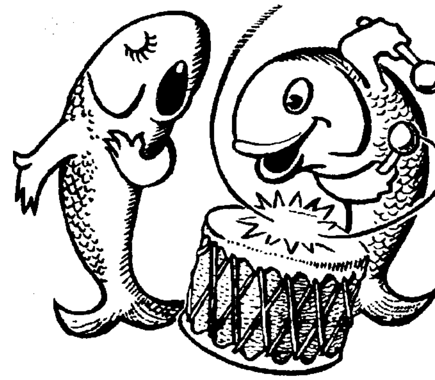
DRUM *and* CROAKER



A Highly Irregular Journal for the Public Aquarist

Volume 54 *January 2023*

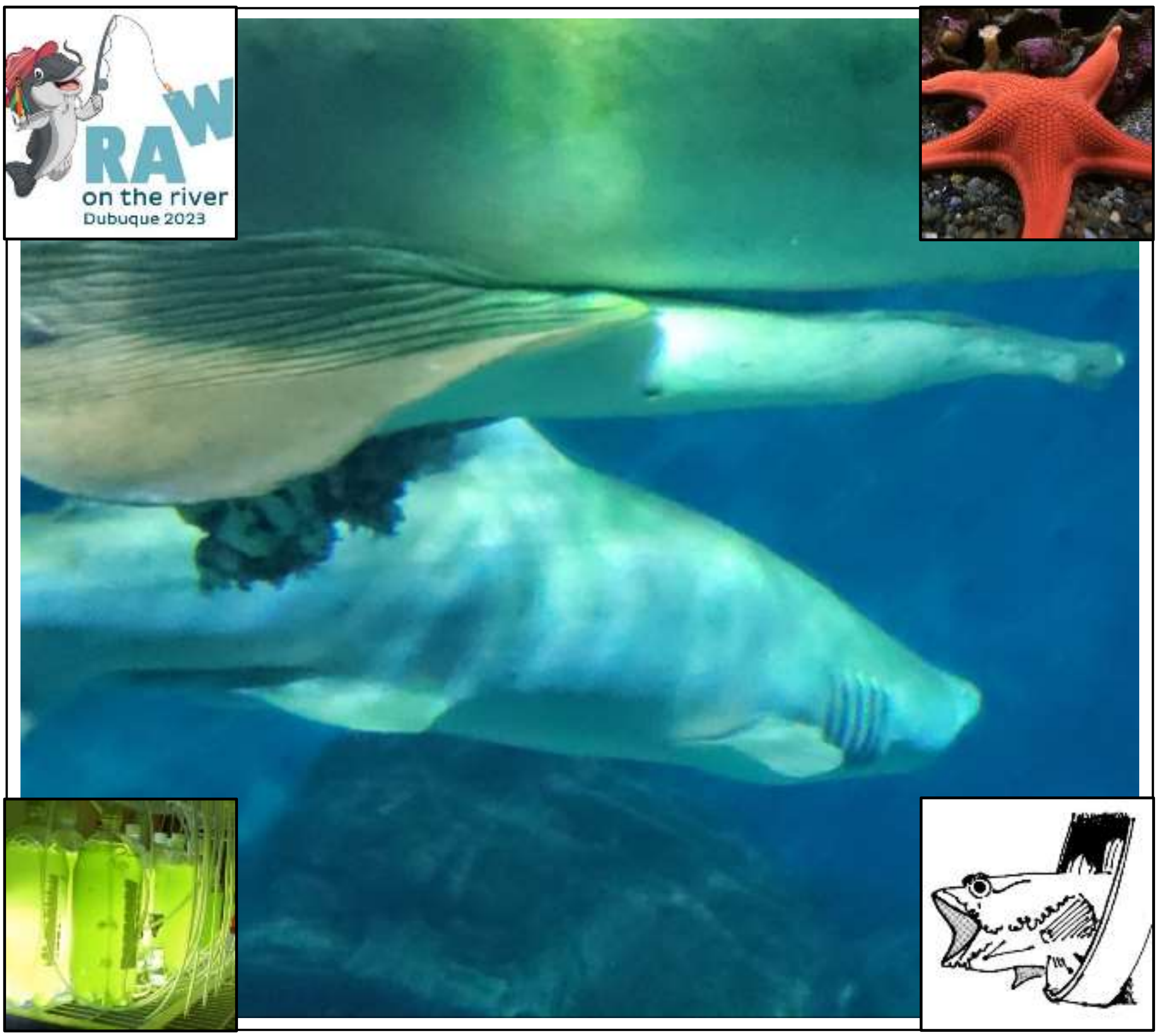


TABLE OF CONTENTS
Volume 54, 2023

2 **Letter from the Editor – The Great Migration**

5 **Drum and Croaker 50 Years Ago**
Steven L. Bailey

8 **Novus Aquas (New Waters)**
A New Feature Looking at the Future of Public Aquariums
Barrett L. Christie

12 **A Novel Approach to Managing Sea Star Illness and Injury Through Complete Immune Support**
Tiffany Rudek and Evonne Mochon Collura

18 **How to Break All the Rules and Still Succeed at Algae Culture**
Barrett L. Christie

26 **RAW 2023 Announcement:**
RAW on the River, The National Mississippi River Museum & Aquarium, Dubuque, Iowa, USA ***(Note a Change from the Original Venue)***

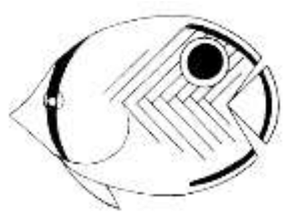
28 **Management of Infection Following Injury to the Spiral Colon in a Sand Tiger Shark, *Carcharias taurus***
Sandi Schaefer-Padgett, Bert Sadler, Rachel Stein, and Barrett L. Christie

37 **An Ethogram for the Oyster Toadfish, *Opsanus tau*: Sampling the Behavioral Diversity of a Common Benthic Fish**
Bernd H. A. Sokolowski and Peter J. Mohan

55 **The Aquarium Symposia from the 1958 to 1970 Meetings of the American Society of Ichthyologists and Herpetologists**

65 **Guide to Authors**

67 **Some Useful Links: Aquality Chapters and the 2022 International Aquarium Conference Abstracts**



Main Cover Photo: Male Sand tiger Shark, “Nacho,” showing spiral colon issue. Sandi Schaefer-Padgett
Inset Cover Photo of Sea Star: Barrett Christie; **Inset Cover Drawing:** Pete Mohan
Interior Gyotaku: Bruce Koike
Interior Line Art: Craig Phillips, D&C Archives

A LETTER FROM THE EDITOR

THE GREAT MIGRATION: IMPACTS ON HUSBANDRY STAFFING AND PUBLICATIONS

First, let me welcome new regular contributors, Steve Bailey (aka "Bailey") and Barrett Christie. I look forward to your perspectives on our past and future.

I started thinking more deeply about the topic of staff retention after seeing both a sharp increase in job postings and a sudden drop in paper submissions for *Drum and Croaker*. Brian Nelson (personal communication, January 10, 2023) reports that there was a near-doubling of job postings on the Aquaticinfo listserve beginning about a year into the pandemic. In 2018 and 2019 there were about 5.8 unique postings a month. This dropped a bit in 2020 as institutions froze hiring. Postings then rose to 9.6 to 9.9 per month in the past two years. These figures included Aquarist, lab, dive safety and curator positions. My impression is that the figures from the last month or so are even higher.

Random lean years are not unique to *D&C*, and occur every 3-5 years on average. Surprisingly, the main pandemic years of 2020 and 2021 actually resulted in a bumper crop of papers. Was there a backlog of progress to report? Was working remotely conducive to writing? The 2022 downturn in papers received for publication is not isolated to this periodical and pervades the entire animal husbandry field. AAZK's *Animal Keeper's Forum* has also experienced a recent and unusual drop in paper submissions (Shane Good, personal communication, December 30, 2022).

A shortage of papers seems to be one side effect of a wave of understaffing throughout our profession. The sharp increase in job postings on the Aquaticinfo listserv is another. While we have seen shortages of experienced biologists before, those seemed to be attributable to the openings of multiple new facilities and the resulting "giant sucking sound" as transfers to new positions thinned the herd elsewhere. A paper given by Earl Herald at the 1967 ASIH Meeting was entitled "Job Mart for Aquarists." By the late 1950s there were perhaps only 20 public aquariums in North America. In the next decade, that number doubled. The brain drain that started in the mid-1950s as new, larger facilities opened must have only gotten worse. Something similar happened in the late 1980s and 1990s as many more aquariums opened, including some of our largest facilities. The number of aquariums doubled again, and the number of new positions increased even more. The resulting lack of institutional memory at depopulated smaller institutions was a factor in the early success of the Regional Aquatics Workshop (RAW), as networking to regain missing skills became increasingly important.

What is happening today is obviously different. There were many layoffs during the pandemic. A lot of institutional memory evaporated at some facilities. But as rebuilding occurred in 2022, it became obvious that positions lost to the pandemic were not easily replaceable. Why? I've listed some theories below, but I don't think any of these scenarios are new. My impression is that the disruption of normal work over the past few years has just made decisions to leave easier. What's a little more temporary chaos in your life?

- Those laid off have found other work or left the workforce entirely. Some realized their pay check was going entirely to child care, while others were close to retirement anyway. Luring them back may be impossible for financial and/or personal reasons.
- Inflation and vacancies in the retail economy have opened lots of jobs easily paying 25% more than entry-level aquarium or zoo work. These can be especially attractive to new aquarists and keepers who may be saddled with student debt greater or equal to their annual salary. There is probably not much passion involved in warehouse, delivery, or barista work, but it might be less demanding and pays a newly-minted biologist's bills. Zippia (zippia.com) provides some freely-accessible statistics on aquarist and zookeeper employment based on data extracted from hundreds of resumes. Compensation amounts stated there for entry levels are similar to figures I have seen presented from within the industry. A median starting pay rate of not much more than $15/hr is typical, even in areas where the cost of living is high, such as the northeast. It is not surprising that retention of new positions is low and those who leave after a short stint often move on to other fields. A 2021 survey of non-profits (nonprofithr.com) suggests that the most difficult group of employees to retain are those in entry level positions (47% of respondents) and those under 30 years of age (46% of respondents). Zippia.com indicates that nearly a third of aquarists change jobs after a year and other third within two years. Is this due to burnout, financial concerns, or some combination of the two?
- Upper-level aquarists may not be willing or able to wait for in-house management positions to open up, may need more pay to support child care, and may have become tied to a particular locale making relocation difficult. Where science teachers are in demand (work now, get your certification later) or where the local water plant needs a biologist, significantly higher pay may lure them away from their "passion." Nonprofit HR notes that 35% of respondents found that mid-level positions, which would include most levels of aquarists beyond entry-level, were also relatively difficult to retain.
- And…The baby boomers are retiring in droves. While the pandemic pushed some folks to get out sooner than they had planned, over half of this cohort are now over 65. These folks hold lots of institutional memory, and their departure just adds to the brain drain created by younger, experienced folks jumping ship.

What can be done? It has always been said that "you don't get into biology to get rich," but that equation can be nudged toward a place where an expensive education, experience and unique skills are rewarded with commensurate compensation. The National Association of Colleges and Employers (NACE) lists the average starting salary for a B. S. graduate in biology at $40K/yr for 2021. Conservation biologists with a bachelor's degree are in this mean ballpark. Our institutional mission statements point out that this is what zoos and aquariums are all about. Thanks to those institutions that are moving the ball in the right direction. If "a rising tide raises all ships," increases in starting pay will create bumps at higher levels as well, hopefully increasing retention everywhere. It will also encourage minorities and others with less access to generational wealth to enter the field. Aquarium jobs (and those in zoos) are far more complicated than they were decades ago. We're not merely the cleaning and feeding crew. The "tank men" of the 1950s would be amazed at the technology we navigate, the species we propagate, and the complexity of our welfare programs.

We work in this field because we love it, warts and all. While our industry figures out how to pay valuable people a living wage, it will be important to keep sharing information with our peers so we can continue to move our profession forward during these challenging times. Be inquisitive. The husbandry of most species is still an emerging science. Share procedural innovations that will help us all work smarter, not harder. Continue to attend (and present at) conferences and submit papers to *Drum and Croaker* so these advances are retained for future aquarists. You are all more important than ever, and we need to hear your voices and preserve your knowledge.

Anon. National Association of Colleges and Employers. First Destinations Dashboard (for 2021). https://www.naceweb.org/job-market/graduate-outcomes/first-destination/class-of-2021/interactive-dashboard/

Anon. 2021 Nonprofit Talent Retention Practices Survey. Nonprofit HR. https://www.nonprofithr.com/wp-content/uploads/2021/09/Infographic-2021-Retention-Survey-For-Publish.pdf

Anon. Zippia, The Career Expert. *(Accessed January 2023)*
https://www.zippia.com/aquarist-jobs/
https://www.zippia.com/zoo-keeper-jobs/

American Shad, Bruce Koike

DRUM AND CROAKER 50 YEARS AGO

A personal reflection, and then an excerpt from the 1973 Issue (Vol.14 #1)

Selected by Bailey/Steven L. Bailey, Frost Science Museum Aquarium

Following in the footsteps of Rick Segedi as the *D&C* retrospectives contributor is an honor; plain and simple. Rick, as many of you know, has had a stellar career as a public aquarium aquarist, curator, volunteer, tireless industry advocate, and reliable contributor to D&C. Rick is reportedly now "retired from retirement," but don't bet on it; my chips are on the square that says his name as a contributor will appear in these pages from time to time.

I first met Rick while at a Cleveland Metroparks Zoo-hosted Australian lungfish workshop back in the early 90's. During an exhibits' BTS (behind the scenes) tour he introduced himself, and I was in awe. He was well known to a mid-30s New England Aquarium Curator through reputation, writings on aquatic husbandry, and as a well-traveled and accomplished aquarium professional. He was gracious, engaging, friendly, and obviously very busy as a CMZ volunteer: he apologized for breaking away from the conversation due to "wanting the exhibits to look their best," and to give the CMZ aquatics staff an opportunity to participate in the workshop without having to be concerned about the 3Ws - windows, walls, and water. That encounter has stuck with me throughout my career. I was so impressed with the obvious dedication to aquatic duties, the fraternal aura he projected, and a humbleness that effortlessly had him sliding into the next phase of his life-long aquatics calling. As I continue along the span of my career, I'm exceedingly grateful for the Rick Segedi-s whose many accomplishments enabled the golden age of aquariums to be a passion with a paycheque and the furthest vocation possible from a job.

<u>From Vol.14 #1, p.ii</u>

Dr. Earl S. Herald, Director of the Steinhart Aquarium, died in a diving accident at Cabo San Lucas, Baja California on January 16, 1973. A memorial fund in Earl's name has been established at the California Academy of Sciences. Friends wishing to remember him may contribute to the fund. The next issue of Drum and Croaker will be dedicated to Earl Herald. All those who would like to contribute an article to this issue are urgently requested to submit it as soon as possible.

The death of E. S. Herald, PhD in January of 1973 was a gut-punch to the public aquarium industry. Dr. Herald was an accomplished ichthyologist specializing in Syngnathids, a television personality hosting the show "Science in Action," an intrepid field researcher, an effective fundraiser spearheading the renovation of the Steinhart Aquarium, and much the face of the public aquarium industry. Greatly admired by his fellow aquarium directors, he was a catalyst for so many initiatives elevating the profile of public aquaria including assisting the launch of the first International Aquariology Congress held in Monaco, garnering respect for the work of aquarium professionals e.g. successfully having advocated for commensurate compensation for aquarium staffers from the City of San Francisco, and insisting upon exhibit authenticity, species diversity, and accuracy.

The next year's Drum and Croaker was indeed dedicated to him. Some rather moving tributes and recollections were penned. Some photos shared clearly demonstrated that he was a dashing Indiana Jones of aquariology, a thick head of hair, abundantly charismatic, energetic, and succeeding famously at his craft. As Gordon Gunter writes in *D&C* Vol.15:

> *I have known Earl Herald as a professional colleague and friend for thirty years. He was a well-respected ichthyologist who worked hard and was highly cooperative. It is the general opinion of all of his professional associates that he was a good bit more amiable than the average biologist. It is such a terrible pity that he was cut down in the prime of his life when he had so many good years in front of him. Mankind is the loser for that.*

Please reflect on all those who have mentored, guided, inspired, led by example, and been endlessly encouraging to you in your career. If time permits, pull out a notecard and dash off a line or two acknowledging the impact they have had on you while exploring this rewarding and meaningful profession.

From Vol.14 #1, p.7

The close association of a Brewery and an Aquarium is reportedly the dream of many an Aquarist. This announcement on pg.7 illustrates that there might be consequences.

CALGARY AQUARIUM CLOSED (1960-1972)

Calgary Brewing and Malting Co. Limited announced last August that it is embarking on a major program to expand the already well known Horseman's Hall of Fame on the Calgary Brewing grounds. Originally opened in 1963, this exhibit of Western Canadiana has been seen during the last 9 years by more than 3 million people.

As a result of this ambitious plan, the Calgary Aquarium closed permanently on September 5, 1972, in order to provide sufficient space for the Hall of Fame expansion. The invaluable collection of specimens in the Aquarium were donated to Calgary's twin city, the City of Quebec, for addition to its already famous Aquarium.

From Vol.14 #1, pp.31-32

Public Aquarium Professionals' quest to find organizations that provide proper representation and an effective platform for practical communication has a protracted history. Sharing information in this current era has many more options beyond AZA, e.g. the AquaticInfo & EUAC listserves, RAW, AALSO & MACNA annual meetings. A nod to those visionaries of 1973, and comforting to reflect on how much progress has been made since.

AQUARISTS AND THE AAZPA, Don Wilkie, Scripps Aquarium

Professional aquarists of the United States and Canada met for many years with the American Society of Ichthyologists and Herpetologists. In recent years there has been some dissatisfaction with this relationship and an increasing feeling that it would be more appropriate for the aquarists to meet with the American Association of Zoological Parks and Aquariums. This matter came to a head at the 1971 AAZPA meeting in Salt Lake City. The aquarists in attendance, after considerable deliberation on this matter, voted to throw their support behind the AAZPA, promote its annual meeting and attempt to develop a strong *program on aquarium activities at its annual meeting. This decision was well received by President Gary Clarke and the AAZPA Board of Directors, who asked that the aquarists name a representative to the AAZPA Program Committee for the 1972 annual meeting in Portland. Don Wilkie was chosen by the aquarists to fill this role. Gil Hewlitt and Lou Garibaldi were named to assist; forming in essence an aquarium subcommittee. Dan Moreno and Bill Braker were included on the Board of Directors when AAZPA became an independently chartered organization in 1972.*

The AAZPA leadership in 1971 as pictured on the October 1996 Cover of *Communique*, the predecessor to AZA's *Connect* Magazine. Seated (left to right): Gary K. Clarke, Lester E. Fisher, DVM, Margaret A. Dankworth, Robert O. Wagner. Standing (left to right): Daniel H. Moreno (Cleveland Aq.), Frederick J. Zeehandelaar, Earl E. Gaylor, William P. Braker (Shedd Aq.), Lamar Fansworth. Provided by AZA for use by Pete Mohan, 2019.

NOVUS AQUAS (NEW WATERS)

Barrett L. Christie, enteroctopusdofleini@yahoo.com

Since 1994 the pages of *D&C* have featured a recurring column compiled by Rick Segedi with highlights of the journal from 30-50 years prior. This year another legend of our industry, Steve Bailey, begins curating this historical column. This year also marks the beginnings of this new *D&C* regular column looking forward in our industry to accompany this look at our past. It is fitting that we do so, for as the old adage goes: those who are ignorant of the past are doomed to repeat it.

For this inaugural column, let us consider change within our industry. Fifty years ago, Glenn Burghardt (1973) put forth an estimate in the pages of *D&C* that of the 44 public aquariums in the United States there were 389 aquarist positions. Many of us working in aquaria know that we have a niche skillset, but this exemplifies just how small of a community we are.

How many of us are there today?

Many of you (105 institutions) provided data on your staffing levels and approximate replacement rate. These data were averaged for small, medium, and large facilities, and applied to the number of U.S. aquaria listed by Mohan (2020). Today there are roughly 1700 aquarist positions in the US, a growth of about 230% since 1973, or 4.6% per year. But this is only part of the story, we have an equal number of other positions working in other capacities, but equally integral to our missions. The ranks of aquarium professionals now include terrestrial animal keepers, trainers, divers, life support operators, water quality technicians, veterinarians, and others. As our knowledge of aquatic animal care has grown, these specialty positions have become increasingly common in the modern public aquarium, and factoring these in our community has grown by an order of magnitude in a half-century.

In 2073 what will our community look like? Is this rate of growth sustainable? If we extrapolate these estimates forward, is it possible there will be tens of thousands of aquarists and other aquatics professionals working in the US (let alone the rest of the world)? I think most seasoned aquarium professionals would tell you that they feel the rate of growth in new facilities will eventually have to taper off, and the boom in new facility construction that recent decades have seen cannot continue forever. But then, one must ask, what does our future industry look like?

If the rate of growth is indeed unsustainable, in what way will the public aquarium evolve? One possibility is in size and scope. Aquaria have trended towards larger and larger displays over time, this is inevitable as we seek new ways to wow those who visit and technology in LSS improves. Will this trend continue indefinitely? There must be some point of diminishing return with size alone, where diversity, color, and morphology of species must come into play. Another is in diversity of species displayed, newfound wonders from far-flung corners of the world. We have access to more species than ever before, yet as Andrews (2018) points out, diversity also has a point of diminishing returns, and biodiversity for its own sake is not a reasonable goal of the modern public aquarium. Diversity, rather, should be thoughtfully approached and in balance with welfare and sustainability concerns, though without excluding the potential for experimentation with novel husbandry techniques to bring new species onto display.

A few trends in collections have been evident over the past few decades, species richness has grown tremendously, but are aquaria on the whole homogenizing themselves? Early aquaria were limited to local fauna, and expeditions were mounted to bring tropical biodiversity to exhibit. Fast forward a century and certain staples have become ubiquitous, the omnipresent *Rhinoptera* touch tank, Indo-Pacific living reefs, seahorses, *et cetera, et cetera, ad infinitum*. The pet trade we have come to rely on as a source of "instant biodiversity" for our collections also has served to homogenize them, to a degree. In recent decades, aquaria have not had to contend with operations similar to the roadside "zoo" that has diluted the image of reputable zoological gardens. Using the same vendors we rely on, anyone with enough funds could open an "aquarium" in a retail space, trotting out a "greatest hits" collection…a touch tank, corals, seahorses, *et cetera*, to a largely indiscriminate public. As we thoughtfully contemplate our diversity and the sustainability of our collections, we must make sure they are tied to mission and a sense of place. We face an uphill battle to define our institutions once again as centers of inquiry, research, and conservation. But the resurgence of these core values looking to the future will be essential to establishing our credibility with the general public.

Another way aquaria can evolve is in quality, which is incredibly difficult to define. Establishing ourselves as biological experts is certainly an imperative, and to this end we must be active in field conservation and in publishing research. Thoughtfully curated collections, with a balance of "greatest hits" and unique local fauna or species from relatively unknown habitats is one hallmark of a quality aquarium.

One thing that is certain is change. The aquarist of 2073 will likely have better tools and resources. We have orders or magnitude more information now than in 1973, but with turnover and loss of institutional knowledge in our field accelerating will new aquarists be prepared to assimilate it? Change will undoubtedly be a constant. Despite some people's visceral reactions to change, it is not necessarily our adversary. However, there is one thing that has been a constant foe to aquarists since the first aquariums opened to the public, and will certainly continue to be the biggest challenge in the future: entropy. Increasing entropy, and our ability to manage it, will shape the aquarium industry in decades to come.

Entropy is inherent in aquariums, and indeed in life itself. Assuming the base status of the universe to be a state of maximal disorder towards which the world as we know it will inevitably slide, we can think of the very existence of life itself as a revolt against the very fabric of the universe. Biological life, and by extension all its myriad forms that captivate us as aquarium biologists are an ever-evolving revolution against the inevitability of entropy. Aquariums that seek to display wonderous forms of this life to visitors are not only a microcosm of the ecology we seek to inspire our visitors with, but a microcosm of the struggle between the splendor of life itself and the tendency for all of it to devolve into chaos.

This microcosm of conflict, the struggle between life and chaos is central to our role as aquarists. Pumps wear out, metals corrode, fiberglass delaminates, and living animals eventually die. These manifestations of entropy are well known to all of us. But as we all well know, but for our daily intervention, everything in our aquariums is *trying* to die every minute of every day. Constant attention to water quality, nutrition, animal behavior, health, the function of life support systems, and thousands of other variables is all that stands between a thriving living collection and chaos. At its core, our job is to proactively make a sequence of decisions and actions each day that keep chaos at arm's length. We stand between the animals in our charge and entropy.

In the context of entropy, life is an unnatural deviation from the norm, perhaps that explains our fundamental affinity for life, what E.O. Wilson described as biophilia. But if life itself is a grand rebellion against entropy, then life contained in glass boxes is the radical fringe of this insurrection against thermodynamics. The complexity needed to sustain life in an aquarium is orders of magnitude greater in such an artificial environment. This has been a fundamental truth about this hybrid of art and science we call animal husbandry since the first public aquaria opened. With the passage of time, we have refined our techniques through better and more complex equipment and better understanding of animal physiology, water chemistry, et cetera. The technology we now use routinely in aquariums was unfathomable to the curators of the first public aquaria, they offer us a much greater margin of safety in the eternal struggle against entropy, however this increased complexity also offers many more ways things can go wrong. Chaos will always find its way into the equation, and we must be even more vigilant to keep it at bay.

Our profession as aquarium biologists is rooted in science, and builds upon itself traditionally in the same manner, with one interesting deviation. We can only learn so much from what is committed to paper, but to become an aquarist is to undertake an apprenticeship. Institutional knowledge is transferred and experience is gained over time. Much of the experience we have and routinely share with each other at conferences, on Aquaticinfo, or in the pages of *D&C* is hard-won knowledge. The paths to our successes are lined with our failures, and we often learn more from what *didn't* work the way we expected than what ultimately did work.

As such the burden to improve the quality of future aquaria lies with us. To continually grow our profession in either size or quality we must commit our failures and successes to the permanent record as well as to institutional memory, and we must rigorously mentor the next generation of aquarists to carry forth these traditions, and further improve the standards and quality of animal care.

We must also provide the resources to retain this institutional knowledge, and advocate for pay equity so that future generations of aquarists are compensated as professionals and can afford to remain in this field. Thus, the challenge for us is to retain the fruits of this apprenticeship model of aquarist training, as much as it is to cultivate them. There may be far more of us in 2073, but we will undoubtedly still be a small, close-knit community, and the responsibility of ensuring the quality of aquarists working 50 years hence is shaped by our actions each day right now in mentoring and teaching staff, newcomers to the industry, and each other.

Entropy is equally present in the human equation as well, after all.

References

Andrews, C. 2018. Public Aquariums in the 21st Century- What's Next? Drum and Croaker. 49: 11-17.

Burghardt, G. 1973. Estimate of Aquarist Positions in the United States. Drum and Croaker 14: 27-28.

Mohan, P. 2020. Trends in Aquarium Openings and Closing in North America 1856 to 2020. Drum and Croaker. 51: 86-98.

Arctic Grayling, Bruce Koike

A NOVEL APPROACH TO MANAGING SEA STAR ILLNESS AND INJURY THROUGH COMPLETE IMMUNE SUPPORT

Tiffany Rudek, Tiffany.rudek@aquarium.org,
Evonne Mochon Collura, Evonne.Mochon-Collura@aquarium.org

Oregon Coast Aquarium, 2820 SE Ferry Slip Rd, Newport, OR, USA

Sea star populations, wild and in aquariums, were severely impacted by disease in 2014. New medical management practices became paramount and evolved quickly as the causes were unknown and difficult to identify. In some cases, animals expired within hours of showing first symptoms while other stars appeared unaffected.

Within the aquarium industry a number of standard medications were applied in an effort to slow or reverse illness. These included freshwater dips, formalin baths, Baytril® injections, chlorhexidine irrigations and TMP-S (trimethoprim sulfa) baths. Decline or recovery varied greatly and aquarists adapted treatments by varying dosages, durations and ultimately ruling out ineffective methods. At the Oregon Coast Aquarium, at least nine of the major exhibits housed a variety of Pacific Northwest sea star species and symptoms of Sea Star Wasting Disease (SSWD) began in April 2014. Through collaborative efforts with other affected aquariums, the Oregon Coast Aquarium Aquarists and Veterinary Staff tried multiple treatments, submitted tissue samples to diagnostic labs and pursued answers that seemed to produce more questions.

In 2020, the focus shifted from triage response for specific symptoms to an environmental perspective – the environments within the animal and surrounding the animal. This combination of factors allows the animal to properly utilize its own immune system to focus on healing instead of expending excessive energy combatting other possible stressors. With this, the exact causative agent of the animals' decline whether it be viral, bacterial, parasitic, or general stressors such as pH or temperature does not matter because all possible issues are addressed and corrected within the same protocol.

Materials and Methods

The treatment consists of three parts to reduce the overall stress on the animal and provide the best chance of recovery. The goals include immune support coupled with removal and control of parasites, fungus, and harmful bacteria.

Part 1: Opportunistic Parasite and Fungal Removal

Microscopic ciliates found naturally in the salt water environment play a role in breaking down detritus and physical waste, most notably *Uronema* spp. Immunocompromised sea stars typically attracted *Uronema* which contributed greatly to their decline. Eliminating the ciliates slowed and sometimes completely halted tissue sloughing, slime production, and tissue degradation (commonly described as "melting.")

It has also been seen in many soft bodied invertebrates that fungus will begin to grow on patches of necrotic tissue, so this part of the treatment covers the removal of that factor as well. At this time, there is no consistent evidence of fungal growth playing a large role in sea star decline;

however, the treatment addresses all possible affecting factors in any case, so it is included. The treatment for the removal of both ciliates and fungus is the same and performed in the same step of treatment.

Evolution of the Ciliate Removal Treatment

Ciliate removal began before the animals were placed into the quarantine system. Animals were placed in 4–5-minute fresh water dips with dechlorinated water at the same temperature as their origin tank. This was highly effective at removing ciliated parasites from their skin but it caused osmotic stress that contributed to further decline in some cases. Alternatively, stars were lifted out of water and lesions were irrigated with fresh water using a curved tip syringe. This was found to be less effective for ciliate removal than the fresh water baths, and the animals were still stressed from being out of water. Both of these treatments were discontinued in favor of another method - a 10-minute bath in a solution of sea water mixed with 3% human treatment grade hydrogen peroxide at a dosage of 1ml/L. The bath did a decent job of removing algae, fungus, and external ciliates from the stars without causing excessive stress. However, the peroxide was not as effective at overall ciliate removal when compared with the fresh water. Due to peroxide's bubbling reaction with the internal fluids, it was not an option for stars with open body cavity wounds including arm drops. In such cases, protocols shifted away from full submersions in any solution to 20% betadine irrigations focused on the wound sites. This method kept the wound sites clean but did not remove ciliates elsewhere so they recolonized quickly which required excessive irrigations. It was also decided that having the star out of water for any length of time was causing unnecessary stress. When none of these methods proved to be effective in all situations, a better solution was necessary.

Sea stars were given short baths in iodine-based solutions mixed with sea water and were removed before they flushed their internal body cavity with the solution. This was tested with povidone iodine solution diluted to 0.25ml of iodine per 100ml of sea water for 5 to 10 minutes depending on animal reaction and stress levels. It effectively removed all ciliates and fungus from the body of the stars. This treatment was quickly changed to using a readily available commercial aquarium product, Seachem® Reef Dip™. The Reef Dip product is an iodine-based solution with a skin coat additive that is marketed to treat corals with fungal infections, and the addition of the skin coat additive was helpful in reducing the harshness of the iodine to the tissue of the stars. Iodine is highly effective in removing bacteria, ciliates and fungus, but without skin coat support it can sometimes irritate fragile, newly healing tissue. This protocol has proven effective in all cases used at this time.

Treatments began with a 10-minute submersion in 10ml of Reef dip/1 gallon of sea water without aeration, followed by a thorough rinsing with sea water. The star was removed using a laminated paper card to prevent injuring the tube feet and scooped into the bath container underwater. After the end of the bath treatment, the container was flushed with clean sea water until no Reef dip solution remained. The stars were transferred back to the holding tank and allowed to leave the container on their own, or were gently scooped out while underwater and allowed to reattach to the tank wall. At no point were the stars ever removed from the water entirely. In some cases, when the stars were excessively producing mucus, the dosage was doubled to 20ml/gallon to achieve the same results. This became the standard dose for *Dermasterias imbricata* when they were in the early stages of treatment and producing higher amounts of slime on the tissue surface, but was reduced to 10ml/gallon when the wounds were nearly healed. Other stars tolerated 20ml/gallon but sometimes exhibited signs of stress after 5 minutes and the baths were ended earlier. The best tolerated dosage for *Pisaster ochraceus, Solaster stimpsoni, Henricia leviuscula, Luidia foliolata, Evasterias troschelii* and *Pycnopodia helianthoides* was 10ml/gallon.

In most cases, the stars did not show any signs of stress while in the bath and tolerated the full 10 minutes while fully relaxed. A lethargic star who was exhibiting initial illness signs like limb torsion, skin sloughing, lost limbs, lethargy, and skin lesions was usually observed to relax in the baths and exhibited no signs of stress and sometimes regained energy. Signs indicating stress included deflation/inflation, excessive arm torsion, arms curled up over the middle of the body, and an attempt to exit the container. If any stress was observed, the bath was immediately ended and flushed and the stars relaxed again. Close observation was necessary during the bath period because the length of time varied slightly from 5 to 10 minutes depending on the severity of the stars' health. Animals with improved healing and strength attempted to leave the container. Several *Pisaster ochraceus* exhibited this behavior as their recovery neared completion.

The dips were performed on stars as necessary, with less severe cases only needing one dip at the beginning of treatment, whereas more severe cases with lesioning throughout the body needed an average of two dips weekly for 2 weeks in order to achieve the same results. In addition to the dips, the tank was scrubbed with a melamine sponge and all food debris was siphoned out daily to discourage any microbial and ciliate growth.

<u>Part 2: Immune Support and Stress Reduction</u>

The second portion of the treatment consisted of managing the water environment that the sea star resided in during treatment to maximize immune function and reduce stress. A star with open wounds or a dropped arm needed to expend energy regulating their water vascular system in order to maintain internal homeostasis. The pH of the water was extremely important when open wounds or lesioning was present in the stars. A lower pH environment allowed ciliates and bacteria to flourish and multiply faster than water at a higher pH level. This was tested by utilizing a *Uronema* trap with a small piece of tissue inside that was allowed to colonize over a 48-hour period at a certain pH. At the end of the period, the number of mixed species of flesh-eating ciliates in each trap was counted and averaged. The pH of the trial system was then elevated, and the experiment was repeated. It was found that a pH between 7.2 and 7.7 were optimal for excessive ciliate and bacterial flourishing, whereas a pH between 7.8 and 8.1 showed lower ciliate and bacterial growth on the tissue. This was noteworthy because Oregon Coast Aquarium's sea water intake is located where the Yaquina Bay meets the Pacific Ocean and its pH naturally varies throughout the year, often measuring 7.2-7.7 for long periods of time.

While harmful microorganisms are thriving, the star's immune system and body is put under stress due to the lower pH levels, leading to an immunocompromised condition. It was noted that internal fluids of sick stars with SSWD contain lower levels of calcium compared to the internal fluid levels of healthy stars (Wahltinez et al., 2020). The internal fluid composition of the stars is a more concentrated composition of the elements found in sea water. This was compared to the incoming natural sea water, and there were many periods of time throughout the year when sea water contained drastically lower levels of calcium, magnesium, strontium, iodine, and other trace elements. Therefore, the deficiency of these critical elements in the natural sea water could impact immune function during low pH events and microorganism overgrowth.

Correcting all of the above-mentioned environmental factors at the same time was possible with the use of a sea water buffer formulated for coral reef systems. Because the trace elements were so critical to the immune function support, buffering to adjust for pH and alkalinity with sodium bicarbonate and soda ash (sodium carbonate) alone were insufficient. Seachem® Complete Reef Buffer™ produced the highest quality results and was easily accessible.

The optimal pH level for a sick star was determined to be 8.1 based on the observed physical body responses of the sea stars that had dropped limbs. When the pH level reached between 8 and 8.1, the stars with open body wounds began to relax into a neutral body position and behaviors associated with attempts to regulate and flush the internal vascular system halted. To accomplish this without causing unnecessary stress, the pH of the quarantine tank was raised by no more than 0.2 daily from the pH of the stars' original system until the optimal pH was reached. After the pH was at the optimal level, it was checked daily and Reef Buffer was added as needed to prevent fluctuations and maintain consistent levels of trace elements. Tank water was changed (25%) and re-dosed every other day to manage presence of stress hormones, saponins and general bioload. The buffer was always dosed into the sump of the tank to allow thorough mixing before reaching the animals.

Part 3: Bacterial Control

In cases of SSWD, injury and illness, the threat of a secondary bacterial infection posed a large risk to the animal's chance of recovery. The ability to control harmful bacteria was critical to the success of the treatment. In the past, utilizing antibiotics was sometimes effective in eliminating harmful bacteria but came with the negative consequence of destroying beneficial bacteria. The destruction of the beneficial internal bacteria caused excessive stress on the animals and sometimes worsened the overall condition. Ultimately, antibiotics were replaced with probiotics.

A probiotic has been formulated for use with marine invertebrates, created for the commercial shrimp aquaculture industry. Ziegler® Rescue™ Probiotic inhibits harmful bacterial strains including *Vibrio spp.* while also providing beneficial probiotics to the animals.

Zeigler Rescue was delivered into the tank near the animals at an initial dose of 15ppm for the first three days of treatment to establish a high probiotic concentration in the tank, and then reduced to 10ppm for the next 28 days. If a star dropped an arm at any point in the treatment, the dosage was increased to 15ppm again for another three days then reduced to 10ppm again. The probiotic was always dosed 30 minutes after the buffer to ensure that the buffer was thoroughly mixed throughout the tank. Additionally, the UV filtration and protein skimmers were always turned off to ensure none of the probiotic was lost from the water. On days when the 25% water change was performed, the probiotic was always added after the water change. The longest period of time that the probiotic was used during the treatment was 90 days. As soon as the wound sites were fully sealed the probiotics were halted.

The temperature of the water in the treatment tank was reduced to between 50 and 51 degrees Fahrenheit to slow bacterial replication in the water. This was performed by setting the treatment tank to the same temperature as the star's initial system so no temperature stress occurred, and then lowering it by 1 degree Fahrenheit daily until it reached the desired range.

Incomplete Success

Two of the stars treated in the early stages of the treatment did not recover but played a large role in the development of a more effective protocol. One *Pisaster ochraceus* began treatment in the severe stage of SSWD with extreme *Uronema* infestation before the Reef Dip iodine baths were included in the treatment. The failure of recovery was a notable part of the developmental process of the treatment because it showed that treating for bacteria and immune support alone was not enough to recover the animal if the ciliate parasitism was not also controlled.

A second case involved a single *Henricia leviuscula* in the severe stage of SSWD with extreme open body cavity wounds at the central disk and no dorsal tissue on two of the arms. The animal was actively sloughing tissue before it was placed into the reef dip bath. After the animal was dipped and placed into the reef buffer and probiotic solution, the tissue loss halted entirely and it regained a strong grip to the tank walls. The progression of the wasting that typically would have ended in death within 24 hours was slowed by a total time period of two weeks. The damaged arms were ultimately dropped after a week and small amounts of tissue sloughing resumed after two weeks despite the immune support and bi-weekly reef dips. Due to the initial severe damage to the central body that included the digestive tract, prognosis was poor and the star was euthanized. This was the first case where the tissue melting symptom of SSWD was halted, and it became possible to prolong the life by a significant amount of time.

Food Refusal

In cases where damage to the oral cavity was present, the stars often refused food for long periods of time. A syringe was outfitted with a small catheter tube, then loaded with a mixture of INVE Aquaculture Selco® and sea water to deliver food near the external radius of the mouth. The stars would sit in the Selco mixed water for 45 minutes to an hour with aeration if the animal was showing no signs of stress. It was dosed at a bath rate of 1ml per 500 liters of sea water. The product had to be fully removed at the end of the hour to prevent fouling. After 30 minutes, the stars presented a "flushing behavior" where they began to deflate and inflate their bodies. During these internal water changes, animals incidentally pulled small amounts of Selco into the stomach. This was performed once per week on stars whose wounds had completely sealed over already but were still refusing food after a month. It was not done on any animals with unhealed wounds. Later into the treatment progress, the product Seachem® Reef Plus™ vitamin and amino acid supplement for corals, was added to the Selco bath at a dose of 0.5ml per 5 gallons.

This technique was very effective in keeping stars alive until they began to accept food again. The most notable case involved a *Pycnopodia helianthoides* who had sustained oral damage and lost half of its arms due to stress. It received weekly Selco soaks for 9 months until its oral cavity and stomach healed enough to accept food again.

Results

In all cases, the combination of the three factors successfully halted the progression of wasting significantly. A total of 17 stars across 7 species received the treatment, with 15 fully recovering. This included three *Pycnopodia helianthoides* who made a successful recovery. In the severe case of the *Pycnopodia helianthoides* that lost half of its limbs, all of the limbs were able to regrow over a one-year timespan.

Citations

Wahltinez, S. J., Newton, A. L., Harms, C. A., Lahner, L. L., & Stacy, N. I. (2020, February 20). *Coelomic fluid evaluation in Pisaster ochraceus affected by sea star Wasting syndrome: Evidence of osmodysregulation, calcium homeostasis derangement, and coelomocyte responses*. Frontiers. Retrieved December 26, 2022, from https://www.frontiersin.org/articles/10.3389/fvets.2020.00131/full

Giant Pacific Octopus, Bruce Koike

HOW TO BREAK ALL THE RULES AND STILL SUCCEED AT ALGAE CULTURE

Barrett L. Christie, Director of Animal Husbandry, bchristie@maritimeaquarium.org

The Maritime Aquarium at Norwalk, 10 N. Water St., Norwalk, CT 06854

Introduction

Algae culture has gotten a reputation as a finicky beast, subject to the whims and seemingly supernatural divinations of forces beyond our control. The exponential growth behavior of algae and possibilities for contamination by bacterial flora, crustaceans, viruses, yeasts, and other microorganisms provide a myriad of ways things can go horribly wrong. In response to this, those intensively culturing microalgae often become fanatical about sterilization and aseptic technique to mitigate the avenues for catastrophic failure of their cultures.

I went the opposite direction.

When faced with these challenges in attempting to provide algae for a freshwater mussel (Unionidae) propagation program from 2008-2016 I threw out the "rule book" of generally accepted best practices. The fastidious and tedious nature of constantly growing pure cultures from plates, only to inevitably struggle to see many of them crash prematurely, led me to draw upon my experience in propagating another group of microorganisms, namely *Saccharomyces* and *Brettanomyces* spp. for the fermentation of beer. Brewer's yeast are often cultured in sterile environments for the first few stages of exponential growth, but in large-scale culture some amount of contamination is generally acceptable, though inoculations (pitches) are sized large enough to allow the yeast to dominate the medium. The techniques used by brewers are adapted to minimize contamination by allowing for rapid proliferation, taking advantage of the fact that the most resilient microorganisms have mechanisms in place to allow them to adapt the culture medium to their specifications and outcompete other flora, and large inoculations allow individual species to quickly dominate the medium, excluding competitors.

These algae methods described below have evolved over time, and rely on trial and error to find species most adaptable to culture. I start with mixed cultures of wild algae with boatloads of bacterial, fungal, protozoal, and zooplankton contamination. I don't sterilize anything in these methods (other than cleaning bottles with bleach), the culture water is straight from a well through a dirty spigot and I don't even bother to filter the air feeding them. Sure, I've tried these other methods and been a slave to aseptic technique, but eventually found that once the right species of wild algae are found and they are adapted to culture, sloppier methods can still be successful. And yes, it is indeed possible to maintain stable cultures being this lazy, if one is persistent enough!

Description of Methods

One starts with mixed cultures from the wild instead of a pure starter culture that has been plated and isolated. A 5-gallon bucket of water from a stream or other natural body of water that is especially green is brought back from the field. At the aquarium the water is run through a 250μm filter sock into a (clean) bucket, then through a 125μm sock into another bucket, and finally through a 25μm sieve into another (food-grade) plastic bucket. This excludes most larger zooplankton (but not their eggs) and most large protozoa and particulate matter.

The filtered algae-containing water is modified to become an enrichment medium (ESM) with the goal of selecting for algae and selecting against bacteria with the addition of antibiotics and inorganic carbon, and adjustment of the pH to 4.0-4.5 with HCl. Erythromycin (500mg) is added to stop reproduction of most Gram- species and ampicillin (200mg) is used to kill most Gram+ bacterial flora. The low pH also inhibits bacterial growth of many species, and sodium bicarbonate (600mg) and sodium carbonate (100mg) are provided as an inorganic carbon source. The bucket is stored in a refrigerator for 24-48h, during which time most microorganisms will concentrate at the bottom, allowing the water above to be decanted, greatly concentrating the solution.

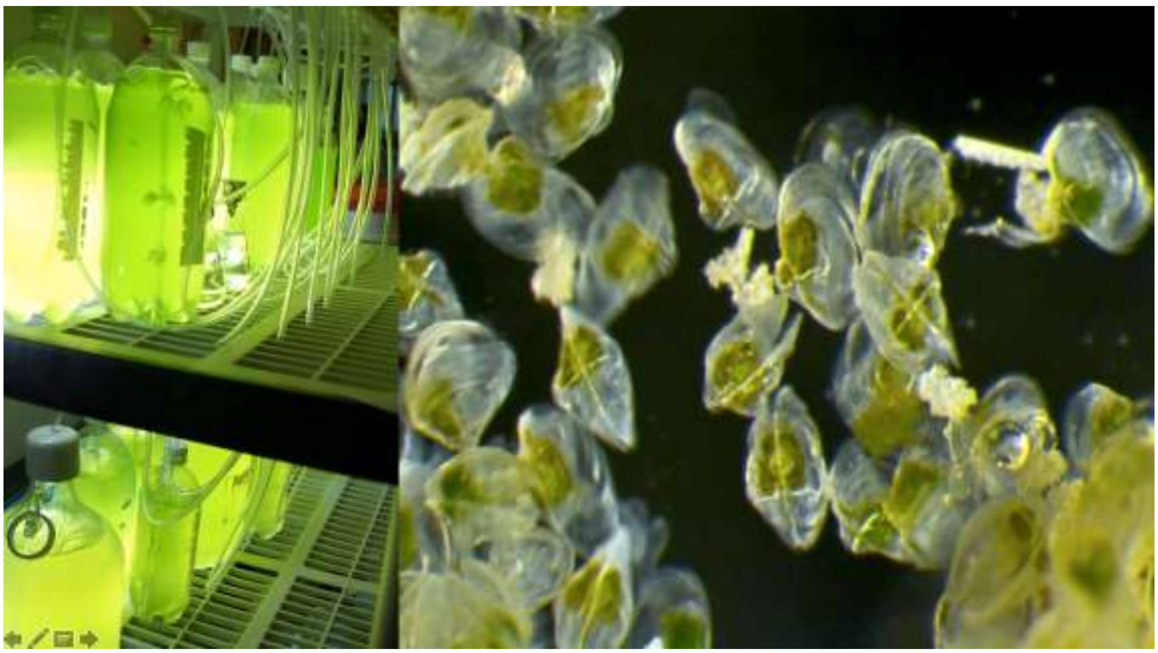


Figure 1. Algae culture setup producing microalgae "the wrong way" (left) with unsterilized media, mixed cultures, and boatloads of contamination. From 20 liters of culture 5,000 ml of extremely dense greenwater could be harvested daily to feed freshwater mussels (right). Note guts packed green in these juvenile *Lampsilis teres* (Unionidae) from filter feeding on eight species of microalgae of wild origin.

After concentrating and separating the algae sieve to 25µm again, and use this green water as an inoculum for multiple small starter culture bottles. Add 33-50% green water to the starter cultures and fill the rest with a modified Guillard's f/2 medium containing no organic carbon (ASM f/2) but containing carbonates and bicarbonates as an inorganic carbon source, and antibiotics to suppress bacteria. Note that formulations for the media described are included as an appendix for reference. Place the starter cultures on a rack with intense lighting and gentle aeration (a mix of 2:1 actinic:white works extremely well), without B-vitamins growth will be sluggish, but the large percentage of inoculant and antibiotics will favor the growth of algae and suppress bacteria and other heterotrophic microflora. As cultures turn dark green, re-sieve to 25µm every 24h to remove crustaceans that may have hatched. This stage will require trial and error until species are found that are most adaptable to being maintained in culture.

After about a week, for any cultures that do start to grow and turn dark green, continue to sieve to remove any zooplankton, and double the culture medium by transferring into a larger vessel with ASM f/2 medium, when these cultures start to grow, sieve again, and double the culture again to a full 1000-2000ml culture vessel with standard G f/2 medium. At this stage with added B vitamins growth should be explosive compared to the ASM f/2 medium.

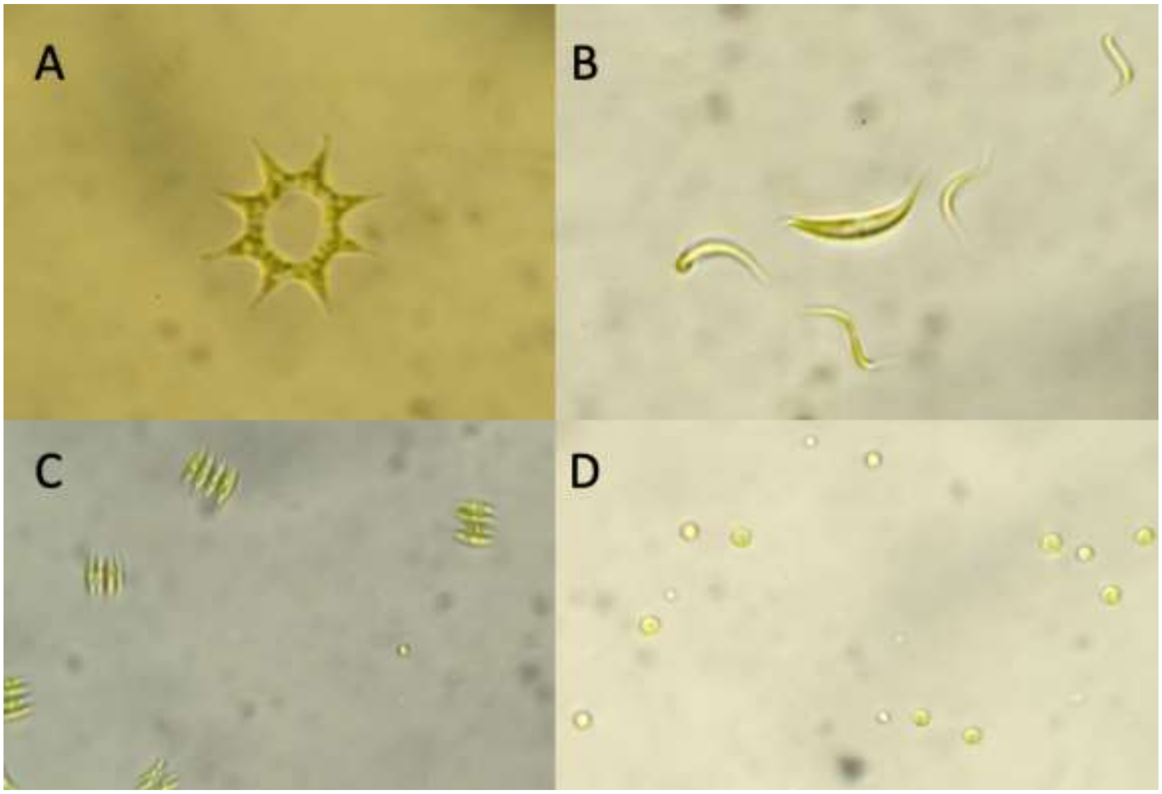


Figure 2. Various species collected from the wild that adapted very well to culture and grew vigorously (A) *Asterionellopsis* sp. -or- *Pediastrum* sp. (B) *Ankistrodesmus* sp. (C) *Scenedesmus cf. quadricauta* (D) *Pseudotetracystis* sp. Magnification 400x.

Once cultures are off and running with standard G f/2 medium continue to re-culture aggressively with large (33-50%) inoculants of the prior culture, once every 72h, each time sieving to 25µm. After about a month sieving can be discontinued. The cultures will grow stronger and stronger with each re-inoculation. If excessive contamination of biofilm starts to form, or lots of bacteria are seen under the microscope, you can switch back to ASM f/2 for a few cycles to suppress them, before switching back to standard G f/2 medium for vigorous growth. Multiple attempts will likely need to be made before algae species that adapt readily to culture are found. Trial and error are necessary, but persistence will eventually result in extremely strong cultures.

These methods use the same principals as artificial cycling of nitrifying bacteria in biofilters, namely suppression of heterotrophic bacteria and other microflora by limiting organic carbon. Many algae species, similar to nitrifying bacteria, can also utilize carbonates and bicarbonates (in addition to CO_2) as a carbon source for growth and reproduction (Dickman, 1973; Chi et al., 2011). Many species of nitrifying bacteria will also undoubtedly be present in these water samples, however as the modified ASM f/2 medium only contains nitrate, the AOB/NOB species will be limited in their growth. Denitrifying species will similarly not be able to proliferate to exploit the NO_3 present because the culture is never anoxic, thus only a limited set of autotrophic bacteria and the desired algae species will be favored in culture.

Continue to re-culture often, each time with a large inoculum (33%+) of the prior algae bottle, this will allow the algae to quickly become the dominant organism each time a culture is re-started. It is important to continue to frequently re-start cultures to keep the algae in the exponential growth phase, even if this produces more algae than is needed. Surplus algae can be refrigerated and kept for 5-10 days. If desired, the dominant species can be plated onto agar and isolated for a more traditional aseptic approach, but if they are frequently re-cultured it is possible to create stable cultures with minimal sterilization. Bottles can be cleaned with bleach to dissolve biofilms, but sterilizing of culture water and neutralizing with sodium thiosulfate is unnecessary with strong cultures.

Using these methods, I have sustained monocultures of 12+ species and various polycultures of mixed algal species for several years, some of these species shown in Fig. 2. Some of these cultures persisted and were re-cultured for over 500 generations, with periodic return to ASM f/2 culture to reduce contamination levels. In general, this autotrophic 're-set' is needed about once every 75-100 re-cultures. It is also worth noting that the success of these unorthodox autotrophic selection methods may have been in part due to the hardness of the well water being used. The water of the Dallas Aquarium at Fair Park well is extremely hard, with significant amounts of not only calcium, but also bicarbonate and sulfate. A formulation to re-create this water from RO/DI is included as an appendix to this study.

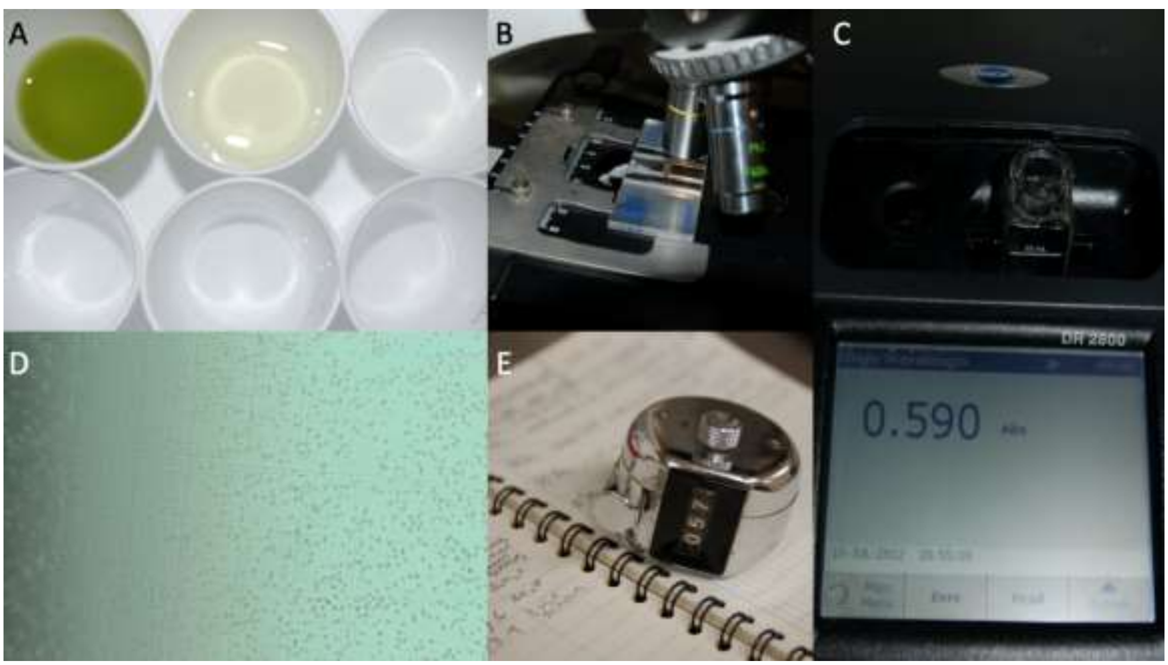


Figure 3. Quantification of algal cell density using a spectrophotometer. Serial dilutions of culture are prepared (A) and counted using a hematocytometer (B,D,E), these same dilutions are placed in cuvettes and absorbance measured (C). Once regression functions are calculated cell densities can be rapidly measured from cultures through absorbance.

Spectrophotometric Algal Cell Counts

Measurement of culture density can be done in several ways. Direct counts of cell density using microscopy in a Neubauer hematocytometer is the lowest cost, simplest method.

Commercial hatcheries often use flow cytometers to automate the process in large-scale microalgae culture applications. But estimation of cell density by proxy through absorption measurements on a spectrophotometer can enable rapid assessment of numerous samples (Fig. 3). Each species of microalgae will have its own unique absorbance curve and specific range where absorbance has a linear relationship with cell density.

Using the methods described above many algal species will eventually become dominant and account for 90-95% of a given culture, however it is also common for polycultures to develop where two species reach a détente of sorts, and each accounts for roughly half of the cells present. In these cases absorption curves will need to be constructed specific to the polyculture, as absorption vs. cell density relationships will vary considerably from the characteristics of either species in monoculture. Figure 4 shows absorbance relationships for three species of freshwater algae and two polycultures.

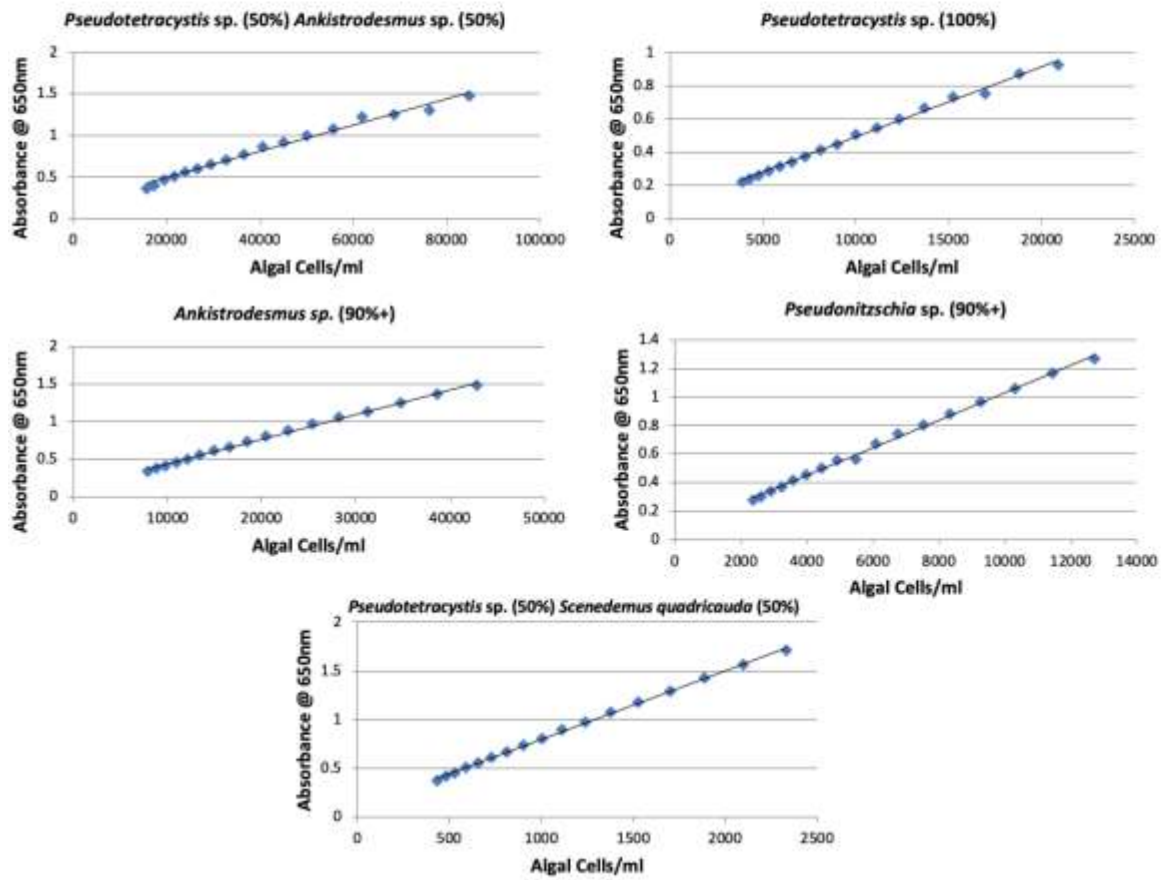


Figure 4. Linear regressions for various mixed-culture, pure-culture, and nearly pure-culture wild algal spp. R^2 >0.995 for each of these cell density vs. absorbance regressions. Measurements taken at λ=650nm, near-peak absorbance for chlorophyll-a. Constructing absorbance curves for various algal species can enable rapid quantification of cell density using a spectrophotometer.

Procedure for Constructing Absorbance Curves

The following are the specific steps to gather absorbance vs. cell density information to construct curves for rapid cell measurements:

1) Turn off aeration and let culture bottle stand 1-2 minutes to allow large flocculants and particles to settle, decant microalgae from the top of the culture bottle.
2) Collect another volume of culture water equivalent to the volume of all dilutions planned (e.g. if using n=20 25ml serial dilutions collect 500ml) and filter to 0.45µm to remove all cells from the water. (*this water will not contain algae but will contain color-influencing compounds from lysed cells, using this for dilution eliminates errors from matrix effect that will result if DI water is used for dilutions*).
3) Create serial dilutions, using the filtered water and original sample, at least 6, but as many as desired, more data will result in better results from regression analysis.
4) Swirl dilutions to homogenize, sample with pipet, and using a Neubauer hematrocytometer determine the cell density in each dilution and record algal counts.
5) Set wavelength of spectrophotometer (λ) to 650nm (*this is near-peak absorption for chlorophyll-a; alternatively a wavelength scan can be performed to identify absorption peaks unique to the species being analyzed, if desired*)
6) Measure absorbance for each dilution, record values.
7) Using an Excel spreadsheet (or your preferred flavor of stats software) run linear regressions on the cell counts vs. absorbance. There should be a strong linear relationship between the columns of data.
8) If $R^2<0.95$ eliminate data points at the high-end and/or low-end of the regression until $R^2>0.95$ (or $R^2>0.99$ for more precise estimations). This determines the range where absorbance can be used for rapid cell counts. Some cultures may require slight dilution for measurement in practice, as they grow to densities above the effective range.

Once regressions are confirmed to high statistical power ($R^2>0.95$-0.99), tables can be made to rapidly translate absorbance measurements into cell counts. Cell count estimates can be used for dosing microalgae precisely to avoid overfeeding and maximize culture efficiency. For applications like bivalve propagation where precise feeding is required, and multiple cultures are being harvested daily, this will save considerable time compared to conducting manual counts and performing dilution calculations.

Literature Cited

Chi, Z., J.V. O'Fallon, S. Chen. 2011. Bicarbonate Produced from Carbon Capture for Algae Culture. Trends in Biotechnology. 29(11): 537-541

Dickman, M. 1973. Changes in Periphytic Algae Following Bicarbonate Additions to a Small Stream. Journal of the Fisheries Board of Canada. 30(12): 1-17.

Guillard, R.R.L. and J.H. Ryther. 1962. Studies of Marine Planktonic Diatoms I. *Cyclotella nana* Hustedt and *Detonula confervacea* Cleve. Canadian Journal of Microbiology. 8:229-239

Appendix: Algal Growth Media Recipes

Below are the specific formulations used to create algal growth and selection media described in this study. Also included is a salt formulation to re-create the well water used in the methods described above to mimic the well at the Dallas Aquarium that has been supplying the facility with (extremely hard) water for 86 years.

Standard Guillard's f/2 Formulation (G f/2) – per 1000ml

Guilliard's f-medium (Guillard and Ryther, 1962) is typically diluted to 50% for algae culture and known commonly as f/2 medium. Concentrations shown per one liter of medium.

$NaNO_3$……………………………………………………………...……75mg
$NaH_2PO_4{\bullet}H_2O$…………………………………………………………5mg
Trace Metals Solution (in DI H_2O)
- $ZnSO_4{\bullet}7H_20$……………………………………...……………23µg
- $MnSO_4{\bullet}H_2O$……………………………………………………152µg
- $NaMoO_4$………………………………………………………...…7µg
- $CoSO_4{\bullet}7H_2O$…………………………………………………….14µg
- $CuCl_2{\bullet}2H_2O$………………………………………………………7µg
- $Fe(NH_4)_2(SO_4)_2{\bullet}6H_2O$…………………………………………...5mg
- $Na_2EDTA{\bullet}2H_2O$…………………………………….…..………..5mg

B-Vitamins Solution (in HEPES buffer)
- Cyancobalamin (B12)……………………….…...……………….…135µg
- Biotin (B7)……………………………….…………………….....25µg
- Thiamine (B1)………………...…………………………...…..335µg

Notes: Trace Metals Solution and B-Vitamin Solution are typically prepared as stock solutions and added in liquid form. Trace metal salts are heated and solution prepared in DI water, stored refrigerated. B-Vitamin solution is typically prepared with 50 mM HEPES buffer and filter-sterilized to 0.23µm for microbial stability in storage. 30g salts per 1000ml medium can be added for marine algal species. Silica salts may be added for diatom culture.

Water Salts to Recreate Dallas Aquarium Well Water

Per 1.0-gallon RO/DI H20:
- $CaCl_2$…………………………………………………………………...150mg
- $CaSO_4$……………………………………………………………….....5.75g
- NaCl……………………………………………………………...…250mg
- $NaHCO_3$……………………………………………………..…..1.75g

Notes: the resulting solution will have a calcium level of approximately 460 mg/l calcium, 170mg/l sodium, 90mg/l chloride, 1070mg/l sulfate, and 300mg/l bicarbonate. This simulates the water chemistry of the well at the Dallas Aquarium at Fair Park which produces extremely hard water, with large quantities of calcium, sulfate, and inorganic carbon present as bicarbonate.

Autotrophic Selection Modified Guillard's f/2 Formulation (ASM f/2)

This is a modification of standard Guillard's f/2 medium that eliminates organic carbon sources to prevent growth of heterotrophs, while still providing a source of nitrate, phosphate, and inorganic carbon for algal species. Concentrations shown are per one liter of medium.

$NaNO_3$……………………………………………………………..……75mg
$NaH_2PO_4 \bullet H_2O$…………………………………………………………5mg
Trace Metals Solution (in DI H_2O)
- $ZnSO_4 \bullet 7H_20$……………………………………..……………23µg
- $MnSO_4 \bullet H_2O$……………………………………………………152µg
- $NaMoO_4$…………………………………………………………..….7µg
- $CoSO_4 \bullet 7H_2O$……………………………………………………14µg
- $CuCl_2 \bullet 2H_2O$……………………………………………………7µg
- $Fe(NH_4)_2(SO_4)_2 \bullet 6H_2O$…………………………………………...5mg
- $(NaPO_3)_6$…………………………………….…..………………10mg

$NaHCO_3$…………………………………...….……………………..158mg
Na_2HCO_3…………………………………………………………..….28mg
Erythromycin………………………………………………………....25mg
Ampicillin……………………………………………………………….10mg

Notes: Elimination of B-vitamins to exclude organic carbon will lead to less vigorous growth but suppress heterotrophic flora. Note that sodium EDTA as a chelating agent in the trace metals solution is replaced here by sodium hexametaphosphate, a polyphosphate agent commonly used in domestic water treatment. An addition of sodium bicarbonate provides an additional inorganic carbon source.

Enrichment and Selection Medium (ESM)

Per 5.0-gallons H_2O from wild containing algae:
Erythromycin……………………………………………………......500mg
Ampicillin……………………………………………………………...200mg
$NaHCO_3$……………………………………………………..……600mg
Na_2CO_3……………………………………………………...…….100mg
HCl added to adjust pH to 4.0-4.5

Notes: Antibiotics and low pH suppress both Gram+ and Gram- bacterial flora, sodium carbonate and sodium bicarbonate offer an inorganic carbon source. Water should be kept at 0-8^{o}C for 24-48 hours to allow algae to settle to bottom and for bacteria to die off.

General Notes: The f/2 formulations were modified from data at the University of Texas Culture Collection Of Algae (https://utex.org/) which has a large amount of information available on algae culture.

RAW
on the river
Dubuque 2023

THE NATIONAL MISSISSIPPI RIVER MUSEUM & AQUARIUM

Port of Dubuque, Iowa

Registration:

To register for the Regional Aquatics Workshop, click the link below and follow the instructions. https://75747.blackbaudhosting.com/75747/RAW-Conference

The registration link will show a price of $175 initially, but if you register before March 31, 2023, you will receive a discounted $150 early bird rate at checkout. Starting April 1, 2023, the price will be $175 as listed.

To pre-purchase a t-shirt for $20, 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. https://75747.blackbaudhosting.com/75747/RAW-Conference—T-Shirt

Contact:

If you have any problems or questions regarding registration or hotel reservations, reach out to Marilyn Snyder at msnyder@rivermuseum.com or 563-557-9545 x201.

Schedule:

The rough agenda will be as follows:
Sunday, May 7 – Aquatic TAG steering committee meetings
Monday, May 8 – Aquatic TAG public meetings
Tuesday, May 9 through Friday May 12 – RAW proper
Tuesday, May 9 evening – Icebreaker at the National Mississippi River Museum & Aquarium

Conference Hotel:
Grand Harbor Resort & Waterpark, $119 per night plus 12% tax and 16% service fee.
To reserve your room at the Grand Harbor Resort, call 563-690-4000 and ask for the block of rooms reserved for Regional Aquatics Workshop. The only way to receive the discounted rate is to call. If the initial block of rooms fills up, we will add more to meet demand, so let us know if you run into trouble.
(Overflow hotels include the Holiday Inn and Hotel Julien, each within walking distance of the conference venue and the River Museum.)

Conference Venue:
Grand River Center, Dubuque, Iowa, right next door to the Grand Harbor Resort, the National Mississippi River Museum & Aquarium, and Diamond Jo Casino.

Airport Info:
Preferred: We recommend people fly into the Eastern Iowa Airport in Cedar Rapids, Iowa (CID) where Travel Dubuque will arrange for a shuttle service from there to Dubuque (82 miles).
Alternatives: You can also fly into MLI Moline, IL (80 miles), MSN Madison, WI (98 miles), or Chicago, IL (177 miles from ORD O'Hare, 199 miles from MDW Midway) and arrange your own ground transportation from there.

Travel Estimates:
Flight Costs to Cedar Rapids, Eastern Iowa (CID) from Sample Locations
(Priced on December 26, 2022 and based on Arrival May 6 and departure May 12th)

- Orlando (MCO) ~$455+
- Los Angeles (LAX) ~$454+
- Houston (IAH or HOU) ~$423+
- Boston (BOS) ~$428+
- Seattle/Tacoma aka Sea-Tac (SEA) ~$496+
- Cleveland Hopkins (CLE) ~$395+

Driving Times to Dubuque from Selected Cities (hours)

- Minneapolis, MN 4.6
- Chicago, IL 3.1
- Cleveland, OH 8.8
- Pittsburgh, PA 10.5
- St. Louis, MO 5.0
- Omaha, NE 5.0

MANAGEMENT OF INFECTION FOLLOWING INJURY TO THE SPIRAL COLON IN A SAND TIGER SHARK, *Carcharias taurus*

Sandi Schaefer-Padgett, Bert Sadler, Rachel Stein, and Barrett L. Christie
sschaefer@maritimeaquarium.org

The Maritime Aquarium at Norwalk, 10 N. Water St., Norwalk, CT 06854

Introduction

Eversion of the spiral colon in elasmobranch fishes is known from at least 19 species (Crow et al. 1990, Henningsen et al. 2005, Clark et al., 2008; Christie, 2012; Wirsema et al., 2015; Rangel et al., 2021). See Table 2 for a summary. This behavior seems to be associated with expulsion of indigestible particles from the lower GI tract, and may very well be performed in most elasmobranch species (Christie, 2012). Trauma to the everted spiral colon has been associated with high incidence of acute mortality (Crow et al., 1991; Christie, 2008) as the tissue is highly vascularized and bites or damage often lead to the animal bleeding out rapidly. There are, however, some records of animals surviving long enough that medical intervention can be attempted, and sharks have been known to survive these incidents (Crow and Brock, 1993).

Sharks in general have shown to be incredibly durable animals, able to survive perforations of the gastrointestinal (GI) tract in some cases (Kessel et al., 2017), however despite these cases there are also incidences of morbidity and mortality arising from such trauma (Borucinska et al., 2001; Otway et al., 2021). The tissue of the GI tract is highly vascularized to aid in nutrient absorption and transfer, often leading to exsanguination following trauma, and it is also home to a dense microbial flora, presenting high likelihood of infection and sepsis secondary to perforations or injury. Contrast radiography has been used in some cases to visualize the extent of GI damage following damage to the spiral valve (Schaller and Dunker, 2005).

Bites to the everted spiral colon from conspecifics are the most common source of trauma recorded in the literature (Crow et al., 1991; Schaller and Dunker, 2005; Christie, 2008), however bites from other fishes have equal potential for serious medical complications. Weideli and Papastamatiou (2021) documented chasing and attempted biting of everted spiral colons from wild sharks, and numerous species have been observed performing similar behaviors in captive settings (Christie, pers. obs.). In general, triggerfishes of the family Balistidae have been anecdotally observed as a taxon prone to investigative biting behavior in aquaria, and are widely regarded as a beautiful nuisance in aquarium collections. Certain taxa known for investigative biting and possessing prominent dentition, such as the triggerfishes, pufferfishes, snappers, and others may pose a greater risk to sharks when sensitive tissues such as the lower GI are exposed for periodic flushing.

Case Report

A male sand tiger shark, *Carcharias taurus* (Accession no. MARITIME/00549) with the house name "Nacho" has been in the collection of the Maritime Aquarium (TMA) since being collected as a young-of-the-year in Rhode Island in the 1990's. In captivity, *C. taurus* has exhibited dominance hierarchies and sexually-motivated aggression between males (Claus et al., 2021). Given the ethological characteristics of sexual hierarchies described by Claus et al. (2021)

this individual seemed to be the dominant male of the 3.3.0 group housed at TMA as evidenced by his interactions with other males, but also with his corresponding high rate of copulation with the female population. Specimen 00549 routinely was observed engaging in more frequent copulatory behavior, and bore more mating scars during the breeding season than the other male *C. taurus* in the TMA collection. The body condition of this animal has been cyclical and predictable, annually increasing in mass approximately 10% between each November-January, largely inappetent and decreasing in body condition February-March, and finishing the breeding season in March or April approximately 10% below baseline weight (Figure 1). This individual also has been noted to display intestinal eversion behaviors with increasing frequency during and after the breeding season (Figure 2).

Figure 1. *Carcharias taurus* MARITIME/00549 at the end of the 2020 breeding season, note slender body condition and conspicuous lacerations from encounters with subordinate males and copulatory activity with female conspecifics.

On May 15th 2020, while the aquarium was closed amid the SARS-CoV-2 lockdown, a grey triggerfish, *Balistes capriscus,* was observed biting at the everted spiral valve of *C. taurus* 00549, the damage resulted in lacerations to the colon and bleeding into the water (Figure 4). The triggerfishes were immediately removed from the exhibit, and at the veterinarian's direction a course of oral antibiotics (cephalexin 25mg/kg PO) was started to mitigate infection. After 4 days the animal had refused food, and thus antibiotics were not being received. An injection of enrofloxacin (Baytril®) was given (5mg/kg IM) of the 1st and 5th day after trauma in the hopes that the animal's appetite would return and medications could be administered orally. After 28 days the animal was still inappetent most days, coloration was poor, and swimming was labored. An additional enrofloxacin injection was given, along with ampicillin sodium (10mg/kg). The animal resumed feeding sporadically, and oral cephalexin (25mg/kg PO q3d) was resumed.

By 101 days after the initial trauma the animal had shown some improvement in color and swimming behavior while on oral antibiotics, but was displaying signs of cachexia (wasting and loss of muscle mass due to an underlying disease process) and had lost an estimated 15-20% of its body weight (see Figure 3). The specimen was caught and given a physical examination that was largely inconclusive, but bloodwork showed elevated WBC counts indicative of an infectious process, and elevated hematocrit suggesting dehydration. The skin also was beginning to show signs of dermal turgor (wrinkles) from dehydration and loss of muscle mass.

Enrofloxacin has been widely used in elasmobranchs (Mylniczenko and Clauss, 2017), but is known to cause a sterile abscess in some cases, owing to the high pH of the injectable formulation. Additionally, repeated handling for injections is known to carry risks such as added general stress, inflammation of injection sites, and increased morbidity from handling (Steeil et al., 2014). After consultation with colleagues, the aquarium's veterinarian prescribed a relatively new antibiotic, cefovecin (Convenia®) known to have a long half-life in some species, requiring a single dose for an effective course of antibiotic therapy in some species. Following administration of cefovecin the appetite of the animal returned almost immediately, allowing for consistent administration of oral antibiotics. Over the next 45 days the animal began to gain weight, both body condition and integument color and skin texture improved significantly, and the course of oral antibiotics was discontinued. At 215 days from the initial trauma the animal again began to refuse food, on day 225 a second cefovecin injection was administered along with vitamin B-Complex as an appetite stimulant and for general nutritional support, and a 21-day course of cephalexin followed (25mg/kg PO q3d). After this the animal continued to gain weight, and after nearly 250 days of medical intervention to combat the infections resulting from the spiral valve trauma the specimen was back to a normal body condition and behavior (Figure 3). The pharmaceuticals used during this protracted recovery are summarized in Table 1.

Table 1. Medications Used to Control Infection in *Carcharias taurus*

Day	Drug	Type	Dose	Route	Frequency
1	Enrofoxacin	Antibiotic	5mg/kg	IM	Once
5	Enrofoxacin	Antibiotic	5mg/kg	IM	Once
8-20	Cephelexin	Antibiotic	25mg/kg	PO	q3d
28	Enrofoxacin	Antibiotic	5mg/kg	IM	Once
28	Ampicillin	Antibiotic	10mg/kg	IM	Once
30-90	Cephelexin	Antibiotic	25mg/kg	PO	q3d
101	Cefovecin	Antibiotic	8mg/kg	IM	Once
102-145	Cephelexin	Antibiotic	25mg/kg	PO	q3d
225	Cefovecin	Antibiotic	8mg/kg	IM	Once
225	B-Complex	Vitamin	5mg/kg	IM	Once
226-247	Cephelexin	Antibiotic	25mg/kg	PO	q3d

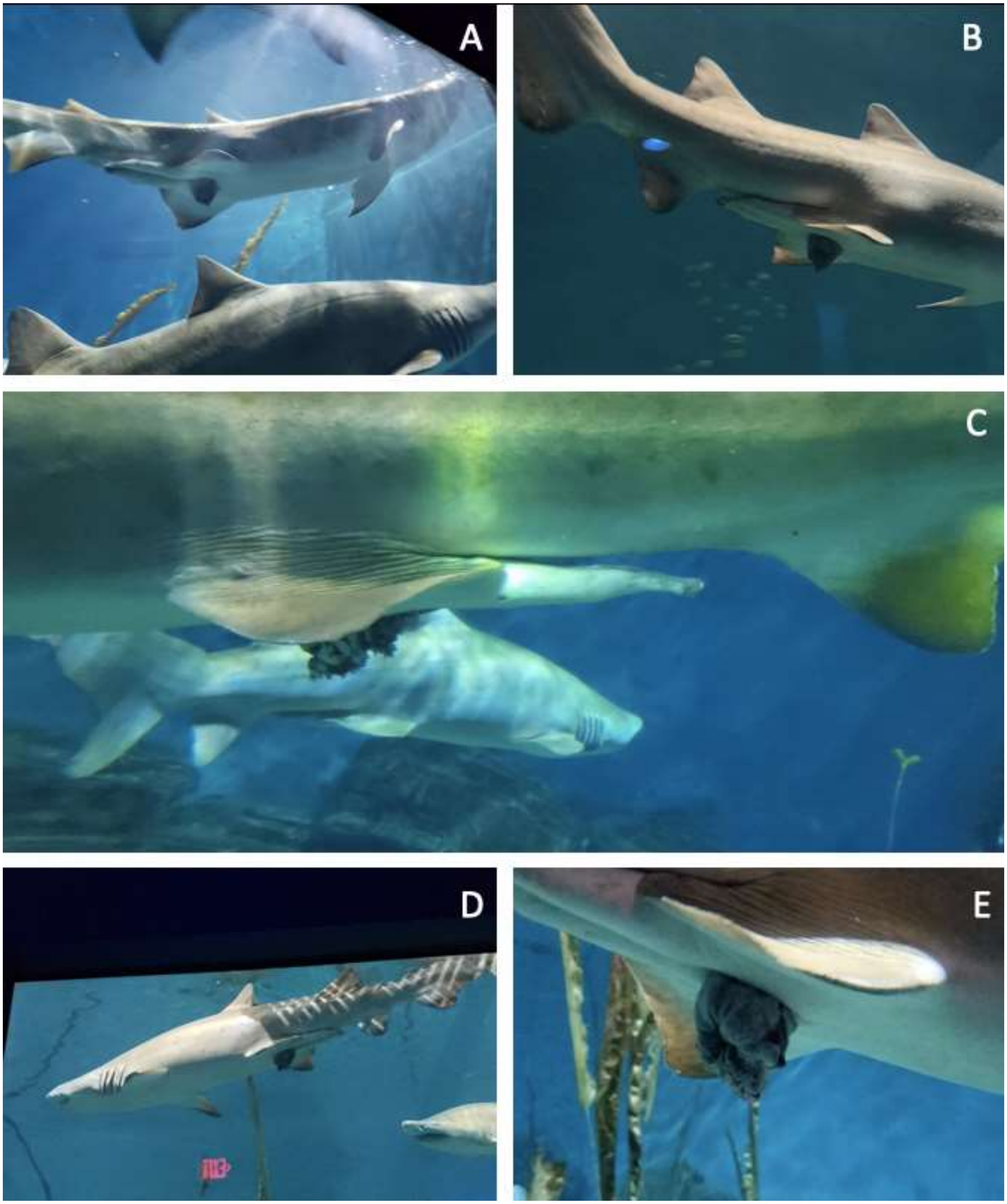


Figure 2. Intestinal eversion in *Carcharias taurus* MARITIME/00549 “Nacho” at various time points. A- November 2018, B- February 2020, D- December 2019, and E- April 20. Photo C shows the everted spiral valve immediately after bite trauma in May 2020.

Table 2. Recorded Instances of Intestinal Eversion in Elasmobranchs to Date from the Literature

Species	Common	Captive/Wild	Location*	Reference
Orectolobiformes: Ginglymostomidae				
Ginglymostoma cirratum	Nurse Shark	Wild	Brazil	Rangel et al., 2021
Lamniformes: Odontaspidae				
Carcharias taurus	Sand Tiger Shark	Captive	TMA	*Present Study*
Carcharhiniformes: Carcharhinidae				
Carcharhinus galapagensis	Galapagos Shark	Captive	UZG	Crow et. al., 1990
Carcharhinus leucas	Bull Shark	Captive	SWC, OEA	Crow et. al., 1990
Carcharhinus melanopterus	Blacktip Reef Shark	Captive	WAQ, SWC	Crow et. al., 1990
Carcharhinus obscurus	Dusky Shark	Captive	OEA	Crow et. al., 1990
Carcharhinus plumbeus	Sandbar Shark	Captive	OEA, NAIB	Crow et. al., 1990
Negaprion acutidens	Sicklefin Lemon Shark	Captive	WAQ	Crow et. al., 1990
Negaprion brevirostris	Lemon Shark	Captive	SWC	Crow et. al., 1990
Rhizoprinodon terranovae	Atlantic Sharpnose Shark	Captive	MGA	Christie, 2012
Triaenodon obesus	Whitetip Reef Shark	Captive	WAQ	Crow et. al., 1990
Carcharhiniformes: Sphyrnidae				
Sphyrna tiburo	Bonnethead Shark	Captive	DAFP	Christie, 2012
Hexanchifromes: Hexanchidae				
Notorynchus cepedianus	Broadnose Sevengill Shark	Wild	S. Africa	Wiersma et al, 2016
Carcharhiniformes: Triakidae				
Mustelis canis	Dusky smoothhound	Captive	MSA	Christie, 2012
Squaliformes: Squalidae				
Squalus acanthias	Spiny Dogfish	Captive	MSA	Christie, 2012
Pristiformes: Pristidae				
Pristis pectinata	Smalltooth Sawfish	Captive	NAIB	Henningsen et. al., 2005
Rajiformes: Rhinobatidae				
Rhinobatos rhinobatos	Common Guitarfish	Captive	ZMA	Christie, 2012
Myliobatiformes: Dasyatidae				
Hypanus americana	Southern Stingray	Captive	DTA	Christie, 2012
Myliobatiformes: Potamotrygonidae				
Potamotrygon motoro	Motoro River Ray	Captive	DAFP	Christie, 2012
Myliobatiformes: Myliobatidae				
Manta birostris	Giant Manta	Wild	Hawai'i	Clark et.al., 2008
Rhinoptera bonasus	Cownose Ray	Captive	DAFP	Christie, 2012
Aetobatus narinari	Spotted Eagle Ray	Wild	Brazil	Rangel et al., 2021

**Legend of aquarium facilities for captive records: UZG = Ueno Zoological Gardens, Tokyo; SWC = Sea World San Diego; OEA = Okinawa Expo Aquarium; WAQ = Waikiki Aquarium, Honolulu; NAIB = National Aquarium in Baltimore, MGA = Moody Gardens Aquarium, Galveston; DAFP = The Dallas Aquarium at Fair Park; MSA = Maine State Aquarium West Boothbay Harbor, ZMA = Zoomarine Mundo Aquático, Albuferia; DTA = Downtown Aquarium Houston; TMA = The Maritime Aquarium, Norwalk.*

Figure 3. Body condition of *Carcharis taurus* 00549 before (A,C) and after (B,D) cachexia induced by infection secondary to spiral valve laceration. Note the wrinkles present on the skin (D) from dehydration.

Discussion

Cefovecin is a relatively new third-generation cephalosporin antibiotic with a broad spectrum of antimicrobial action and pharmacokinetic data shows a very long terminal half-life in some species (Wernick and Müntener, 2010). In species where the drug persists for an extended time in an animal the use of cefovecin is advantageous because of decreased risk factors associated with handling and repeated injections (Steeil et al., 2014). This is an especially attractive possibility in elasmobranch husbandry and medicine, because of both the difficulty and greater physiological stress from handling which can lead to lactic acidosis and death in many species. While quite promising, it is still unknown how the drug will be metabolized or eliminated across various shark species. Among aquatic and marine species there are reports of cefovecin usage in sea otters (Lee et al., 2016), rockfishes of the genus *Sebastes* (Seeley, 2016), horseshoe crabs, *Limulus polyphemus* (Steeil et al., 2014), and whitespotted bamboo sharks, *Chiloscyllium plagiosum* (Steeil et al., 2014). In rockfishes the terminal half-life of cefovecin was 32.5h (Seeley et al., 2016), however in whitespotted bamboo sharks it averaged only 2.0h (Steeil et al., 2014), suggesting varying effects between fish species.

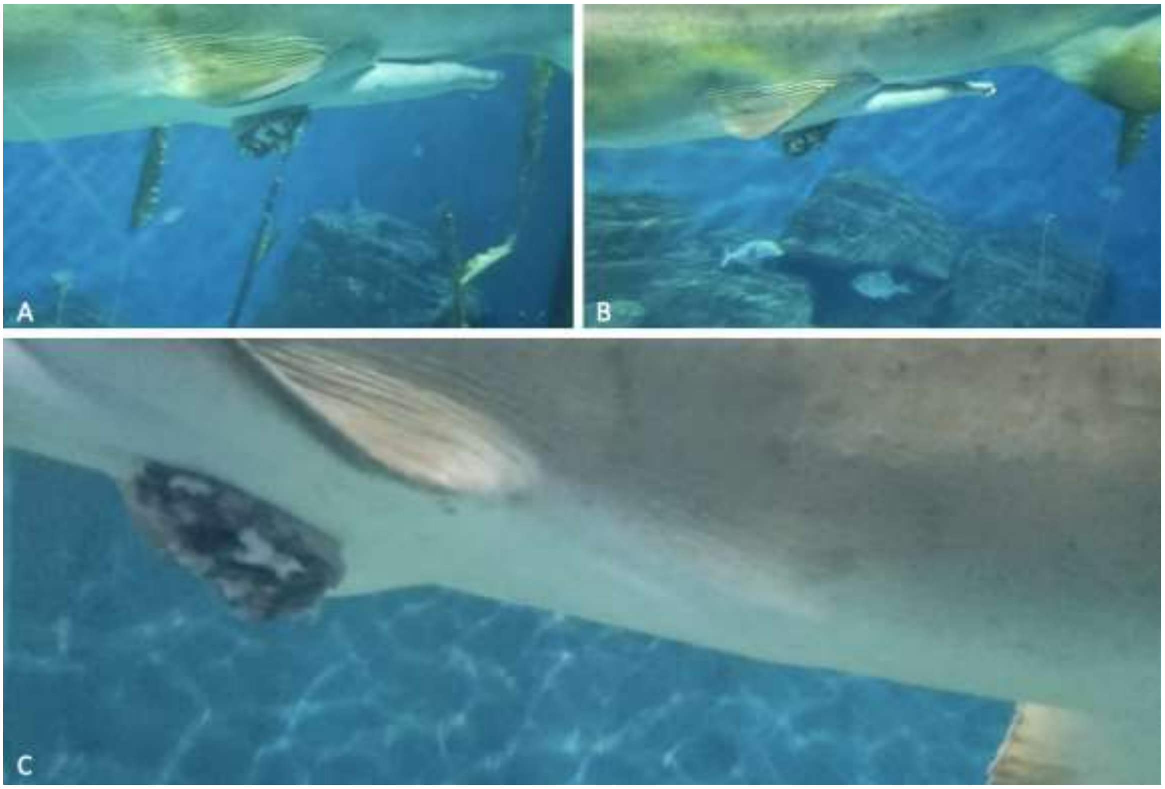


Figure 4. Probably more lacerated shark butts than you needed to see. The photos above show the extent of the lacerations to the spiral valve the day it was bitten. Note the offending triggerfish (B), and the visible tears to the lining of the lower GI. While these wounds were superficial enough so as not to result in exsanguination, they allow for rapid and severe infection from the resident gut microflora.

It is not unheard of for elasmobranchs to survive trauma to the spiral valve, and Crow and Brock (1993) document a similar case in *Carcharhinus melanopterus*, which was managed with gentamycin sulfate. In this case the protracted inappetence, dehydration, and loss of body condition took 250 days of medical intervention to resolve, aided by the use of a novel drug in sand tiger shark medicine. It is unknown if cefovecin persists as long in *C. taurus* as in some other species, but anecdotally in this case it seemed to have remarkable effects and warrants further investigation by clinicians working with aquarists on the management of elasmobranchs in aquaria. To the knowledge of the authors this case study represents the first description of the use of cefovecin in *C. taurus*, as well as the first written record of intestinal eversion in the species, though it has been anecdotally observed by aquarists at many facilities.

Acknowledgements

Thanks to our amazing colleague Dr. Charles Duffy, V.M.D. of the Norwalk Veterinary Hospital and Maritime Aquarium for his tireless dedication to the TMA animal collection, and for the idea to use cefovecin in managing infection in this shark.

References

Borucinska, J., Martin, J. and Skomal, G., 2001. Peritonitis and pericarditis associated with gastric perforation by a retained fishing hook in a blue shark. Journal of Aquatic Animal Health, 13(4): 347-354.

Christie, B.L. 2008. The Natural History and Aquarium Husbandry of the Atlantic Sharpnose Shark, *Rhizoprionodon terraenovae.* Drum and Croaker 39: 19-39.

Christie, B.L. 2012. Intestinal Eversion in the Atlantic Sharpnose Shark, *Rhizoprionodon terraenovae*- A Behavior Ubiquitous among Elasmobranch Fishes? Gulf of Mexico Science. 30(2): 50-53.

Clark, T.B., Papastamatiou, Y.P. and Meyer, C.G., 2008. Intestinal eversion in a free-ranging manta ray (*Manta birostris*). Coral Reefs, 27(1): 61-61.

Claus, E., Henningsen, A., Shivji, M. and Wetherbee, B., 2021. Sexual conflicts in sand tiger sharks *Carcharias taurus* (Rafinesque, 1810) in an artificial environment. Journal of Zoo and Aquarium Research, 9(3): 161-170.

Crow, G.L., J.C. Howe, S. Uchida, S. Kamolnick, M.G. Wisner, and J.N. Caira. 1990. Protrusion of the Valvular Intestine Through the Cloaca in Sharks of the Family Carcharhinidae. Copeia 1990(1): 226-229.

Crow, G.L., J.A. Brock, J.C. Howe, and B.E. Linnon. 1991. Shark Bite Wounds of the Valvular Intestine: The Cause of an Acute Mortality Syndrome of Captive Blacktip Reef Sharks, *Carcharhinus melanopterus*. Zoo Biology 10: 457-463.

Crow, G.L., and Brock, J.A. 1993. The use of gentamicin sulfate therapy in a captive blacktip reef shark (*Carcharhinus melanopterus*) with intestinal biting syndrome. Zoo Biology 12: 479-482.

Henningsen AD, B.R. Whitaker, and I.D. Walker. 2005. Protrusion of the valvular intestine in captive smalltooth sawfish and comments on pristid gastrointestinal anatomy and intestinal valve types. Journal of Aquatic Animal Health. 17(3): 289-95.

Kessel, S.T., Fraser, J., Van Bonn, W.G., Brooks, J.L., Guttridge, T.L., Hussey, N.E. and Gruber, S.H., 2017. Transcoelomic expulsion of an ingested foreign object by a carcharhinid shark. Marine and Freshwater Research, 68(11): 2173-2177.

Lee, E.A., B.A. Byrne, M.A. Young, M. Murray, M.A. Miller, and L.A. Teil. 2016. Pharmacokinetic indices for cefovecin after single-dose administration to adult sea otters (*Enhydra lutris*). Journal of Veterinary Pharmacology and Therapeutics. 39(6): 625-628.

Mylniczenko, N. and Clauss, T., 2017. Pharmacology of elasmobranchs: updates and techniques. The Elasmobranch Husbandry Manual II: Recent Advances in the Care of Sharks, Rays and Their Relatives, pp.289-302.

Morales, P. and F. Dunker. 1999. Suspected Intestinal Torsion in a Blacktip Reef Shark (*Carcharhinus melanopterus*). Journal of Zoo and Wildlife Medicine. 30(1): 170-172.

Otway, N.M., West, G.J., Gore, D.B. and Williamson, J.E., 2021. Hook-shaped enterolith and secondary cachexia in a free-living grey nurse shark (*Carcharias taurus,* Rafinesque 1810). Veterinary Medicine and Science, 7(1): 240-250.

Rangel, B.S., Vignoli, R., Xavier, N. and Bettcher, V.B., 2021. New records on intestinal eversion in a free-ranging nurse shark (*Ginglymostoma cirratum*) and a free-ranging, breaching-spotted eagle ray (*Aetobatus narinari*). Environmental Biology of Fishes, 104(10): 1353-1357.

Schaller, P. and F. Dunker. 2005. Use of Contrast Radiograph to Determine Trauma to the Valvular Intestine in the Blacktip Reef Shark. Drum and Croaker 36: 23-28.

Seeley, K.E., K.N. Wolf, M.A. Bishop, M. Turnquist, and B. KuKanich. 2016. Pharmacokinetics of long-acting cefovecin in copper rockfish (*Sebastes caurinus*). American Journal of Veterinary Research. 77(3): 260-264.

Steeil, J.C., Schumacher, J., George, R.H., Bulman, F., Baine, K. and Cox, S., 2014. Pharmacokinetics of cefovecin (Convenia®) in white bamboo sharks (*Chiloscyllium plagiosum*) and Atlantic horseshoe crabs (*Limulus polyphemus*). Journal of Zoo and Wildlife Medicine. 45(2): 389-392.

Weideli, O.C. and Papastamatiou, Y.P., 2021. Intestinal eversion in a pelagic shark evokes chase and bite attempts by large-bodied carangid fish. Journal of Ethology, 39(1): 125-127.

Wernick, M.B. and C.R. Müntener. 2010. Cefovecin: a new long-acting cephalosporin. Journal of Exotic Pet Medicine. 19(4): 317-322.

Wiersma, G., Verhoog, P., Fowler, S. and Meekan, M., 2016. The first field observation of intestinal eversion by a shark. Marine Biodiversity, 46(1): 17-18.

Black Rockfish, Bruce Koike

AN ETHOGRAM FOR THE OYSTER TOADFISH, *Opsanus tau:* SAMPLING THE BEHAVIORAL DIVERSITY OF A COMMON BENTHIC FISH

Bernd H. A. Sokolowski[1] bsokolow@usf.edu
Peter J. Mohan[2] p.mohan@akronzoo.org

[1]University of South Florida, Morsani College of Medicine, Depts. of Otolarygology-HNS, Molecular Pharmacology and Physiology
[2]Akron Zoological Park

Introduction

A more extensive version of this paper was initially submitted for publication in a scientific journal in 1989. At the time, ethograms were becoming unfashionable and no longer suitable as subjects for stand-alone publications. Rather, they were viewed simply as tools to be used with other forms of behavioral research. As we were focused on new academic and curatorial responsibilities, we never got around to resubmitting it elsewhere.

In recent years zoo and public aquarium communities have shown an increasing interest in identifying positive and negative indicators of animal welfare (Miller *et al*., 2020). One positive indicator is an animal's ability to exhibit their natural range of behaviors at normal frequencies (Vicino and Miller, 2015). The development of an ethogram is the first step in understanding the diversity of behaviors exhibited by any species. While much focus has been placed on zoo mammals, aquarium husbandrists are now also developing welfare metrics and will therefore need to create ethograms.

The adult oyster toadfish, *Opsanus tau*, is normally solitary, migrating to shallow waters in late spring and summer to spawn, and then returning to deeper water for the winter (Gray and Winn, 1961; Gudger, 1908). The male secures a nest upon arriving at the spawning site and protects his shelter against any intruder including other toadfish (Gray and Winn, 196l). The male attracts the female who lays eggs in the shelter, which are then guarded by the male (Herald, 1967). Vocalizations play an important role in these interactions among *O. tau* and other fishes inhabiting turbid oyster reef habitats. Oyster toadfish produce a grunt of approximately 90 Hertz (Hz) in agonistic situations and the male "boatwhistles" at approximately 200 Hz during spawning season (Fish, 1972). Other oyster bed fishes such as the striped blenny, *C. bosquianus* (Tavolga, 1958) and naked goby, *G. bosci* (Mok, 1981) also use acoustic signaling.

While its moderate size and relatively sedentary lifestyle have made it a good subject for behavioral research, this sturdy fish also found early acceptance for a variety of anatomical and physiological studies (*see* Robinson and Schwartz, 1965). If the zebrafish is considered the white rat of freshwater fishes, the toadfish may be considered the zebrafish of marine fishes. The distinctive boatwhistle vocalization produced by males, its function, and the mechanics of its production have been involved in many early and more recent studies (Demski and Gerald, 1972; Fish, 1972; Gray and Winn, l961; Rice *et al*., 2011; Rosner *et al*,. 2018; Winn, 1972). The swimbladder muscle associated with sound production is the fastest known in vertebrates (Rome, 2006). Their auditory [*see* Edds-Walton, 2016 *for review*] and vestibular systems (Highstein *et al*., 1996; Steinacker *et al*., 1997) have been extensively studied both anatomically and physiologically, including at microgravity on the space shuttle (Boyle *et al.,* 2001). Moreover, their unusually large eggs make them easily collectable and resilient for raising through hatching and into older post-hatched ages for various studies of development (Sokolowski, 1986; Galeo *et al*., 1987; Sokolowski and Popper, 1987, 1988; Tracy, 1961).

The oyster toadfish exhibits many of the physical and behavioral adaptations associated with both intertidal species (Gibson, 1986; Horn and Gibson, 1988) and other oyster bed inhabitants. The present study may therefore serve as a baseline for those interested in developing ethograms of other bottom-dwelling species. Sympatric species with similar behavioral repertoires include: the striped blenny, *Chasmodes bosquianus*; the naked goby, *Gobiosoma bosci* and the clingfish, *Gobiesox strumosus*. Common adaptations include: 1.) some means of preventing dislodgement from shelter sites (attachment organs or wedging behaviors), 2.) paternal care of eggs deposited by one or often several females, 3.) cryptic coloration, 4.) scaleless epidermis with provision for generous mucus secretion, 5.) locomotor patterns characterized by short hops along the substrate, and 6.) tolerance of salinity, temperature, and O_2 extremes. Due to similarities in behaviors with the sympatric *C. bosquianus*, a previous ethogram developed for this species (Phillips, 1971a) was used as a starting point for this *O. tau* ethogram.

Methods

Twelve toadfish (5.7.0) were collected from the Chesapeake Bay aboard the *RV Aquarius* (Chesapeake Bay Laboratories (CBL)). They were held together at 20°C in a 190 L tank with a salinity of 17-20 parts per thousand at the University of Maryland, College Park. Individuals ranged in Total Length (TL) from 22.5-25.5 cm with a Head Girth (HG) ranging from 17-19 cm. Fish were distinguished by unique body markings such as scars or by different colored threads placed through their dorsal fins.

Prior Residency Analyses

A “prior residence” study was used to elicit behavioral responses from “resident” fish and “intruders” that were added later. Equipment for this study consisted of two 57 L observation tanks kept at the same temperature and salinities as the holding tank. Clay flower pots (16 x 14 cm or 14 x 12 cm) resting on their side were used as shelters. Two light reflector bowls (25 cm), each containing one 100-watt Norelco® tungsten light, were fastened to the sides of the tank and served as a light source. A tripod-mounted 35 mm Leica® M3 camera with either a 50- or 135-mm lens loaded with ASA 160 color tungsten film was utilized for documenting the behaviors. Prints of these original photographs were recently scanned and enhanced using Adobe Photoshop® to reduce water haze, camera movement, etc. The prior resident situation was created by first placing a solitary fish (resident) in a 57 L tank for either 24, 48, 96, or 210 hours. A second fish (intruder) was then introduced for 15 to 60 minutes. Residents were exposed to one or two intruders separately during a single day. The present paper describes the behaviors observed during these lab studies and the associated field work.

Field Observations

Field observations were made from February to October using SCUBA. The study site was the CBL pier, which had previously been used for various marine life studies and juts out into the tidal Patuxent River. An artificial oyster reef had been created over the years as ropes suspending oyster trays failed, dumping their contents. Fish were found in 1.5-4.0 m of water in shelters formed by other pier-associated debris, such as terra cotta tiles, concrete blocks, Plexiglas sheets, PVC tubes, logs, and old oyster culture trays. Fish behaviors were recorded using a Nikonos® IV-A camera, with a 35 mm lens and ASA 64 or 200 Kodak Ektachrome® film. Lighting was provided either ambiently or by an SB 101 Nikon® strobe.

As noted earlier, the ethogram for *Opsanus tau* was constructed based on the definitions and categories of behavior presented by Phillips (1971a) in his analysis of the behavioral repertoire of *Chasmodes bosquianus*, a blenny that is sympatric with the oyster toadfish. In the present paper, "positions", which he defined as "the relationship between the animals' body and the direction of gravity", are included with non-social behaviors.

Results

Non-Social Behaviors

The following behaviors were observed in isolated and social conditions, but were not contingent on social interaction. As described by Phillips (1971a) "a position is the relationship between the animal's body and the direction of gravity."

Stand - The body is maintained parallel to the substrate and the pelvic fins are extended perpendicular to and slightly supporting the body above the substrate (Figure 1). The tail may be laterally flexed or elongated so that the ventral side of the caudal and anal fins touches the substrate. Standing may also take place in a vertical position in a tank or against some object in the field. In this position the tail is bent into an “L” position on the substrate with the weight of the fish displaced on the lower half of the “L.” Either the pelvic or pectoral fins are used to prop the head portion of the body vertically against a wall or object. Observations of vertical standing could occur either on the substratum of the environment or the head of another toad fish. The latter situation can be part of "stacking" behavior.

Figure 1. Example of Stand behavior. B. Sokolowski.

Rest - This behavior is generally observed when the fish is in a shelter. The pelvic fins are folded and the body lies flush against the substrate. The dorsal fins are either erect or folded depending on the state of arousal of the animal.

Inverted Down - The dorsal part of the body faces the substrate while the ventral part of the body lies against the roof of the shelter. Inverted-Down is also observed during social situations. These include competition for shelters and Cohabitation by females depositing eggs on the roof of the nest, the most common place to find eggs (Figure 2).

Figure 2. Two toadfish Cohabiting in a large jar. The upper animal is exhibiting Inverted Down behavior. P. Mohan

Fast Forward Swim - Fast forward swimming consists of lateral undulations of the entire tail portion of the body and the caudal fin. The dorsal, anal, and pectoral fins may be either folded, as during an escape response, or the dorsal and anal fins are spread while the pectoral fins are folded back. The pelvic fins are used to push the body off the bottom prior to fast forward swimming. The maximum observed swimming distance for a toadfish pursued by a diver was approximately four meters.

Suck - By opening its mouth and flaring its opercula, the fish creates a vacuum and takes in food or other particles. If edible, the objects are swallowed; otherwise, they are expelled.

Expulsion - The animal blows jets of water from its mouth. Phillips (1979) also observed this in toadfish directing non-edible particles away from themselves.

Fan - The pectoral fins alternately move rostral to caudal. This behavior occurs while the animal is in its shelter or is attempting to move its body into position while standing.

Burrow - The fish attempts to maneuver its body into an opening either head, tail, or side first by moving its body in a side-to-side motion.

Forward Shelter Entry - The fish swims head first into the shelter and makes a 180° turn inside. Head-out is the preferred resting position.

Backward Shelter Entry - The fish backs tail first into the shelter using fanning movements of the pectoral fins, while the tail is extended and the caudal fin is either in a closed or open position.

Body Out - The anterior portion of the body extends approximately a third to one-half of the way out of the shelter in either a resting or standing position. This shelter orientation effectively combines the head-out and part-out orientations described by Philips (1971a).

Body In - The fish is totally enclosed in the shelter in a Rest or Stand position. The tail may be straight or flexed towards the anterior end of the body (Figure 3).

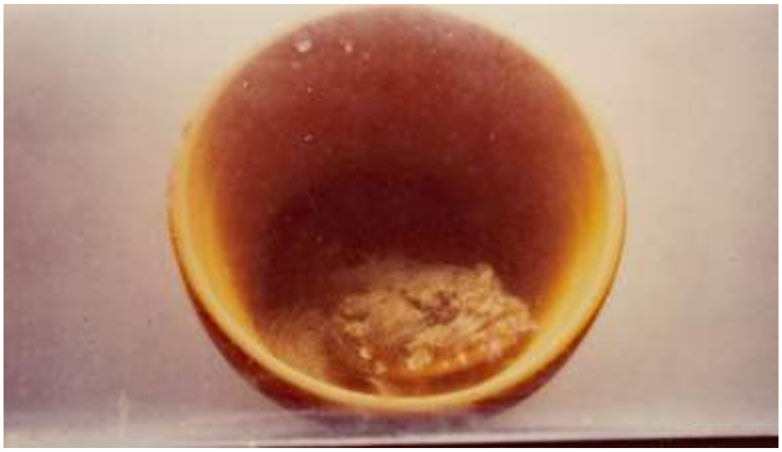


Figure 3. Body In position in a shelter. B. Sokolowski.

Social Behaviors

The following behaviors are displayed with or towards another fish. These include behaviors that could contribute to both positive and negative welfare in an aquarium situation.

Stack - Individuals pile one on top of the other, reaching stack heights of up to four fish. This behavior is observed in captive fish in the absence of any kind of shelter.

Ram - Ramming was observed in relation to stacking behavior in the laboratory. A fish swims into the bottom of a stack in an apparent attempt to dislodge the lower members of the stack or Burrow beneath them.

Cohabit - The resident of a shelter shares it with a conspecific (Figure 4). Males share their shelters with females, other males, and other species such as the American eel *Anguilla rostrata*.

Figure 4. Cohabiting toadfish in a discarded container in the Pawtuxent River at California (Seven Gables), MD. P. Mohan.

Mouth Open - The mouth is opened widely so that the white lining of the oral cavity is clearly visible to the intruder of a particular territory (Figure 5). This behavior was also elicited towards the investigators while pursuing toadfish.

Figure 5. Mouth Open in both the resident fish (R) and intruder (L). B. Sokolowski.

Jaw Grip - Gripping occurs after Mouth Open so that the lower jaw of one fish is held by the second while the upper jaw of the second is held by the first.
Jaw Hold - Jaw hold is similar to Jaw Grip except that one fish does not clamp down on the upper jaw of the other (its jaws remain open). Jaw Hold was never observed to take place on the upper jaw (Figure 6).

Figure 6. Jaw Hold. Resident (R) and intruder (L). B. Sokolowski.

Jaw Shake - Shaking occurs with Jaw Grip and is displayed by vigorous side to side shaking of the head of the fish displaying Jaw Grip.
Bite - Biting is directed towards the lower jaw, tail, or lateral side of another fish and is distinctly more transitory than the jaw closure observed during Jaw Hold or Jaw Grip.
Lunge - The animal moves rapidly forward approximately one body length. Lunging is accompanied by Mouth Open and may result in Pushing or Biting.
T-Formation - The body of one fish is horizontally perpendicular to, and with head facing, the body of another fish. T-formation was observed in conjunction with Lunging of one fish towards the operculum of a second fish.
Push - Pushing accompanies Jaw Grip or Jaw Hold and is displayed by one or both fish shoving the other in a backward direction (Figure 7).
Fin Spread - All fins are in an elevated position so that all the patterns are clearly visible. Pectoral fins are held in a forward position, dorsal fins are fully elevated, and the caudal fin is maximally unfolded (Figure 8). This action pattern occurs in the context of agonistic encounters or when the fish attempts to wedge itself in an enclosed shelter, while the investigator attempts to dislodge the animal from its shelter.

Figure 7. An example of the result of Push behavior in conjunction with Open Mouth. B. Sokolowski.

Figure 8. Lateral view of Fin Spread accompanied by Mouth Open. B. Sokolowski.

Fin Fold - All fins are partially or maximally depressed.
Dorsal Spine Elevation - The dorsal spines, which compose the first dorsal fin, are erect. Dorsal Spine Elevation can be displayed alone or in conjunction with elevation of the second dorsal fin or Fin Spread (Figure 8).
Opercular Flare - Both opercula are flared open. This behavior occurs with Fin Spread and Arched Body.
Retreat - Retreating occurs when one fish increases the distance between itself and a conspecific by backing away while still facing the other individual.
Flight - A fish increases the distance between itself and another individual by rapidly swimming away from the aggressor.
Tail Beat - The tail is rapidly and laterally undulated against the side of another fish which lies parallel to the initiator in either the same or opposite direction.
Arched Body - The body is held in an arched position so that the mouth and caudal fin are at the lowest point of the arch while the area directly caudal to the dorsal fin (D1) is at the apex of the arch. The entire dorsal portion of the head is in maximal view to the other fish during an agonistic encounter. (Figure 9)

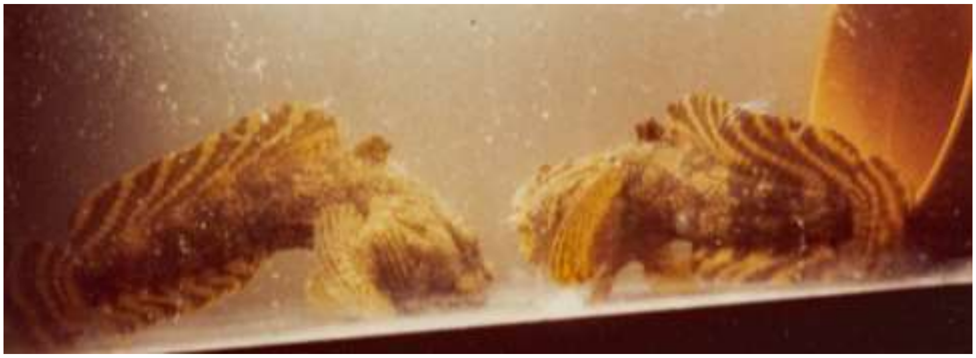


Figure 9. Arched Body displayed by an intruder (L) in a frontal orientation. B. Sokolowski.

Squeeze - The bodies of two fish lie parallel to one another while one fish moves in a back-and-forth motion in an attempt to displace a second fish from the corner of a shelter. This action pattern usually occurs during competition for a shelter (Figure 10).

Figure 10. Squeeze behavior in progress. B. Sokolowski.

Slow Approach - The approach is displayed by the intruder in the prior resident situation. The intruder approaches the resident in short distances over a period of 15-20 minutes. Slow Approach was not displayed by the resident.

Direct Approach - The intruder in the prior resident situation approaches the resident within two minutes. This approach was followed by direct entrance into the shelter of the resident (Figure 11).

Discussion

Non-Social Behavior

Stand and Rest represent orientations maintained by toadfish when they are inactive. Inverted-Down is most often seen during mating while females are depositing eggs in a nest. Deposition of the eggs on the "ceiling" of a shelter is probably conducive to keeping detritus off the developing embryos. This position is also incidental to competition for shelters between conspecifics and is only seen in relation to shelter use.

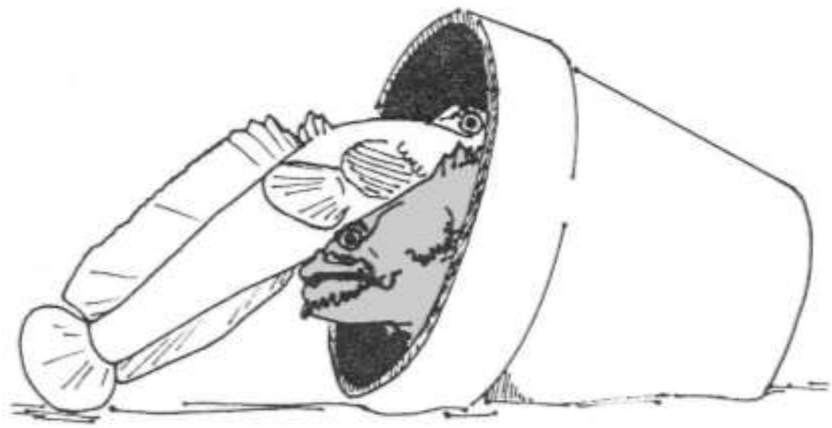


Figure 11. Direct Approach of intruder into an occupied shelter. P. Mohan.

The Expulsion behavior displayed by *O. tau* is also observed in other fishes including *C. bosquianus* (Phillips, 1971a) and in burrowers such as the Atlantic yellowheaded jawfish, *Opistognathus aurifrons* (Mohan, personal observation). This behavior is involved with keeping the shelter free from debris but may also occur when potential food items are tested for palatability.

Fanning behavior aerates and removes detritus from the eggs (Van Iersal, 1953). Gudger, (1908) observed the behavior in a male toadfish that was guarding eggs. However, we have observed this behavior by females outside of the context of egg maintenance. The fact that male blennies also occasionally perform the Fan behavior in a nest without eggs (Phillips, 1971a) and that non-parental fanning is observed in *Gasterosteus aculeatus* (Sevenster, 1961) suggest that this behavior is conducive to shelter maintenance, irrespective of whether eggs are present.

Burrowing behavior has been adapted to utilize non-natural materials, due to the abundance of human refuse and the great loss of oyster and grass beds in the Chesapeake Bay. Most nests observed by the authors have been under or inside concrete blocks, bricks, boards, tin cans, jars, terra cotta drain tiles and other assorted garbage which appear to have no biological counterpart (Figure 4). Historically toadfish may have burrowed under oyster clumps, sunken logs, and rocks. Orth (1975) also reported that toadfish burrow under the exposed fringes of *Zostera* beds.

The type of shelter entry used by the toadfish may depend on the size of the cavity and on the motivational state of the animal. Animals engaging in agonistic interactions are more likely to use Backward shelter entry to attain cover. Whether a toadfish exhibits Body Out versus In, while inside a shelter, may also reflect motivational state. Phillips and Swears (1979) reported seeing toadfish partially out of their shelters while watching potential prey items. Fish responding to intruders also exhibit Body Out. When inactive during the day toadfish are usually found in the Body In orientation.

Social Behavior

Stack and Ram appear to be behaviors that result from insufficient shelter availability in laboratory aquaria, but might also lead to cohabitation in natural situations where dominance is unclear, where shelters are a limiting resource, or where communal shelter utilization is otherwise advantageous. The last example occurs in several situations. During mating several males may cohabit a single nest while one male is the primary sound producer (Sokolowski, personal observation). This sort of communal sharing of a nest may provide an alternative reproductive strategy for some males. Fine *et al*. (1984) hypothesized this behavior based on measurements of the size of sonic motor nucleus (SMN) cells in the medulla of *O. tau*. The SMN innervates the swim bladder muscles and regulates sound production such as the boatwhistle call of the male. These authors suggested that males with larger SMN cells boatwhistled more frequently when compared to similar body-sized males with smaller SMN cells. Consequently, since animals with smaller cells may call less frequently, they may use alternative reproductive strategies such as cohabitation (Fine *et al*., 1984), a behavior we observed in the field. However, anatomical studies of this nucleus are needed from cohabiting *O. tau* to verify this hypothesis. Parasite or satellite male populations are found also among other fish populations during mating (Barlow, 1967). Cohabitation may also occur when two conspecifics share contiguous nest cavities during overwintering. Other species, such as the cunner, *Tautogolabrus adspersus* (Green and Farewell, 1971), share shelters while overwintering and juvenile channel catfish, *Ictalurus punctatus*, actively seek shelter together as a school during the winter (Brown *et al.*, 1970). At the low metabolic levels experienced by *O. tau* at winter temperatures, the motivation to seek shelter is probably stronger than the desire to defend a shelter from con- or contraspecifics. We also observed *O. tau* sharing shelters with the American eel, *Anguilla rostrata,* although we did not determine the significance of this cohabitation. However, during one October dive we found a dozen eels and a half dozen toadfish sheltering under a single 61 x 76 cm structure. This was at the usual time when toadfish became scarce at our shallow study site as they moved to deeper water. As noted above for other temperature fishes, cohabitation in winter may signal positive welfare even though it would result in aggression and negative welfare at other times of the year. Likewise, Stack behavior can be a sign of either positive or negative welfare. Most readers will be familiar with the group resting habit of nurse sharks (Castro, 2000, for example), which would be considered a positive behavior. Stacking due to a lack of available shelters in an aquarium could be considered a negative behavior.

While aggression between conspecifics is a natural behavior, there is often insufficient opportunity for losing fishes to retreat in an aquarium environment. This will have a bearing on the number of animals of a particular species or sex that can be held in any aquarium, and the number of shelters that need to be provided. In the present study, the elicitation of threat postures (a term used to cover all types of responses seen in fighting and territorial behavior) was related to the defense of a shelter (territory). These behaviors only occurred in the large, communal tank upon the introduction of a shelter. There will be some vigorous debate among husbandrists about whether allowing threat displays and even other more serious levels of natural aggression constitutes positive welfare in the sense that the full range of natural behaviors is allowed to be exhibited. It is interesting that different social phenotypes (e.g., winners, losers) show different patterns of gene expression in the social decision-making network (SDMN) of the zebrafish brain. This network underlies the expression of social behavior in these teleosts (Teles *et al*., 2016). Attention to animal health is always likely to supersede any perceived benefit derived from intense "natural" agonistic behavior.

Barlow (1962) suggested that the hostility level observed between two fish is indicated by different levels of fighting. The most advanced stage of fighting in the nandid, *Badis badis*, is exemplified by mouth locking, which is equivalent to Jaw Grip in *O. tau*. The lowest stage of *B. badis* fighting involves displays, or “color fights,” in which animals approach conspecifics and change their coloration. Toadfish also use displays such as Fin Spread, Dorsal Spine Elevation, Opercular Flare, and Arched Body in lower-level confrontations. Additionally, two levels of physical fighting were exhibited, although they do not appear to result from habituation, since intermediate stages did not occur between the levels. The lower level is characterized by Tail Beating and Squeezing and is preceded by Drive-In by the intruder into an occupied shelter. Entrance into the shelter elicits Squeezing and Tail Beating by the resident. In contrast, an intense level fight between two toadfish might proceed along the following lines. An intruder on the boundary of a territory displays Fin Spread. The resident shows Head Out of the shelter and fixates on the intruder. The intruder employs a Slow Approach towards the resident. Once the intruder is within one body length the resident displays Fin Spread and Raised Operculum. Arched Body may be displayed by either one or both fish. If the intruder approaches within approximately half a body length, the resident displays Open Mouth which may result in an Open Mouth display by the intruder. Open Mouth may result in Jaw Locking which passes to Pushing. Pushing may result in Jaw Grip or a Bite which passes to Retreat or Flight. Low level fighting in *O. tau* resembles "intraterritorial" fighting" in cichlids, while high level fighting resembles "boundary fighting" (Baerends and Baerends-Von Roon, 1950). Appeasement may also be displayed by Fin Folding or Jaw Hold and a decrease in motion, which may be followed by erection of the dorsal spines alone. When only one combatant engages in Jaw Grip, the resulting Jaw Hold seems to serve a similar function as the reduction of motion observed by Wickler (1957) in fighting blennies (*Blennius fluviatilis*).

The positioning of several threat postures (Fin Spread, Mouth Open, Arched Body) occurs in either a lateral or frontal display, indicating different motivational states. A lateral display might indicate a conflict between attack and escape responses, while a frontal display indicates a tendency to attack (Tinbergen, 1953). Fin Spread by *O. tau* occurs as either a lateral or frontal display. Open Mouth and Arched Body occur with Fin Spread in a frontal position only, and often result in physical contact.

Individual recognition of conspecifics may play a role in determining levels of aggression and the types of approach used by an intruder (Phillips, 1971b). This stresses the importance of being able to identify individual fish (where possible) in an aquarium situation, so that their cohabitation can be modified as needed to ensure good welfare of potential combatants. Marler (1956) suggested that the type of aggressive behavior shown by territorial birds may depend upon whether the approaching individual is recognized. An attack is forthcoming if the animal is not recognized, whereas displays are performed before a recognized animal (Marler, 1956). Noble and Curtis (1939) suggested that some cichlid fish, *Cichlosoma cutteri* and *Hemichromis bimaculatus*, can recognize individuals. A recent study suggests that the long-term social memory for conspecifics, seen in mammals, is present also in zebrafish (Madeira & Oliveira, 2017). Observations made during the present study suggest that recognition and approach are variables influencing the type of aggression. Direct Approach behavior by the intruder results in shelter entrance and lower stage fighting. Slow Approach with behavioral displays results in either/both intense or less intense fighting. Furthermore, recognition of the intruder by the resident may be the result of a previous encounter in the large holding tank. The readiness to fight is influenced by personal recognition, such that, the loser of a previous fight is less likely to win again (Francis, 1983; Bakker and Sevenster, 1983) and retires quickly upon meeting the victor (Barlow, 1962). Therefore, if the resident recognized an intruder as a previous victor in the large tank, the aggression displayed by the resident towards the intruder may be less intense. The differences in aggression can be time dependent, depending on previous outcomes (Hsu *et al*., 2006; Lan and Hsu, 2011). Recognition is also important in light of differences in androgen and corticosteroid levels between winners and losers. These differences in hormonal levels may last up to 14 days (Hannes, 1984; Harding, 1981) although no differences are measured between individuals that have established dominance-subordinate hierarchies (Hannes, 1984; Ramenofsky, 1984).

Comparisons with Intertidal Species

The similarities between the behavior patterns of tide pool teleosts and fishes found on oyster-dominated substrates, and the comparable phylogenetic make-up of the two groups, suggest that the two habitats provide similar challenges to resident species. Kortmulder (1986) described several mechanisms that could be responsible for apparent convergent evolution in behavior and color patterns in distantly related *Barbus* spp. Demersal spawning in protected cavities, punctuated locomotion, cryptic coloration, and evolutionary scale loss might be expected in any topographically complex habitat where unpredictable water movement and predation on eggs and nest guardians are important selection pressures. The authors have observed egg predation to occur by contraspecifics within minutes of removal of a *C. bosquianus* from its nest cavity as well as predation of *G. bosci* on *O. tau* eggs that were dropped during egg collection by the investigators. This behavior contrasts with the relatively lax parental vigilance observed by Allen (1975) in the demersal spawning pomacentrid, *Amphiprion chysopterus*. This anemonefish deposits its eggs in relatively calm, deep forereef or lagoon areas, where egg predation is probably ameliorated by good line of sight vision allowing parents to recognize egg predators before they approach the nest. *A. chysopterus* parents may spend no more than one third of their time involved with nest maintenance during the first 5 days of development (Allen, 1975). The turbid water conditions commonly found on oyster reefs interferes with the early identification of potential egg predators, contributing to the high level of nest vigilance seen in male *O. tau* and other sympatric species. In addition, the turbidity and low O_2 may not only contribute to this vigilance but also to Fanning behavior.

Poor visibility may also reinforce the use of auditory communication in courtship and territorial behavior. As mentioned previously, sonic signaling occurs for *O. tau* in spawning and agonistic behaviors (Fish, 1972). Other oyster bed species such as *C. bosquianus* (Tavolga, 1958) and *G. bosci* (Mok, 1981) also use acoustic signaling. These vocalizations apparently evolved to facilitate the maintenance of territories and to attract mates in the visually limiting conditions that prevail in estuarine environments. The elaborate color and motor displays seen in many tropical groups are probably muted in their Chesapeake Bay relatives because line of sight vision is limited by low (0.1 - 2.0m) visibility and the physically heterogeneous, but topographically flat, bottom structure. However, recent evidence indicates that female damselfish (*Pomacentrus parititus*) can differentiate between the mating calls of conspecific males (Myrberg, 1986). This evidence suggests that females use male calls for more than attraction to a nest site, but may also use calls for mate assessment (Myrberg 1986). Interestingly, *O. tau* males can jam the boatwhistle calls of a congener, thus making the mating call less attractive to females (Mensinger, 2014)

Conclusion

The behavioral repertoire of the oyster toadfish is a close match for other benthic species living in similar habitats. Behaviors described here will also be found in many other species and some are common to most fishes regardless of their lifestyle.

What was old is new again. Ethograms were an integral part of early studies of animal behavior. They have now become an important tool in allowing us to qualify the behavioral diversity of each of the fish species held in our facilities. It is not necessary to start from scratch when developing an ethogram for each species. A deep dive into older scientific literature will reveal many exhaustive studies on the topic. One example is Baerends and Baerends-Von Roon (1950), a 242-page analysis of cichlid behavior. You do not necessarily need to dig into university library "stacks" to access this old but valuable information. For example, the above paper and many others are available as read-only documents via a free membership to JSTOR (https://www.jstor.org/). Google Scholar® is a good place to start a general search. Some papers will be available as downloadable PDFs. You may find citations in papers that will lead you to information on other species. When searching the literature, you may not find an existing ethogram for a particular species, but related species and those with similar lifestyles will have been documented and offer convenient starting frameworks for creating new ethograms.

Acknowledgements and Comments

We would like to thank Dr. Tim Tricas for his constructive criticism of an early version of the original manuscript and Dr. Douglas Martin for allowing us the use of the facilities at the Chesapeake Biological Laboratories back in the late 1970s and early 1980s. In addition, we would like to thank the late Dr. Eugenie Clark for the use of her laboratory to document these observations. Pete Mohan would also like to thank his coauthor, friend, and dive buddy for agreeing to resurrect this material for the use of the public aquarium community.

We have attempted to update citations when relevant. Ethograms have continued to be published for other species, since we wrote the original paper in the late 1980s, albeit at a reduced frequency. Seek and ye shall find.

Those interested in propagating this species may wish to consult Mensinger et al. (2001) and Mensinger and Tubbs (2006).

Literature Cited

Allen GR. 1975. The Anemonefishes: Their Classification and Biology. 2nd ed. TFH Publications, Neptune City, NJ 352 pp.

Baerends GP and Baerends-Von Roon JM 1950. An introduction to the study of the ethology of cichlid fishes. Behav Suppl #l: 242pp.

Bakker TCM and Sevenster P 1983. Determinance of dominance in male sticklebacks (*Gasterosteus aculeatus*). Behav 86, 55- 71.

Barlow GM 1962. Ethology of the Asian teleost *Badis badis* III. Aggressive behavior. Z Tierpsychol 19, 29-55.

Barlow GW 1967. Social behavior of a South American leaf fish, *Polycentrus shomburgkii*, with an account of recurring pseudofemale behavior. Am Midi Natl 78, 215-234.

Boyle R, Mensinger AF, Yoshida K, Usui S, Intravaia A, Tricas T, Highstein SM. 2001. Neural readaptation to Earth's gravity following return from space. J Neurophysiol 86(4), 2118-2122.

The authors in 1986. Bernd Sokolowski (L) and Pete Mohan (R).

Brown BE, Inman I, Jearld A, JR. 1970. Schooling and shelter seeking tendencies in fingerling channel catfish. Trans Amer Fish Soc 99, 540-545.

Castro J. 2000. The biology of the nurse shark, *Ginglymostoma cirratum*, off the Florida east coast and the Bahama Islands. Environ Biol Fishes, 58, 1-22.

Demski L, Gerald J. 1972. Sound production evoked by electrical stimulation of the brain in the toadfish (*Opsanus tau*). Anim Beh 20, 507-513.

Edds-Walton PL. 2016. What the toadfish ear tells the toadfish brain about sound. Adv Exp Med Biol 877, 197-226.

Fine ML, Economos D, Radtke R, McClung JR. 1984. Ontogeny and sexual dimorphisms of the sonic motor nucleus in the oyster toadfish. J Comp Neurol 225, 105-110.

Fish MP. 1972. Sound production of the toadfish (*Opsanus tau*). In: Behavior of Marine Animals, Current Perspectives in Research, Vol. 2. (Winn HE. and Olla BL, eds.) Plenum Press, New York and London.

Francis RC. 1983. Experiential effects in agonistic behavior in the paradise fish, *Macropodus opercularis*. Behav 85, 292-313.

Galeo AJ, Fine ML, Stevenson JA. 1987. Embryonic and larval development of the sonic motor nucleus in the oyster toadfish. J Neurobiol 18(4), 359-373.

Gibson RN. 1986. Intertidal teleosts. Life in a fluctuating environment. In: The Behavior of Teleost Fishes (Pitcher TJ., ed.) Johns Hopkins University Press, Baltimore, pp. 388-408.

Gray G, Winn HE. 1961. Reproductive ecology and sound production of toadfish (*Opsanus tau*). Ecol 42, 274-282.

Green JM, Farewell M. 1971. Winter habits of the cunner, *Tautogolabrus adsperius*, (Walbaum, 1792) in Newfoundland. Can J Zool 49, 1497-1499.

Gudger EW. 1908. Habits and life history of the toadfish (*Opsanus tau*). Bull U.S. Bur Fish 28, 1073-1109.

Hannes RP. 1984. Androgen and corticoid levels in blood and body extracts of high and low-ranking sword tail males (*Xiphophorus helleri*) before and after social isolation. Z Tierpsychol 66, 70-76.

Harding CF. 1981. Social modulation of circulating hormone levels in the male. Ann Zool 21, 223-231.

Herald ES. 1967. Living Fishes of the World. Doubleday and Co., New York. 281 pp.

Highstein SM, Rabbitt RD, Boyle R. 1996. Determinants of semicircular canal afferent response dynamics in the toadfish, *Opsanus tau*. J Neurophysiol 75(2), 575-596.

Horn MH, Gibson RN. 1988. Intertidal fishes. Sci Amer 258(1), 64-70.

Hsu Y, Earley RL, Wolf LL. 2006. Modulation of aggressive behaviour by fighting experience: mechanisms and contest outcomes. Biol Rev Camb Philos Soc 81(1), 33-74.

Kortmulder K. 1986. Similar behavior and color patterns in three not closely related *Barbus* species. Is evolutionary convergence a likely explanation? Behav 98(1-4), 180-212.

Lan YT, Hsu Y. 2011. Prior contest experience exerts a long-term influence on subsequent winner and loser effects. Front Zool 8(1), 28.

Madeira N, Oliveira RF. 2017. Long-term social recognition memory in Zebrafish. Zebrafish 14(4), 305-310.

Marler P. 1956. Behavior of the chaffinch, *Fringilla coelebs*. Behav Suppl 5, 1-184.

Mensinger AF, Stephenson KA, Pollema SL, Richmond HE, Price N, Hanlon RT. 2001. Mariculture of the Toadfish *Opsanus tau.* Biol. Bull. 201: 282-283.

Mensinger AF, Tubbs ME. 2006. Effects of Temperature and Diet on the Growth Rate of Year 0 Oyster Toadfish, *Opsanus tau*. Biol. Bull. 210: 64–71.

Mensinger AF. 2014 Disruptive communication: stealth signaling in the toadfish. J Exp Biol. 217(Pt 3), 344-350.

Miller LJ, Vicino GA, Sheftel J, Lauderdale LK. 2020. Behavioral diversity as a potential indicator of positive animalwWelfare. Animals 10, 1211.

Mok HK. 1981. Sound production in the naked goby, *Gobiosoma bosci* (Pisces, Gobiidae). A preliminary study. In: Proceeding in Life Sciences, Hearing and Sound Communication in Fishes (Tavolga WN., ed.) Springer Verlag, New York, 447-455.

Myrberg A, Mohler M, Catala JD. 1986. Sound production by males of a coral reef fish (*Pomacentrus partitus*): its significance to females. Anim Beh 34, 913-923.

Noble GB, Curtis B. 1939. The social behavior of the jewel fish, *Hemichromis bimaculatus*. Bull Amer Mus Nat Hist. 76, 1-46.

Orth RJ. 1975. The Role of Disturbance on an Eelgrass, *Zostera marina*, Community. Ph.D. Dissertation, University of Maryland, College Park. 115 pp.

Phillips RR. 1971a. The relationship between social behavior and the use of space in the benthic fish *Chasmodes bosquianus* L. (Teleosti, Blennidae): Ethogram. Z Tierpsychol 29, 11-27.

Phillips RR. 1971b. The relationship between social behavior and the use of space in the benthic fish *Chasmodes bosquianus* L. (Teleosti, Blennidae): II. The effect of prior residency on social and enclosure behavior. Z Tierpsychol 29, 389-408.

Phillips RR, Swears SB. 1979. Social hierarchy, shelter use, and avoidance of predatory toadfish (*Opsanus tau*) by the striped blenny (*Chasmodes bosquianus*). Anim Behav 27, 1113-1121.

Ramenofsky M. 1984. Agonistic behavior and endogenous plasma hormones in male Japanese quail. Anim Behav 32, 698-708.

Rome LC. 2006. Design and Function of Superfast Muscles: New Insights into the Physiology of Skeletal Muscle. Annu. Rev. Physiol. 68: 22.1–22.29

Rice AN, Land BR, Bass AH. 2011. Nonlinear acoustic complexity in a fish 'two-voice' system. Proc Biol Sci 278(1725), 3762-3768.

Robinson PF, SCHWARTZ FJ. 1965. A revised biliography of papers dealing with the oyster toad fish, *Opsanus tau*. National Resources Institute, Chesapeake Biological Laboratory, Solomons, MD. Contribution No. 284. 18 pp.

Rosner E, Rohmann KN, Bass AH, Chagnaud BP. 2018. Inhibitory and modulatory inputs to the vocal central pattern generator of a teleost fish. J Comp Neurol 526(8), 1368-1388.

Sevenster P. 1961. A causal analysis of a displacement activity (Fanning in *Gasterosteus aculeatus* L.). Behav Suppl 9, 1-170.

Sokolowski BH. 1986. Development of the otolith in embryonic fishes with special reference to the toadfish, *Opsanus tau*. Scan Electron Microsc (Pt 4), 1635-1648

Sokolowski BH, Popper AN. 1987. Gross and ultrastructural development of the saccule of the toadfish *Opsanus tau*. J Morphol 194(3), 323-348.

Sokolowski BH, Popper AN. 1988. Transmission electron microscopic study of the saccule in the embryonic, larval, and adult toadfish *Opsanus tau*. J Morphol 98(1), 49-69.

Steinacker A, Monterrubio J, Perez R, Mensinger AF, Marin A. 1997. Electrophysiology and pharmacology of outward potassium currents in semicircular canal hair cells of toadfish, *Opsanus tau*. Hear Res 109(1-2), 11-20.

Tavolga WN. 1958. Underwater sounds produced by males of the bleniid fish, *Chasmodes bosquianus*. Ecol 39(4), 759-760.

Teles MC, Cardoso SD, and Oliveira RF. 2016. Social plasticity relies on different neuroplasticity mechanisms across the brain social decision-making network in zebrafish. Front Behav Neurosci 16;10, 16.

Tinbergen N. 1953. The herring gull's world. Collins, London.

Tracy HC. 1961. Development of the spinal neural crest, nerves, and muscles in the toadfish (*Opsanus tau*). J Comp Neurol 116, 291-315.

Van Iersal JJA. 1953. An analysis of the parental behavior of the male three-spined stickleback (*Gasterosteus aculeatus*). Behav Suppl 3, 1-159.

Vicino G, Miller LJ. 2015. From Prevention of Cruelty to Optimizing Welfare: Opportunities to Thrive. In Proceedings of the International Ethological Conference, Cairns, Australia, 19 September.

Wickler W. 1957. Vergleichende Verhaltensstudien an Grundfischen. I. Beitrage zur Biologie, besonders zur Ethologie von *Blennius fluviatilis*. Also in vergleich zu anderen Bodenfischen. Z. Tierpsychol 14, 393-428.

Winn HE. 1972. Acoustic discrimination by the toadfish with comments on signal systems. In: Behavior of Marine Animals, Current Perspectives in Research, Vol. 2, Vertebrates (Winn HE and Olla BL, eds.) Plenum Press, New York, 361-385.

Largemouth Bass, Bruce Koike

THE AQUARIUM SYMPOSIA FROM THE 1958 to 1970 MEETINGS OF THE AMERICAN SOCIETY OF ICHTHYOLOGISTS AND HERPETOLOGISTS

Editor's Note:

It is good to know where we came from. Many of the topics discussed 50-60 years ago are still on our radar.

Since the 2022 RAW abstracts were actually included in that year's issue of D&C (due to the early timing of the meeting), I decided to substitute the remaining meeting notes for the Aquarium Symposia from Copeia, the journal of the American Association of Ichthyologists and Herpetologists (ASIH). See the previous two issues of Drum and Croaker to review the presentations given at the 1955 to 1957 meetings.

Readers will note the conspicuous absence of papers in certain years. This seems to be attributable to meeting locations that were not near public aquariums. You will also notice that many of these topics, including papers by the same presenters, can be found in Drum and Croaker from this period.

For sixteen years (1955-1970), the North American public aquarium community often held the "Annual Aquarium Symposium" in conjunction with the annual meeting of ASIH. These symposia are the earliest regular meetings of public aquarium professionals from this part of the world of which I am currently aware. As noted in the 2021 issue's "Drum and Croaker 50 Years Ago," these annual aquarium presentations moved to AAZPA (now AZA) in 1971. Many of the early founders and contributors to Drum and Croaker (D&C) were presenters at these conferences. AZA's website states that they maintain proceedings starting in 1974. It would be interesting to see what was presented in 1971-1973.

Much of the content below was transcribed directly from the issues of Copeia in which it was originally recorded.

Indiana University, Bloomington, Indiana, August 25-28, 1958.
Copeia 1958 (4): 350.

"Those members who were able to arise very early in the morning and who did not have other (AIBS) sessions to attend participated in a field trip to the Spring Mill State Park, about 40 miles south of Bloomington. There they visited Twin Caves and caught and examined blind fish. The parties in individual cars then divided, some returning to Bloomington, others visiting the rugged scenic country of Southern Indiana. During the evening the annual Symposium on Aquaria was held with Dr. Earl S. Herald acting as co-ordinator. The program presented at this meeting follows:"

Longevity Among Aquarium Fishes. Sam Hinton, Scripps Institution of Oceanography.

Comments of Keeping the Pilot (Blackfish) Whale in Captivity. F. G. Wood, Jr., Marineland, Florida.

Relative Performance of Different Depths of Filtrant in Sand Filters. William Kelley, Cleveland Aquarium.

Principles and Practices of Water Management in Public Aquariums. James W. Atz, New York Aquarium, N. Y. Zoological Society.

The Shedd Aquarium's New Railroad Collecting Car, The Nautilus. William Braker, Shedd Aquarium, Chicago, Illinois.

Cinematographic Review of External Features of Various U. S. Aquariums. Earl S. Hearld, Steinhart Aquarium, California Academy of Sciences.

Sharks and New Field Collecting Techniques. Captain William Gray, Seaquarium, Miami, Florida.

Deterioration of Cement Aquariums Due to Salt Water Corrosion. William Braker, Shedd Aquarium, Chicago, Illinois.

New Exhibition Methods at the Vancouver Aquarium. Murray Newman, Vancouver Public Aquarium, Stanley Park, Vancouver, B. C.

The First Year of Operation of the New York Aquarium (color film review). James W. Atz, New York Aquarium, N. Y. Zoological Society.

San Diego State College, Sand Diego, California. June 17-21, 1959
Copeia 1959 (4): 361.

"During the evening the ichthyologists transferred their activities to the Scripps Institution of Oceanography, at La Jolla, where they conducted two separate but concurrent sessions. The first of these, with Mr. Sam Hinton presiding, was devoted to an "Aquarium Symposium" where the following subjects were presented:"

Observations on the Behavior of Captive Pilot Whales. David Brown, Marineland of the Pacific, California.

Maintenance of Sharks in Captivity. Eugenie Clark, Cape Haze Marine Laboratory, Placida, Florida.

The Relative Ability of Various Species to Survive under Aquarium Conditions. Murray Newman, Vancouver Public Aquarium, Vancouver, B. C.

Problems in Redesigning a Multisystem Aquarium within an Existing Structure. Earl S. Herald and Robert P. Dempster, California Academy of Sciences, San Francisco.

The National Aquarium. Craig Phillips, U. S. Fish and Wildlife Service, Washington, D. C.

Optical Properties of Aquaria and Their Applications to Lighting and Design. William Kelley, Cleveland Public Aquarium, Cleveland, Ohio.

Candiru (Parasitic Pygiid) Feeding on the Gills of Living Goldfish. (Filmed for the first time.) Daniel Moreno, Cleveland Public Aquarium, Cleveland, Ohio.

Public Aquarium Workshop, A Project of the American Association of Zoological Parks and Aquariums. William Kelley, Cleveland Public Aquarium, Cleveland, Ohio.

General discussion of exhibit labels and aquarium instructional techniques.

Chicago Natural History Museum, Chicago, Illinois. June 17-19, 1960.
Copeia 1960 (4): 399.

June 17th: "During the afternoon the ichthyologists met in the Auditorium of the Shedd Aquarium where, with William Braker presiding, the following papers were heard:" This session appears to contain both scientific and aquarium-related talks. Only those relevant to aquarium are included here.

A Technique for Fish Photography. John E. Randall, University of Miami.

The Technique of Anesthetizing and Operating on Large Sharks. Perry W. Gilbert, Cornell University.

Collecting Specimens for the Vancouver Public Aquarium. Murry A. Newman, Vancouver Public Aquarium.

Tests of the Efficiency of Sand and Gravel Filters for Aquariums. Robert P. Dempster, Steinhart Aquarium, California Academy of Sciences.

The Spawning and Early Development of the Parrotfish, *Sparisoma rubripinnis*. John E. Randall and Helen A. Randall, University of Miami.

Observations on the Behavior of Young Gars (*Lepisosteus*). George A. Moore and Carl D. Riggs, Oklahoma Agricultural and Mechanical College and University of Oklahoma.

The New National Fish Hatchery Aquarium at Welaka, Florida. Craig Phillips, National Aquarium, Washington, D. C.

Aggressive Behavior of Some Species of Lionfish (*Pterois*). R. M. Segedi, Cleveland Aquarium.

An Aquarium Exhibit with Polarized Light. W. E. Kelley, Cleveland Aquarium.

University of Texas, Austin, March 30 to August 3, 1961
Copeia 1961 (4).

No papers from any session are listed in the journal's meeting summary for 1961.

Willard Hotel, Washington, D. C., June 14-17, 1962.
Copeia 1962 (4): 866.

June 14th: "Two concurrent sessions were presented during the evening, the first of which was presided over by Craig Phillips, and was devoted to aquaria. Papers presented included:"

Bill Kelley, Cleveland Aquarium, "The Voices of American Alligators During the Mating Season."

Ken Morrison, National Aquarium, "A Brief History of the National Aquarium."

William Hagen, U. S. Fish and Wildlife Service, Washington, D. C., "The New National Aquarium."

James W. Atz, New York Aquarium, "The Walrus and Other Pinnipeds in Captivity."

Frank Powell, Aquarama, Philadelphia, "the Philadelphia Aquarama."

Lee Finneran, New England Aquarium Corporation, "Educational and Moral Responsibilities of Future Aquariums."

Henry Goodridge, Rockport, Maine, "Capture and Raising of Atlantic Harbor Seals."

William B. Gray, Miami Seaquarium, "Unusual Specimens in Aquaria."

Earl S. Herald, Steinhart Aquarium, San Francisco, "The New Steinhart Aquarium."

Murray Newman, Vancouver Aquarium, Vancouver, British Columbia, Canada, "Plans for Expanding the Vancouver Aquarium."

F. G. Wood, Jr., Marine Studios, Marineland, Florida, "The Common Names of Fishes."

Don Olsen, Chicago, Illinois, "The Seven Seas Exhibit of the Brookfield Zoo."

University of British Columbia, June 17-21, 1963.
Copeia 1963 (4): 716-717.

Kenneth S. Norris, University of California (Los Angeles), "Progress at the Mission Bay Oceanarium and the Oceanic Foundation - Sea Life Inc. Complex at Hawaii."

Elmer Taylor, Calgary Aquarum, "Transporting Sea Water to an Inland Aquarium."

Perry W. Gilbert, Cornell University, "Technique of Handling and Operations on Sharks of Medium and Large Size."

Donald W. Wilkie, Philadelphia Aquarama, “Techiques Employed in Transporting Octopus Specimens.”

William Braker, Shedd Aquarium, Chicago, “Collecting and Transporting Hawaiian Fishes.”

James Land, Oakbay Aquarium, Victoria, “A New Concept in Aquarium Construction and Exhibition.”

L. C. Finneran, New England Aquarium, Boston, “Educational Exhibits Planned for the New England Aquarium.”

William E. Kelly, Cleveland Aquarium, “Ideal Configurations for a Semi-Closed Circulating Aquarium System.”

Carl Tuthill, Scripps Institute of Oceanography, “A New Type of Slurp-Gun.”

W. B. Gray, Miami Seaquarium, “The Capture of the White Porpoise.”

Ross F. Nigrelli and Henry Vogel, New York Aquarium and N. Y. City Dept. of Health, “Tuberculosis of Fishes and Other Cold-Blooded Vertebrates with Special Reference to Mycobacterium fortuitum from Fish and Human Lesions.”

R. M. Segedi, Cleveland Aquarium, “Some Notes on Anaerobic Bacterial Activity as a Source of Toxicity in Aquariums.”

John H. Prescott and David C. Powell, Marineland of the Pacific and Steinhart Aquarium, “Air Embolism and Gas Supersaturation Problems in Public Aquaria.”

Burton Clark, Miami Seaquarium, “Certain Aspects of Aquarium Management.”

James A. Thompsen, Tube Turns Plastics, Inc., “The use of UPVC Piping Systems.”

Murray A. Newman, Vancouver Public Aquarium, “A Pictorial View of Some of the World’s Great Aquariums.”

Moorehead-Biltmore Hotel, Moorehead City, NC, August 31- September 4, 1964.
Copeia 1964 (4): pp. 740-741.

While a specific “Aquarium Symposium” was not featured, a related session on September 3rd seems to have been inspired by public aquarium personnel. “At 8:00 PM, a symposium entitled ‘Techniques for handling fishes in small laboratories and experimental situations’ was presented in Vanity Hall, with John G. New presiding over the following contributions:”

“Water Quality Requirements of Fresh and Brackish Water Fishes,” Neal R. Foster, Cornell University and the Academyof Natural Sciences of Philadelphia.

“The Microcosm Concept and Small Marine Aquaria,” James W. Atz, Yale University.

“A Temperature-Controlled, Salt-Water Circulating Apparatus for Developing Fish Eggs and Larvae,” William E. Fahy, Institute of Fisheries Research, Moorehead City, North Carolina.

“Manipulation of Light and Temperature to Facilitate Handling Fish for Experimental Purposes,” Clark Hubbs, University of Texas.

Additionally an aquarium-specific paper was presented the next day:
“Behavior of Pacific Bonito *Sarda chiliensis* (Cuvier) at Marineland of the Pacific,” John J. Magnuson and John Prescott, Marineland of the Pacific.

University of Kansas, Lawrence, KS. June 16-20, 1965.
***Copeia 1*965 (4).**

No aquarium-related papers appear in the meeting summary for this conference.

Miami, Florida, June 19-25, 1966.
Copeia 1966 (4): 899.

Two sessions were held on the afternoon of June 19th and the evening of June 20th to lead off the conference schedule. Burton Clark of the Miami Seaquarium presided and provided introductory remarks.

June 19:

Vincent F. Penfold, Vancouver Public Aquarium, “The New Vancouver Aquarium.”

Donald W. Wilkie, Scripps Institution of Oceanography, “Quinaldine: Some Information on its Use with Marine Fish.”

E. Ledecky-Janecek, Cincinnati Zoo, “A New Approach to Aquascaping.”

David Miller, New England Aquarium Corp., “Through a Glas Darkly.”

Gene Goetz, Miami Seaquarium, “Nutrition, the Feeding Habits of Fishes in Captivity.”

Warren Zeiller, Miami Seaquarium, “Incubation and Hatching of the Eggs of the Texas Skate (*Raja texana*).”

Charles J. Kulp and Warren Wisby, National Fisheries Center, Washington, “Effects of Recirculatio Rates on Ammonia Levels of Closed System Marine Aquaria.”

Warren Zeiller and Billie M. Bevan, Miami Seaquarium, “Ultraviolet Irradiation for Disease Control in Marine Aquaria.”

June 20:

Edward J. Peterson, National Fisheries Center, Washington, “A Meal-Gelatin Diet for Aquarium Fishes.”

Vincent F. Penfold, Vancouver Public Aquarium, “A Unique Aquarium Educational Facility.”

Donald W. Wilkie, Scripps Institution of Oceanography, “An Experiment in Dolphin Transport.”

James W. Atz, American Museum of Natural History, “Phermones and Related Substances in Aquaria.”

Earl S. Herald, Robert P. Dempster, and Phillis J. Ensrud, Steinhart Aquarium, California Academy of Sciences, “Analysis of the Effects of *Homo sapiens* on an Alligator Wishing Well.”

San Francisco, California, June 18-23, 1967.
Copeia 1967 (4): 877.

Two sessions were held on the first day of the conference.

AM: Earl S. Herald, presiding and providing introductory remarks and a salute to the new aquariums.

Daniel H. Moreno, “The (New) Cleveland Aquarium.”

Murray A. Newman, “The (New) Vancouver Public Aquarium.”

Paul L. Montreuil, “The (New) Montreal Aquarium.”

Ross F. Nigrelli and George D. Ruggieri, New York Aquarium and Osborn Laboratories on Marine Sciences, “Studies on *Cryptocaryon irritans*, a Histiophagous Ciliate of Marine Fishes.

R. Stuart MacKay, Boston University, “Discussion and Movies of a Mouse Breathing Oxygenated Fluid for an Extended Period and Returning to Air Breathing.”

Aquarium Activity Reports and Short Comments.

Earl S. Herald, Steinhart Aquarium, “Job Mart for Aquarists.”

William P. Braker, John G. Shedd Aquarium, “Common Names for Exotic Fishes.”

Earl S. Herald, Steinhart Aquarium, “Jet Aircraft Cargo Temperatures.”

Ross F. Nigrelli, Robert Rucker, Paul Osborn, Sylvan Cohen and Robert P. Dempster. Panel Discussion: Diseases, Care and Treatment of Captive Fishes.

PM: Donald Wilkie, presiding.

Warren Wisby, U. S. National Aquarium, "Research and Development Activities."

Robert P. Dempster, Steinhart Aquarium, William H. Shipman, Naval Radiological Laboratory, and Maurice Rakowicz, Brine Shrimp Sales Co., "The Curse of Copper-Bearing Minerals in Aquarium Sand and Gravel."

Thurston S. Grafton, State University of New York, Buffalo, "Investigation of the Sudden Death of a Female Harbor Seal."

William H. Shipman, Naval Radiological Laboratory, "The Physical-Chemical Basis of Sterilization of Aquarium Water by Ultraviolet, Ozone, and Radioactive Sources."

William B. Gray, Miami Seaquarium, "Unusual Aquarium Animals Found Along the East Florida Coast."

F. G. Wood, Jr., Fred Hubbard, Frey Frye, Sam Ridgway, Thurston S. Grafton, Kent Burgess, and David Kenney. Panel Discussion on "Care, Treatment, and Training of Captive Cetaceans."

Ann Arbor, Michigan, June 16-19, 1968.
Copeia 1968 (4): 895.

Vincent Penfold, Vancouver Public Aquarium, "Transportation and Care of Belugas."

Murry Newman, Vancouver Public Aquarium, "Killer Whales at Vancouver."

Warren Zeiller, Miami Seaquarium, "The Texas Skate (film)."

Dan Moreno and Richard Segedi, Cleveland Public Aquarium, "Some Observations on the Spawning Behavior of the Australian Lungfish *Neoceratodus forsteri*."

Robert Morris, New York Aquarium, "Transporting Killer Whales."

Donald Wilkie, Scripps Institution of Oceanography, La Jolla California, "Educational Programs and Policies at Wayland Vaughan Aquarium."

Warren Wisby, The National Aquarium, Washington, "The National Fisheries Center (film)."

Murray Newman, Vancouver Public Aquarium, "The Vancouver Public Aquarium Education Program."

American Museum of Natural History, New York, NY, June 9-12, 1969.
Copeia 1969 (4): 877.

Walter W. West, National Fisheries Center and Aquarium, “Aquarium Glass: A Structural Component (progress report).”

Lee M. Kindley, Office of Saline Water Research, U. S. Department of the Interior, “Reverse Osmosis for Aquarium Waters.”

Charles Baummer and Lauren D. Jensen, Department of Environmental Engineering Science, Johns Hopkins University, “The Removal of Nitrogenous Wastes from Closed Aquarium Systems.”

“Most Exceptional Fish (seahorse),” Motion picture produced my Miami Seaquarium.

Richard H. Reckaweg, National Fisheries Center and Aquarium, “Use of GPX High-Density Plywood in the Construction of Aquaria.”

Robert A. Morris, New York Aquarium, “Underwater Collecting Techniques Used in the Virgin Islands.”

Murray Newman, Vancouver Public Aquarium, “Research Facilities at the Vancouver Public Aquarium.”

New Orleans, Louisiana, March 26-30, 1970.
Copeia 1970 (4): 802, 804.
Two sessions were hosted by Dan Moreno, Cleveland Aquarium.

March 28:

Warren Zeiller, Miami Seaquarium, Miami, Florida, “To Catch a Meal.”

Peter H. Cohan, Cooperative Scienc Education Center, Oak Ridge, Tennessee, “The Effective Use of Living Reptiles and Fish in he Instructional Program at the Elementary and Secondary School Level.”

R. W. McIndoe, Staff Engineer, Celite Division, New York, “High-Rate Sand Filtration vs D. E. F.” ***(presumably: Diatomaceous Earth Filtration)***

Paul Montreuil, Montreal Aquarium, “Management and Chlorine Dioxide Sterilization of the Dolphin Pool at Montreal Aquarium.”

Murray Newman, Vancouver Aquarium, “Collecting in Fiji.”

Louis E. Garibaldi, National Fisheries Center and Aquarium, “The Application fo Fluidics in the Aquarium.”

Robert A. Morris, New York Zoological Society, “Mission Narwhal.”

Jeff Moore, Dallas Aquarium, “Dallas Aquarium Collecting Vehicle.”

Warren Zeiller, Miami Seaquarium, “The Argonaut (Paper Nautilus).”

Gerrit Klay, Shark-Quarium, “Sharks.”

March 30:

H. E. Kumpf, National Fisheries Center and Aquarium. “The Pert Approach to Aquarium Management.” *(Editor’s Note: “The program evaluation and review technique (PERT) is a statistical tool used in project management, which was designed to analyze and represent the tasks involved in completing a given project.” – Wikipedia, 2023)*

Murray A. Newman, Vancouver Public Aquarium, “The New Whale Pool at Vancouver Public Aquarium.”

H. E. Kumpf, National Fisheries Center and Aquarium, “Operation and Principle of the Protein Skimmer (Foam Dome) in Aquaria.”

Vincent Penfold, Vancouver Public Aquarium, “Care and Feeding of Special Animals at the Vancouver Public Aquarium.”

Elmer H. Taylor, Central Wharf, Boston, Massachusetts, “Opening and Progress of the New England Aquarium.”

Copperband Butterflyfish, Bruce Koike

A BRIEF GUIDE TO AUTHORS
Updated 2023

This guide is intended for those not accustomed to using a "Guide to Authors", as provided by more formal periodicals. Historically only about 15% of *D&C* authors get this correct ☺. Please help me out, folks!

The approximate deadline for submissions is December 21st.

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

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 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:

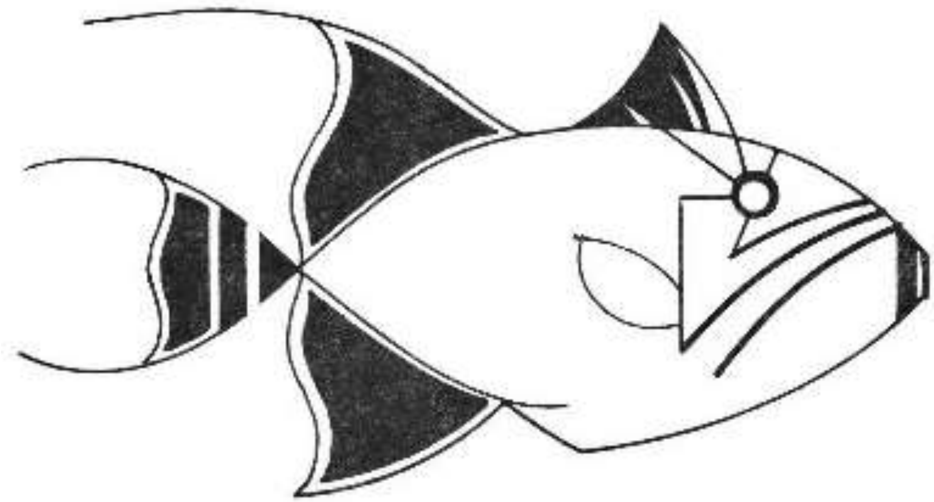


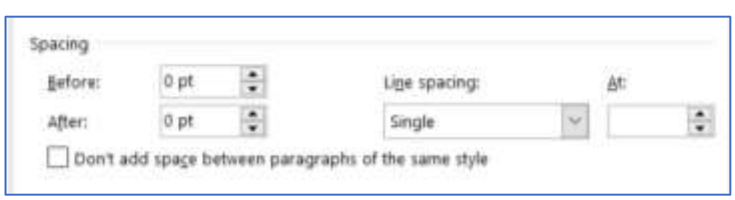
Figure 1. Legends should appear under the photo (such as this drawing by Craig Phillips) or graph in this format in 10-point font, aligned with the sides of the image or figure (center or justify). Very short legends can be centered. Photographs should be pasted into the document in the proper location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

Table Legends

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

Other Things I Whine About

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



- Use the "enter" key for all line spacings ("carriage return" for those who remember typewriters with a slidey thing on top).
- If you submit a table, put the data IN an actual table. Don't use the space bar or tabs to "line up stuff." This formatting can be lost if I have to change margins or otherwise reformat.
- Use the "tab" key to set your 0.5" indent at the start of each paragraph. It's likely your default. Don't use the space bar.
- Use bullets or numbers to make lists. It is easier to reformat these later if needed.

Short Contributions ("Ichthyological Notes")

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

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

Literature Cited

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

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. The formatting used for the journal *Ichthyology and Herpetology* (formerly "*Copeia*") is appropriate. Scroll way down on this page:

https:/www.asih.org/ichsandherps/instructions-to-authors

SOME USEFUL LINKS:
AQUALITY CHAPTERS AND THE 2022 INTERNATIONAL AQUARIUM CONFERENCE ABSTRACTS

Aquality Chapters (LSS Resource)

Chapters are being gradually posted at
https://sites.google.com/view/aqualitywebsite/manual

2022 International Aquarium Conference (IAC) Abstracts

Yeah, no idea why the link includes "2021."
https://www.iac2021.eu/abstract-book/