipe length required do however suggest some design considerations for removal of lanthanum precipitates. These inferences reduce the likelihood of lanthanum breakthrough and the maintenance required to clear fouling while preventing installations of excessive lengths of piping between point of $LaCL_3$ injection and mechanical filtration.

Literature Cited

AALSO. 2020. A Field Guide to Water Quality practices, Common System Components, and Practical Mathematics. Association of Animal Life Support Operators. 281p.

Hayward. 2023. Flow of Water Through Schedule 80 Plastic Pipe. Hayward Flow Control.

Hua, D., Wang, J., Yu, D., and Liu, J. 2017. Lanthanum exerts acute toxicity and histopathological changes in gill and liver tissue of rare minnow (*Gobiocypris rarus*). Ecotoxicology. 26: 1207-1215.

Sun, B., Lu, Y., Liu, Q., Fang, H., Zhang, C. and Zhang, J., 2020. Experimental and numerical analyses on mixing uniformity of water and saline in pipe flow. Water. 12(8): 2281.

US Dept. Interior, Federal Water Quality Administration. 1970. Removal of Phosphate from Wastewater Using Lanthanum Precipitation. Program 17010-EFX.

Young, S.J.F. and Thoney, D.A. 2014. Phosphate Control and Algae Management Using Lanthanum Chloride in Three Marine Aquariums. Proceedings of the Aquality II Symposium. Public Aquarium Husbandry Series.

Zhang, Y. and Wong, K.S. 2012. Lanthanum chloride or lanthanum carboxylate for orthophosphate removal in seawater aquarium- a feasibility study. Ocean Park Hong Kong.

Appendix: Lanthanum Dosing Formula

Aside from how long of a pipe run is needed for lanthanum reaction before filtration, one of the most common questions asked of the author is how to calculate a dosing scheme for lanthanum. This is a complex question, because every system is different and will respond differently to dosing of flocculants. Moreover, there are a number of different commercially available salts used by different manufacturers to create their products, each with varying molecular weights of lanthanum relative to the weight of the overall salt (i.e. hydrated salts, different anions). The following provides an estimation to begin experimentation with lanthanum dosing:

$$\text{Dose in ml} = \frac{\left(\left(V(3.785)\,P_i\right)\left(V(3.785)\,P_d\right)\right)\left(\frac{100}{C}\right)(L_c)}{1000}$$

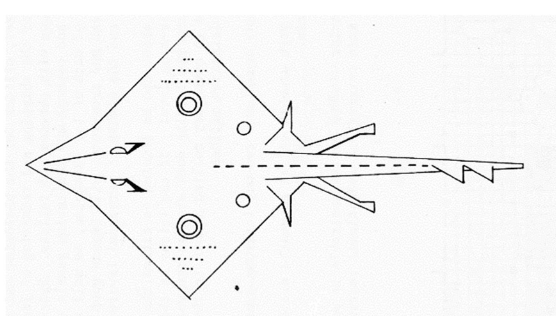
In the above the variables are defined as follows:

- V = volume in gallons
- P_i = initial orthophosphate concentration in mg/L
- P_d = desired orthophosphate concentration in mg/L
- C = percent strength of lanthanum stock solution expressed as a whole number (1-100)
- L_c = correction factor for the type of lanthanum in stock solution (see below)

Correction factors:

- Lanthanum chloride anhydrous ($LaCl_3$) = 1.76
- Lanthanum chloride heptahydrate ($LaCl_3{\bullet}7H_20$) = 2.67
- Lanthanum glycolate ($La(C_2H_3O_3)_3$) = 2.61
- Lanthanum glycolate heptahydrate ($La(C_2H_2O_3)_3{\bullet}7H_2O$) = 3.52
- Lanthanum acetate hydrate ($LaCH_3O_2{\bullet}H_2O$) = 1.46

These dosing estimates may also be approximated from the spreadsheet calculator at this link:
https://www.dropbox.com/s/pejp55kbew94w7x/LaCl3%20Calculator.xlsx?dl=0



POSTER ABSTRACTS from Jelly Camp 2023

October 7-9, Baltimore, MD USA.

Hosted by the National Aquarium and the Smithsonian National Museum of Natural History

Jennie D. Janssen, National Aquarium jjanssen@aqua.org

In October 2023, 65 jelly experts from the United States, Canada, and the Bahamas convened in Baltimore, Maryland, USA to attend Jelly Camp 2023 – a first of its kind conference bringing together jelly aquarists and jelly researchers to advance their collective knowledge of jellyfish through building collaborations between public aquaria and research/academia (Figure 1). The conference was preceded by a 1½ day workshop for the co-authors of the 3rd edition of the Association of Zoos and Aquariums Animal Care Manual for Jellyfish. There were also pre- and post-conference behind of house tours of the Invertebrate Zoology Department at the Smithsonian National Museum of Natural History and the National Aquarium's Animal Care and Rescue Center.

Figure 1. The Jelly Camp 2023 logo. Illustrated by Jelly Camp 2023 attendee Nicholas Bezio (Jelly Camp 2023 attendee) and designed in conjunction with the Jelly Camp 2023 Planning Team to feature local jelly species *Chrysaora chesapeakei* and *Mnemiopsis leidy* below the waves and in a shape reminiscent of a pseudokreisel to combine *in situ* and *ex situ* aspects of jellyfish expertise.

Jelly Camp 2023 provided invaluable opportunities to introduce professionals from aquaria and academia to each other, gain perspectives on building future collaborations, and create a jelly centric network to serve this joint community going forward. Jelly aquarists and researchers interested in joining this network can gain access through Jennie Janssen or any jelly professional already in the network. Presentations and workshops included classroom and hands-on learning to expand and better share our collective knowledge. Workshops were recorded by Animal Professional and are posted on their website www.animalprofessional.com. A serendipitous highlight of the conference was the fortuitous appearance of multiple jelly species in Baltimore Harbor on the day we conducted a jelly collecting workshop on the Aquarium's research vessels R/V Nani and R/V Spartina.

Workshops and the ice breaker event were held at the Institute of Marine and Environmental Technology (IMET) in the Rita Rossi Colwell Center where the National Aquarium (NA) Culture Lab resides. Attendees were invited to tour the NA Culture Lab during the ice breaker event. The following evening's event was held at the National Aquarium where attendees toured the Aquarium as guests before partaking in a private reception and poster session in front of NA's *Jellies Invasion* exhibit. The back-of-house spaces of *Jellies Invasion* were also open for attendees to tour during that evening event. Thirteen posters were displayed throughout the conference at IMET and then at NA for the poster session. Two awards were presented at the closing of Jelly Camp 2023 for the honor of Best Poster.

While there is no plan for Jelly Camp to be a recurring event as a conference, there was great interest in another Jelly Camp conference sometime in the future, if not a recurring conference every few years. Therefore, the National Aquarium will be discussing options for either hosting another Jelly Camp in the future or seeking other potential hosts to pass the mantle to. Regardless, all were encouraged to take home the idea of hosting low stress, local day trip Jelly Camp field trips to visit nearby jellyfish experts build more collaborations regionally. That is, after all, how the idea of Jelly Camp got started between the two host organizations. To follow random happenings around this idea and event, you can search or follow #JellyCamp2023 and/or #thisisjellycamp on social media.

Acknowledgements

Many thanks to all the conference organizers, presenters, and poster judges: Dr. Allen Collins, Annie Blanken, Abigail Balk, Alexandria Caviness, Allissa Lucas, Sharyl Crossley, Dr. Stephanie Allard, Dr. Chad Widmer, Luke Rollins, Dr. Rebecca Helm, Dr. Mónica Medina, Victoria Sharp, Vivian Li, Dr. Abigail Reft, Rachel Stein, Haley Beck, Matt Wade, Barrett Christie, Laren Mellenthin, Danielle Castillo, Stephen Spina, and Dr. Christopher Mah. Thanks also to John and Ryan Gardner of AnimalProfessional.com and Brian Nelson, Jay Bradley, Allan Kottyan, and Meredith Meyers of the National Aquarium Marine Operations team. Special thanks to Rachel Thayer, Sharyl Crossley, and Dr. Allen Collins for serving as our Code of Conduct Points of Contact throughout the conference.

For making Jelly Camp 2023 a reality, thanks go to both host organizations – the National Aquarium and the Smithsonian National Museum of Natural History – for their ideological and financial support, as well as our many sponsors who excitedly stepped up to support this inaugural jelly jam: Immortal Sponsors – Abyzz, Aquatic Equipment & Design, Asahi America, Georgia Aquarium, GLMV Architecture, Gulf Specimen Marine Laboratories, Shedd Aquarium, TJP Engineering; Medusa Sponsors – Exotic Aquaculture, Piscine Energetics, Reynolds Polymer Technology, Satchell Engineering, ZooQuatic Laboratory; Ephyra Sponsors – Dynasty Marine

Associates, Flying Sharks, JelliQuarium by Midwater Systems, Marine Enterprises International, RK2 Systems, Sunset Marine Labs; Polyp Sponsors – Tracks Software, U.S. Mysids.

* * *

Using Scientific Illustration to Help Visualize the Strange and Beautiful Diversity of Jellies

Nicholas Bezio
University of Maryland, College Park, MD USA
Smithsonian National Museum of Natural History, Washington, DC, USA

Science illustration is an important and influential tool for the scientific community's ability to communicate their findings to a wider audience. Through scientific illustration, complex concepts and phenomena that are not easily observable to the naked eye can be displayed in a universal format. Knowledge of deep-sea environments and appreciation for the biodiversity and role zooplankton play in marine ecosystems is severely limited in the general public and, to an extent, among scientists. Furthermore, it is challenging to visualize and comprehend certain organismal groups due to the inherent "strangeness" and abstraction their bodies adapt. Due to these inherent difficulties when studying zooplankton, specifically jellyfish, illustration is often vital to making long-lasting identifications and descriptions of unique biological processes. Over the years, I've collaborated with numerous researchers and citizen scientists worldwide, documenting the biological processes within the jellies and aiding in describing their biodiversity, often finding new species along the way. By utilizing physical specimens or photographs from divers, I work with researchers and citizen scientists alike to create realistic illustrations to bring these beautiful animals to life. The final product is a high-resolution and realistic illustration that is both valuable to researchers with anatomical accuracy (i.e., # of lappets, the diameter of the bell, # of tentacles, etc.) and visually engaging with the general public. This poster features various examples of illustrations I completed in collaboration with numerous individuals worldwide to bring awareness to a wider audience about these beautiful yet fragile animals.

Creating Successful Partnerships with Biological Illustration Programs to Increase Capacity for More Effective Scientific Communication

*Annie Blanken, Jennie Janssen, Jack Cover
National Aquarium, Baltimore, MD, USA

Within the National Aquarium (NA) Animal Care and Welfare Department, there are a variety of natural processes and techniques that are more effectively communicated visually than through written word alone. Since 1994, NA has partnered with the Johns Hopkins School of Medicine's Medical and Biological Illustration graduate program to provide 88 of their students with real-world experience in working with clients to develop illustrations that serve operational and educational needs for the Aquarium. Through this annual assignment, NA's content experts gain experience in conveying their big idea, and if selected, working with the artist in the conceptualization of the illustration, and reviewing the various drafts to ensure accuracy and focus on scope. This partnership has been an effective tool for building science communication skills and mentorship experience for NA content experts and creating beautiful and effective visuals to assist in communicating complex concepts to colleagues, new staff, and the general public. This poster features a step-by-step guide illustrating the in vitro fertilization technique utilized by the

Jellies team at NA, as well as several other illustrations marshalled by the Jellies team over the past decade. Developing similar collaborations with art schools or other artist outlets should be considered by facilities or organizations looking to increase their overall scientific communication capacities.

Using *Gonionemus vertens* Polyps as an Educational Tool to Teach Asexual Reproduction, Population Growth, and Novel Observations on Life History

*Paul Bologna and Angela Chemdlin
ontclair State University, Montclair, NJ, USA

Hydrozoans represent a novel group of organisms that can be used to teach a variety of foundation biological concepts. Many species exhibit a bi-phasic life history which can teach principles of alternation of generations applicable to plants and algae. They also provide an opportunity to discuss sexual vs. asexual reproduction and their impacts on populations and species abundance. Lastly, they can be used in a more experimental pathway to evaluate clonal growth and reproduction coupled with physiological constraints like temperature and salinity. We have developed culturing techniques using *Gonionemus vertens* polyps that allow students to generate clonal growth rates under varying temperatures ranging from 20-24C. Results from large scale class activities indicate that optimal growth was achieved at higher temperatures (24C), compared to slower growth at low and intermediate temperatures. Beyond simple evaluation of clonal growth rates, utilizing classroom research activities has allowed us to observe undiscovered growth patterns and potential stress responses to changing environments. For example, students observed a polyp which was producing a medusa bud, but subsequently reverted into a tissue mass that produced numerous frustules generating new polyps adjacent to the original polyp. Other observations include polyps producing numerous tissue masses underneath themselves, potentially akin to scyphozoan production of podocysts. Continued use of this species as a teaching and research model may help elucidate the mystery of the missing polyps in the field.

What's That on the Aquarium Wall? A New Species of *Scolionema*

*Allen Collins[1,2], Rebecca Helm[1,3], Nicholas Bezio[1,4], Megan O'Grady[1,5]
[1]Smithsonian Institution, Washington, DC, USA
[2]National Oceanic and Atmospheric Administration, Washington, DC, USA
[3]Georgetown University, Washington, DC, USA
[4]University of Maryland, College Park, MD, USA
[5]Loyola University Maryland, Baltimore, MD, USA

The Department of Invertebrate Zoology in the Smithsonian National Museum of Natural History has approximately 50 million specimens in its collection. These are preserved in various way – embedded on permanent slides, formalin, ethanol, dry, etc. – and nearly all dead. At the same time, the department maintains a small facility, known as IZ's AquaRoom, for maintaining living species with simple techniques. More than a dozen species of medusozoan jellyfish are maintained in IZ's AquaRoom, mainly in their polyp phase, isolated in dishes to prevent cross contamination. In addition, the room contains a community tank, most notably inhabited by an octocoral, *Convolutriloba* flatworms, and various algae. On this wall of this community tank a small solitary polyp, provisionally identified as a member of the hydrozoan order Limnomedusae,

was noticed, and removed to a dish containing 33 ppt room temperature water. For months, the polyp occasionally produced creeping frustules. After approximately three months, these frustules began to metamorphose into polyps establishing an asexually replicating culture. Several months later, some of the polyps produced medusae, which were reared to adult. Adult medusae conformed to the Limnomedusae genus *Scolionema*, which presently contains two known species. Genome sequence has been derived from the AquaRoom line of *Scolionema*, and the integration of morphological and molecular data indicate that it represents a species that has yet to be named and described. We are searching available genetic data derived from metabarcoding studies to identify the native range of this species prior to publication.

***Velella velella* Medusae Husbandry and Culture**

Rebecca Helm
Georgetown University, Washington, DC, USA

Velella velella polyps, known as by-the-wind sailors, wash up on beaches around the world, yet we have not developed methods for keeping these or other floating polyps in captivity. One challenge to keeping *Velella* polyps in their complex life cycle, which includes a small medusa. I have collected medusae on two occasions and am working to develop optimal culture conditions. I present my progress and setbacks in maintaining medusae of this species, and seek feedback for future efforts. I hope this work inspires others to join in the adventure to culturing *Velella*.

***Cassiopea xamachana* Microbiome Conserved Across Tissue Types and Florida Sites, But Varies Across Developmental Stages**

*Allison H. Kerwin[1], Aki Ohdera[2], Juliet Bier[1], Devon Goodman[1], Marta Mammone[2], Victoria Sharp[2], Alesandra Escheandia[2], Mónica Medina[2]
[1]McDaniel College, Westminster, MD, USA
[2]Pennsylvania State University, University Park, PA, USA

The upside-down jellyfish, *Cassiopea xamachana*, is a useful model system for tri-partite interactions between the cnidarian host, the photosymbiont, and the bacterial microbiome. The bacterial microbiome of wild *C. xamachana* is largely uncharacterized. To explore the bacterial community, wild medusae (n=6) and larvae (n=3) were collected from two Florida Keys sites. Bacterial community composition was characterized via amplicon sequencing of the 16S rRNA gene V4 region. The medusa bacterial community was dominated by members of the Alphaproteobacteria and Gammaproteobacteria, while Planctomycetota, Actinomycetota, Bacteroidota, and Bacillota were also present. Community composition was consistent between locations and tissue type (arm, bell, and gonad). The larval bacterial community clustered apart from the medusa community in beta diversity analysis and was characterized by the presence of several Pseudomonadota taxa that were not present in the medusa. A bacterial isolate library encompassing much of the amplicon sequencing diversity was developed and tested via metabolic assays. Most characteristics were not correlated with host sex or tissue type, but gelatinase production was more common in lapellet isolates, while lactose fermentation was more common in female oral arm isolates. *Endozoicomonas* sp. was dominant in both amplicon sequencing and in our isolate library, and was equally prevalent across tissue types and sexes. The bacterial composition of *C. xamachana* resembled that of tropical corals, but appears less species diverse.

Characterizing the bacterial consortium of this symbiosis is an important step in the development of this model cnidarian system, crucial as tropical corals come under increasing threats from global change.

Symbionts of Symbionts: The Influence of Microbiomes on Symbiodiniaceae Physiology and Eco-Evolution

*Vivian Yifan Li and Mónica Medina
Pennsylvania State University, University Park, PA, USA

Cnidarians, including the upside-down jellyfish *Cassiopea*, depend on microalgal endosymbionts (Symbiodiniaceae) for resources that support growth, development, reproduction and metabolism. Conversely, Symbiodiniaceae have also evolved a dependence on their cnidarian hosts for a suite of nutrient needs and waste removal processes.

Many species of Symbiodiniaceae have been successfully maintained in culture, *ex hospite*, for decades. Recently, there have also been efforts to generate axenic (bacteria-free) cultures of Symbiodiniaceae. The difficulty of culturing axenic Symbiodiniaceae suggests that bacteria in free-living cultures may act as "nutrient surrogates" for symbionts in the absence of a cnidarian host. Thus, examining how bacterial microbiomes benefit free-living Symbiodiniaceae in culture may provide insight into the various benefits Symbiodiniaceae receive from their cnidarian host *in hospite*.

My research examines relationships between Symbiodiniaceae and their bacterial microbiomes, seeking to answer the question of how Symbiodiniaceae microbiomes influence their physiologies from an eco-evolutionary perspective. I will characterize the microbiomes of 5 Symbiodiniaceae lab cultures spanning a range of physiological traits and phylogenetic placement. I will determine the community structure and composition of the microbiomes of each of these cultures using amplicon sequencing of the 16S rRNA gene. I will then investigate the putative metabolic capabilities of key/core microbiome members that may be providing benefits to Symbiodiniaceae in free-living cultures.

Studying Symbiodiniaceae microbiomes thus generates valuable insight into the health and functioning of cnidarian holobionts, providing new perspectives for conservation in the face of climate change.

Blue Ain't Your Color, But It Might Be Cassiopea's: The Effect of Color on Thermal Tolerance and Clonality in The Upside-Down Jellyfish

*Megan Maloney and Katherine Buckley
Auburn University, Auburn, AL, USA

Some marine cnidarians have complex reproductive strategies which can alternate between sexual and asexual. Despite being clonal, asexually produced cnidarians can exhibit high levels of morphological variation depending on their developmental environment. This can be exceptionally beneficial in unpredictable environments, which are compounded by climate change. Determining trait variability in clonal organisms and how these traits increase fitness enables researchers to better predict who will be successful when faced with global change. The upside-down jellyfish (Cassiopea sp.) are highly tolerant to stressors associated with ever-changing marine environments, particularly extreme temperature variations Additionally, Cassiopea exhibit conspicuous color variation, in both hue and the quantity of colored appendages. However, it is unknown if clonal Cassiopea have variation in coloration at the ephyra stage. Therefore, we aim to determine: (1) if blue coloration varies between ephyra from a clonal line of polyps, (2) if having blue coloration could be advantageous under heat stress as adults and (3) if adults individuals are able to acclimate to increased temperatures. To measure the variation in coloration of clonal ephyra, we stressed polyps to induce strobilation and then quantified color variation. We found morphology differences between clones, and that the presence of blue coloration in adult Cassiopea resulted in higher lethal temperatures. These results suggest that coloration may play a critical role in survival under increased temperatures.

Received Second Place for Best Poster

Fungal Symbiont Diversity in *Cassiopea xamachana*

*Victoria Sharp, Marta Mammone, Mónica Medina

Pennsylvania State University, University Park, PA, USA

Marine fungus is found in every ocean body, yet are relatively understudied as symbionts. Despite their significance in the life cycles of organisms like the bobtail squid and coral, there has been little exploration into the presence of fungus in marine organisms. The upside-down jellyfish *Cassiopea xamachana* is a model organism for many diverse topics such as algal photosymbiosis and bioaccumulation from the environment. We sampled male and female *C. xamachana* adults from 3 locations in Key Largo, Florida, USA and genetically barcoded fungus from 4 tissue compartments and their mucus. Additionally, tissue and mucus from lab-reared *C. xamachana* was taken for analysis. By comparing wild and lab-grown jellies, we explore the possibility of obligate fungal symbionts for this host and the extent that environmental factors play on *C. xamachana* accumulation of endosymbiotic fungus.

Diving Mega Moon, One of the World's Largest Jelly Exhibits

Polly Strott
Georgia Aquarium, Atlanta, GA, USA

Georgia Aquarium in Atlanta is known for its unique and impressive display. Tending to the needs of such exhibits requires a diverse team and creative ways of completing maintenance tasks. Mega Moon, a twenty-five-thousand-gallon habitat with a depth of eighteen feet and a forty-foot-long window, is no exception. The curved front exhibit uses eleven top and bottom spray bars for water movement and eleven standpipes of varying heights, located within the overflow boxes, to pull water into the sump. This massive exhibit houses over four hundred moon jellies and periodic dives are necessary to maintain the system. Due to the number of animals in Mega Moon and the labor required to remove them, the choice was made to dive the tank with the jellies still on exhibit. This was an unprecedented decision for the facility. To facilitate this, aquarists determined that rearranging the location of the two shortest and two tallest standpipes was the simplest and most effective way to shift the moon jellies to one side of the habitat while divers cleaned the other. Working with the aquarium's seamstress, aquarists then created a cloth barrier around a PVC skeleton to sequester the jellies while divers were in the water. The barrier is placed into the exhibit before the dive begins. Divers then secure the barrier to the window and back wall using suction cups. After many iterations, this innovative method of diving with jellies enabled a streamlined deep cleaning of half of the exhibit each month, while also extending the display quality of the moon jellies by minimizing stress and potential damage to their bells.

Severe Seawater Acidification Causes a Significant Reduction in Pulse Rate, Bell Diameter, and Acute Deterioration in Feeding Apparatus in the Scyphozoan Medusa *Cassiopea* sp

*Rachel Thayer[1], Ian Brunetz[2], Shrayen Daniel[2], Karah Nazor Rivers[2], and Cecilia Wigal[3]
[1]Tennessee Aquarium, Chattanooga, TN, USA
[2]McCallie School, Chattanooga, TN, USA
[3]University of Tennessee at Chattanooga, Chattanooga, TN, USA

The detrimental effect of ocean acidification (OA) on marine animals with carbonate exoskeletons or shells is an issue drawing increased attention in marine biology and ecology, yet few studies have focused on the impact on gelatinous organisms like scyphozoan medusae. Here, we examined the physiological tolerance of Cassiopea sp., an abundant genus of scyphozoans valuable for their role as bioindicators and for having similarities to other cnidarians, to OA by conducting three, 12-week trials using CO2 diffusers and electronic pH controllers to incrementally lower the water to test pHs of 7.5 and 7.0. The impact of reduced pH on the survival, pulse rate, bell diameter, and reorientation and settlement abilities of Cassiopea sp. medusae were measured weekly. Cassiopea sp. was tolerant to pH 7.5 while further reduction of the pH to 7.0 resulted in 22.22% mortality rate, which was significantly different from the control and treatment pH 7.5. Significant differences between the treatment pH 7.0 and control first occurred on day 23.5 with a 50% reduction in the pulse rate, and on day 36 with a 16.6% reduction in bell diameter, while pH 7.5 had no effect. By the final time point of 66 days in treatment pH 7.0, there was an 87% reduction in pulse rate and a 36% reduction in bell diameter versus control. Reduced pH 7.0 caused bell malformations, inhibited swimming abilities, and deterioration of the oral arm feeding apparatus, but had no effect on the orientation and settlement assay. Observations indicate that asexual reproduction via planuloid production and strobilation was unaffected by pH reduction, though polyps in treatment pH 7.0 gave rise to ephyrae with inverted bells. Combined, findings from this study demonstrate Cassiopea sp. to be resilient to the end of century ocean acidity prediction of pH 7.6, and vulnerable to more severe OA to pH 7.0.

Received First Place for Best Poster

Tale of Two Seas: Effect of Temperature and Hypoxia Effects on the Metabolic Rates of Salpa fusiformis from the Pacific and Atlantic Oceans.

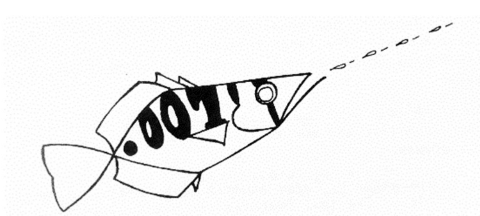
Lloyd Trueblood
La Sierra University, Riverside, CA, USA

Salps are pelagic tunicates vital to carbon cycling. They contribute through filter feeding and converting waste into dense fecal pellets, which rapidly descend to the deep ocean. Despite their importance, there's a notable gap in research focused on salp physiology. Specifically, few studies have delved into how environmental changes, such as variations in temperature and dissolved oxygen, affect their basic physiological processes. In this study, I investigated the effects of temperature and oxygen partial pressure on the metabolism of *Salpa fusiformis* sourced from two distinct regions: the California Current in the Pacific Ocean and the Sargasso Sea in the Atlantic Ocean. For individuals collected from the California Current, routine metabolic rates were 1.66 µmol O_2 g-1 h-1 at 10°C and 3.95 µmol O_2 g-1 h-1 at 17°C, yielding a Q10 value of 3.45. In contrast, those from the Sargasso Sea displayed metabolic rates of 2.35 µmol O_2 g-1 h-1 at 14°C and 6.00 µmol O_2 g-1 h-1 at 21°C, with a closely corresponding Q10 value of 3.81. Individuals from both locations were able to respire oxygen down to lowest detectible levels. This research sheds light on the observed metabolic decrease and subsequent carbon output related to temperature decreases during vertical migration. Furthermore, the study explores their hypoxia tolerance in the context of the distinct oxygen profiles of the two sampled locations.

Cassiopea as a Model to Study Organism-Environment Interactions in Response to Global Change

*Ziyu Wang and Marie Strader
Texas A&M University, College Station, TX, USA

Cassiopea xamachana is an epibenthic Scyphozoan expanding its range in multiple locations globally, likely driven by its high tolerance to multiple abiotic factors. In the Strader Lab, we are developing *Cassiopea* as a model to study interactions between the changing environment and shifts in organismal traits. We have developed a robust husbandry system to utilize *Cassiopea xamachana* to address the following research aims: 1) determine the thermal tolerance limits of *Cassiopea xamachana* at multiple life-history stages to predict current and future distributions in Florida, 2) model range expansion and phenotypic distributions from observation data, and 3) understand the role of the abiotic environment in regulating sleep behaviors. We have found that *C. xamachana* polyps demonstrate cold tolerance up to 13˚C, which may signify potential thermal limits that could constrict their northern range expansion. Further, from iNaturalist observations, we show evidence of range expansion northward in Florida. Finally, we are investigating how sleeping behaviors are modulated by temperature and light regimes.



PAUL L. SIESWERDA
06 MARCH 1942 – 10 DECEMBER 2023

Steven L. Bailey

The public aquarium industry lost one of its most enthusiastic and dedicated members on Sunday December 10, 2023 when Paul L. Sieswerda – aquarist, curator, author, administrator, AZA Bean Award winner, liaison, documentary film subject, researcher, educator, and industry cheerleader – lost his battle with cancer. He was an outstanding mentor, coach, friend, upbeat colleague, fair & just supervisor, passionate idea guy, athlete, and excellent travel companion.

https://gothamwhale.org/, https://obits.robinsonfuneralhome.com/obituary/paul-sieswerda

Paul's (PLS to many) work spanned seven decades [Count 'em: from the '60s to the '20s] at the New England and New York Aquariums, and then on to the non-profit he founded, Gotham Whale. His enthusiasm and drive to make a difference never failed him. He'd roll up his sleeves on "lesser projects" somehow knowing that those in time would create big change. PLS was an inviting and generous colleague; he seemed to never be disappointed if he couldn't quite sell what he was working on to you.

Paul's reflex was to share any discovery, idea, or experience with anyone who was interested. In the '90s PLS encouraged younger curator-colleagues to join him at European Union of Aquarium Curators meetings, single handedly drawing us abroad -- an annual important cross-continent interaction that continues.

A now-retired European Aquarium Director remarked “He was the most ‘European’ of American (curators). Always committed and passionate, … he’ll be sorely missed, not only by his colleagues and friends, but by the profession as a whole.”

A number of folks contributed diverse stories about and remembrances of Paul, including Steve Walker, Steve Spina, Barbara Bailey, Nadia Ounais, John Coakley, and Isabel Koch. Some highlights are shared below:

- After accepting a full-time “Curatorial Department” position and leaving graduate school, an Aquarist who had recently completed their master’s thesis was a bit overwhelmed and considering not finishing up writing. It took a year, but “completing wouldn’t have happened had it not been for Paul…being so encouraging and supportive!”
- A now retired industry professional working with PLS on marine fish propagation in the early days recalls: “Propagating these fishes seems like child's play now, but we had to figure it all out for ourselves!” In those days, correspondence happened through the mail, not e-mail, and took time. They compared notes on such details as phytoplankton foods, larval fish ontogeny basics, filtration techniques to great success in rearing gobeids and amphiprionins.
- Another aquatics profession-lifer recalls PLS creating hand-written exhibit-rounds notes emphasizing the famous “3Ws” aka *Windows, Walls, and Water*. PLS felt that a visitor from a state with no public aquarium at that time might be seeing live aquatic organisms for the first time, and that the curatorial team was obliged to provide the most engaging exhibits and well-cared for animals possible.
- This D&C contributor remembers being forgiven for the 1986 guillotine-esque death of a 36-pound New England lobster during an ill-conceived and executed pre-press event weigh-in. That unique near-career ending debacle ingloriously made the *National Lampoon Magazine Year in Review*! <sigh>

Left: PLS & Capt John Rothchild of Shedd Aquarium’s research vessel Coral Reef II.
Right: PLS & (son) PJ Sieswerda after a dive in NEAq’s Giant Ocean Tank.

PLS fish ID-ing during a NEAq Bahamas Collecting Trip

As these stories illustrate, Paul impacted many who became aquarium industry lifers. His character traits of being humble, self-deprecating, eternally optimistic, infinitely sharing, goofy, and always adventurous is what many of us value most about this profession. Many of us carry on bits of it, including many "Sieswerda-isms" – his quirky phrases, subtle methods for sizing up staffers, and quips. Some favorites:

- "The little things will lead to success."
- his method of getting a read on another person while seated for a discussion by "accidently" edging a pen off the table to see if the intended target sitting with clear line of sight will pick the pen up and return it to you.
- All Aquarists should aspire to "skyrocketing to the middle."

We all stand on the shoulders of those who figured out the basics of our aquariological craft, that now are accepted as givens, and allow the inconceivable to be constantly pursued. Many greats have recently passed who made the "Golden Age of Aquaria" just that. PLS would've resisted being put in that company, but safe to say he was among the group. Paul - Et graviter desiderari.

JOHN CULBERTSON – 1944-2023

Jay Hemdal

John C. Culbertson, 79, of Holland, Ohio, passed away on Saturday December 16, 2023 at home surrounded by family.

In public aquarium circles, John was best known for having brought together a group of four young aquarium curators for a casual meeting at the Columbus Zoo in July 1989. This informal gathering in the kitchen of the Johnson Aquatics Complex (since razed) would evolve later that year into the Regional Aquatics Workshop (RAW).

Left: John at RAW #3 in St Louis, October 1990. File Photo likely taken by Dan Moreno.
Right: John's Business Card

John owned an animal feed company in Holland, Ohio, and also operated a rescue for exotic animals, including "lunker" fish. He would think nothing of driving a Pacu four hundred miles in his van to an aquarium that needed it. A bit less known was his artistic ability, including the image on the next page.

I had known John since the late 1970's, when he and his wife Lois would deliver animal feeds to a pet store that I worked at. We reconnected when I later became the aquarium curator for the Toledo Zoo in the summer of 1989. With his kind assistance, he introduced me to other people in the Zoo business that he knew, something I could not have easily done on my own.
Jay Hemdal


JOHN CULBERTSON

A LITERATURE REVIEW ON THE FASCINATING LARGER PACIFIC STRIPED OCTOPUS

Zalel Schwarz, zaleschwarz@gmail.com

This review was created as part of a course requirement at Oregon Coast Community College, where the importance of a literature search prior to animal acquisition is emphasized. The present paper is a synopsis. More detail can be found in the cited works, particularly Caldwell, et. al. (2015) and Grearson et. al. (2021).

Introduction

The Larger Pacific Striped Octopus is unusual among octopuses, both for reproducing multiple times before senescence, and its social nature. Over the past decade, every few years science communication articles come out, remarking on these unique traits, indicating this animal is of interest to the public, but it seems to be rarely displayed in public aquariums. There is existing information on the captive care of this species, as it has appeared in the aquarium trade (Algae Barn, 2022), and both it and a closely related species, the Lesser Pacific Striped Octopus, has been used as a model organism in laboratories (Grearson et. al. 2021). With the public's enthusiasm to see cephalopods and the unusual traits of this species, it is surprising that it is not displayed more frequently.

Note on Names

The Larger Pacific Striped Octopus, also referred to as an LPSO or the Harlequin octopus, was first described in scientific literature in 1977, but does not yet have an official scientific name (Caldwell et. al. 2015). There is some suggestion to name the species *Octopus rodanichius* in honor of Dr. Arcadio Rodaniche, who first described it (Octolab TV 2020). It is not to be confused with the Lesser Pacific Striped Octopus, *Octopus chierchiae*, which it is related to, though both have similar coloration and an iteroparous life cycle (Caldwell et al 2015).

Natural History

The Larger Pacific Striped Octopus lives in the Eastern Pacific Ocean (Octolab TV 2020) and, as of the 1970s, was common in the Panama Canal, and have been captured in Nicaragua in the 2010s (Caldwell et al. 2015). They have been caught from as deep as 300 meters (Caldwell et al. 2015).

Unlike other species of octopus, LPSOs are social, and groups of several dozen, according to some accounts up to 40, have been observed living in close proximity to each other (Octolab TV 2020) (California Academy of Sciences 2022). Each occupy their own den, which are about a meter apart (Caldwell et. al. 2015).

Larger Pacific Striped Octopus appear to hunt crustaceans and bivalves by sight, with a preference for shrimps (Caldwell et al. 2015), which they hunt approaching shrimp from behind and slowly lowering a single arm past the front of the shrimp before gently tapping on it (Caldwell et al. 2015) (Octolab TV 2020). This light tap in front of the shrimp startles it, and the shrimp will generally flee away from the touch, towards the octopus (Octolab TV 2020). Conversely, they prefer to pounce on crabs rather than using this slow approach (Caldwell et al. 2015). This may be because crab claws can cause serious injury to them (Grearson et. al. 2021), due to their small size (their mantle length is, on average, 11 centimeters) (Octolab TV 2020). Adults tend to take their prey back to their dens to feed and then later eject the remains after swimming a few body-lengths away from the entrance of the den (Caldwell et. al. 2015).

The Larger Pacific Striped Octopus and its relative, *O. chierchiae*, are unusual among octopi for their ability to mate and lay eggs several times before senescing (Rodaniche 1984). Some literature refers to this as being iteroparous (Rodaniche 1991), while more recent descriptions describe it as "continuous spawning", as after the initial mating females will continue to spawn and mate, either with the same or other males, until senescence begins (Caldwell et al. 2015). Mating occurs with a beak to beak "embrace", rather than at a distance, with some antagonistic behavior between the pair, such as grappling, jetting water, and pushing each other, with females often being the aggressor (Caldwell et. al. 2015). Despite these instances of aggression, female LPSOs are not cannibalistic (California Academy of Science 2022). In captivity, male and female pairs of LPSO have been observed sharing a den for several hours at a time over three consecutive days. The pair mated daily and shared food (Caldwell et. al. 2015), and there is some evidence that they may form bonded pairs (Rodaniche 1991). Eggs are laid 14 to 30 days after mating, and a female will often lay multiple (two to three) clutches of eggs, with anywhere between a month to 90 days between clutches (Grearson et. al. 2021). Spawning continues for around six months, with brooding behavior continuing for an additional two months, before senescence begins (Caldwell et. al. 2015). Senescence lasts longer for female LPSOs than males, with females gradually declining in hunting and feeding behavior over the course of two to four months, with males dying one to two weeks after abruptly ceasing to eat (Caldwell et al. 2015). If they have eggs, females will continue brooding behavior throughout senescence (Caldwell et al. 2015).

In *O. chierchiae*, a closely related species, the eggs hatch 40 to 50 days after being laid (Grearson et al. 2021). Some research suggests that LPSO eggs develop more quickly, hatching after about 38 days (Caldwell et al. 2015). The young *O. chierchiae* are bright orange, and only begin to develop their characteristic stripes seven to 14 days after hatching (Grearson et al 2021). Young LPSO show have both black and orange spots on hatching, but there is no discussion of when they develop their characteristic stripes (Caldwell et al. 2015). The young are fairly large and immediately begin a benthic lifestyle (Rodaniche 1984). Growth of *O. chierchia*, measured by mantle length, seems to be linear for the first 150-200 days of life, after which it slows (Grearson et al 2021). Because of the similarities of their life cycle, LPSO growth may be similar.

Housing

In the laboratory, individuals have been kept in small tanks with mesh lids submerged in a larger tank (Caldwell et al. 2015). This system was used to keep individuals separate from each other in case they would behave aggressively but still allow them to see and communicate with each other (Caldwell et al. 2015). However, a group of two females and three males have been housed together in a 100-gallon tank and successfully reproduced there (California Academy of Sciences 2022), and a group of two females to one male has also been kept successfully (Caldwell et. al 2015). For an exhibit, a group could be housed in a tank with a large footprint with sand or sand and mud substrate to mimic their natural habitat (Octolab TV 2020) with a variety of den materials, such as conch and barnacle shells, terra cotta flower pots, glass bottles, and three-to-five-centimeter diameter PVC pipes (Caldwell et al. 2015).

In research settings, water temperature has been kept around 20-23º C (68-73º F) (Caldwell et al. 2015), though hobbyist resources recommend keeping it around 78º F (AlgaeBarn 2022). Salinity should be kept between 34 and 36 ppt and pH should be between 7.7 and 8.2 (Hanlon and Forsythe 1985), though some hobbyists keep it a bit higher, between 8.1 and 8.4 (AlgaeBarn 2022).

LPSOs do not seem to be particular about lighting. They are active only during the day and have been kept in the laboratory with just ambient light (Caldwell et al. 2015).

Feeding

Research is still ongoing as to what foods should be offered to the closely related *O. chierchiae* in captivity. Previous attempts at feeding live brine shrimp, live copepods, and frozen shrimp and fish have failed, though they have fed on live grass shrimp and crabs (Grearson et. al. 2021). While adults have only taken live food, juveniles and hatchlings have taken partial freshly killed grass shrimp, *Palaemonetes* shrimp and *H. sanguineus* crabs (Grearson et. al. 2021).

LPSOs are known for being picky eaters as well (California Academy of Sciences 2022). Hobbyist resources suggest offering a variety of shellfish, such as shrimp and crabs (AlgeaBarn 2022).

Conclusion

While there are still gaps in knowledge about the care and behavior of the Larger Pacific Striped Octopus, it has still been kept successfully in captivity in laboratories and by a few hobbyists. The potential to learn more about its care and behavior, as well as its unusual reproduction and social nature, should earn it the attention of aquarists, and a place on display at more public aquariums.

Literature Cited

Algae Barn. (2022, April 1). Pacific Striped Octopus. AlgaeBarn. Retrieved October 8, 2022. https://www.algaebarn.com/shop/captive-bred/rare-opportunities/pacific-striped-octopus/

Caldwell, Ross, R., Rodaniche, A., & Huffard, C. L. (2015). Behavior and Body Patterns of the Larger Pacific Striped Octopus. *PloS One*, *10*(8), e0134152–e0134152. https://doi.org/10.1371/journal.pone.0134152

California Academy of Sciences. (n.d.). Larger Pacific Striped Octopus. Retrieved May 7, 2022, from https://www.calacademy.org/explore-science/larger-pacific-striped-octopus

Grearson, Dugan, A., Sakmar, T., Sivitilli, D. M., Gire, D. H., Caldwell, R. L., Niell, C. M., Dölen, G., Wang, Z. Y., & Grasse, B. (2021). The Lesser Pacific Striped Octopus, Octopus chierchiae: An Emerging Laboratory Model. *Frontiers in Marine Science*, *8*. https://doi.org/10.3389/fmars.2021.753483

Hanlon, & Forsythe, J. W. (1985). Advances in the laboratory culture of octopuses for biomedical research. Laboratory Animal Science (Chicago), 35(1), 33.

Larger pacific striped octopus. Octolab TV. (2020, January 17). Retrieved April 19, 2022, from https://octolab.tv/species/larger-pacific-striped-octopus/

Rodaniche. (1991). Notes on the behavior of the larger Pacific striped octopus, an undescribed species of the genus Octopus. *Bulletin of Marine Science*, *49*(1-2), 667–667.

Rodaniche. (1984). Iteroparity in the Lesser Pacific Striped Octopus Octopus Chierchiae (Jatta, 1889). *Bulletin of Marine Science*, *35*(1), 99–104.

A BRIEF GUIDE TO AUTHORS

Updated 2024

This guide is intended for those not accustomed to using a "Guide to Authors", as provided by more formal periodicals. Most authors now make a concerted effort to follow this format, and it makes my job much easier. Please help me out, folks!

The approximate deadline for submissions is December 21st.

As always, typical Drum & Croaker articles are not peer-reviewed and content will not be edited, other than to correct obvious errors, clarify translations into English, modify incorrect or cumbersome formatting, or delete superfluous material (photos with no captions/legends, etc.). Other types of contributions (announcements, etc.) may be edited to meet space limitations.

As has always been the case, materials in *Drum and Croaker* may be reproduced unless otherwise specified. Occasionally articles appear in *D&C* that originated elsewhere. These must be obtained with approval from the original authors and publisher, and proof of this is required. Special instructions on the reuse of these papers and how they should be properly cited may be provided.

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 as LOW as practical. High resolution photos make the final PDF file huge and I always compress them anyway.
- **Format the title section with the line spacing set on 1.5 lines (not another method) and using centered, boldface font. Only the title should be CAPITALIZED (except italicized *Scientific namesii*).** When using MS Word, go to the "Home" tab, open the detail on the "Paragraph" section, and choose "1.5 lines" under spacing and make sure the before and after spacing settings are at "zero." In order to preserve single line spacing in a title section (name of paper, multiple authors, multiple institutions) add an "enter" (carriage return) at the end of each line. Turn on the ¶ tool, highlight the symbol at the end of the line of text and choose the line spacing needed. For some additional help with these settings, see "Other Things I Whine About" below.
- Double-space after your "institution name" to begin the body of your text. When correct, the title and headings formatting should look like this:

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

Jill Fishhead, Senior Aquarist jfishhead@fishland.com

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

Text and Heading Format

Headings and text should look like the above heading and this paragraph. Use single spacing with 1" (2.54 cm) margins on ALL sides. Please indent/tab 0.5 inch (1.3 cm) at the beginning of each paragraph (not using the space bar!) and leave a single space between paragraphs. Justify the text (see toolbar options and note how pretty the right margin of this paragraph lines up!). Section headings should be in bold (as above) at the left margin.

Please use the following format for figure legends:

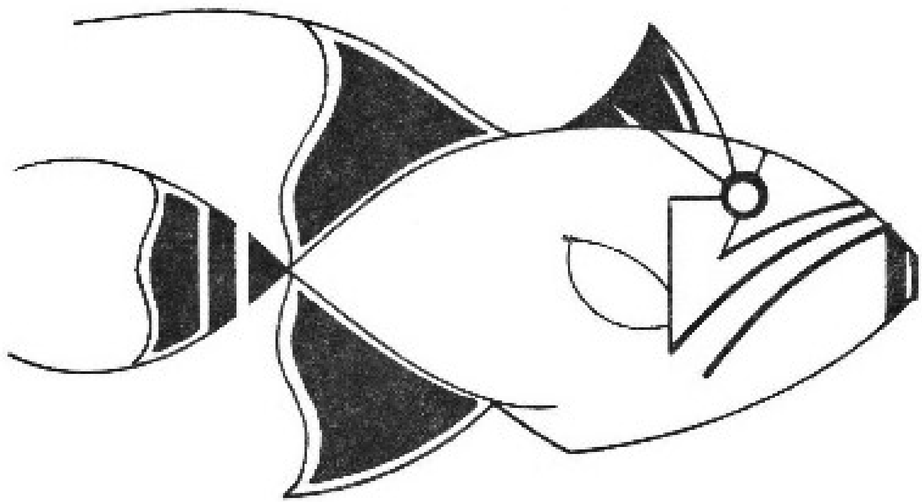


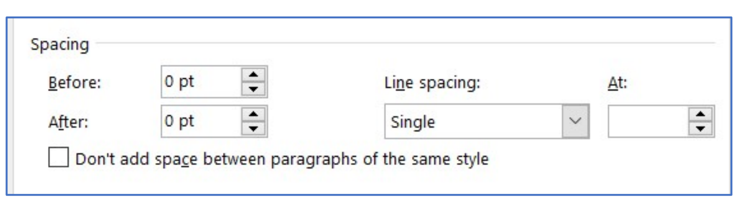
Figure 1. Legends should appear under the photo (such as this drawing by Craig Phillips) or graph in this format in 10-point font, aligned with the sides of the image or figure (center or justify). Very short legends can be centered. Photographs should be pasted into the document in the proper location by the author. 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 you start the document, 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. Center column headings in tables (vertical and horizontal), bold them, and use light grey shading in those cells.
- 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 less than a page in length. A brief bold faced and capitalized title should be centered, the body text should be formatted as above, and **author and affiliation should be placed at the end of the piece** with the left end of each bolded line right of the center of the page. Reformatting that must be done by the editor may reduce a shorter "main" article to a note, or may bump a note up to main article status.

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

Literature Cited

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

When providing full references/citations at the end of a paper, use an accepted standard style. I'm not a stickler about this. Just make sure all the relevant information is present, and stick to one style. APA format is probably most appropriate. You can enter a known paper title in Google Scholar, and click on the "cite" link below the displayed reference. This will produce an APA-formatted citation that you can copy and paste into your paper. You can also use open-source software like Zotero to extract APA formatting from reprints that you have downloaded. The formatting used for the journal Copeia is also appropriate, and was my original preference "back in the day." Scroll way down on this page: https:/www.asih.org/ichsandherps/instructions-to-authors