# DRUM *and* CROAKER

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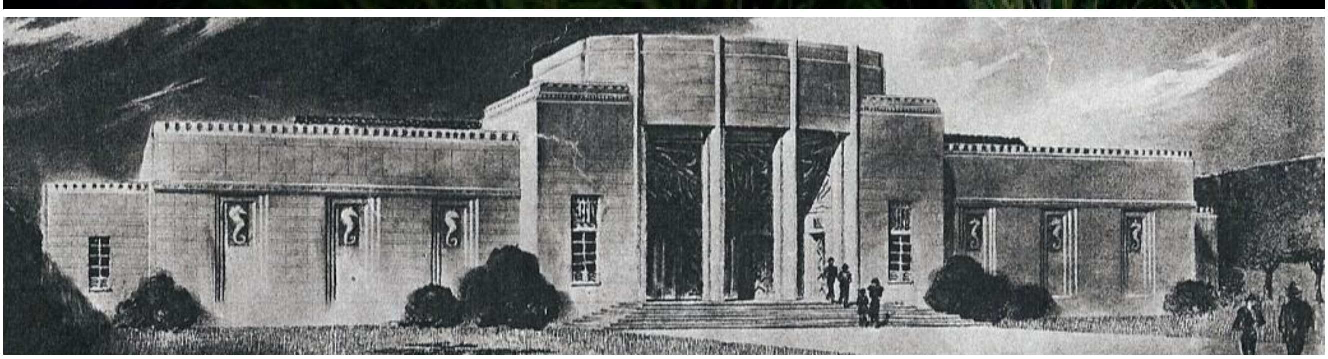

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## DRUM AND CROAKER ~50 YEARS AGO

*Excerpts from the March 1965 Issue, Edited by John H. Prescott*

**Richard M. Segedi**

**The Dallas Aquarium -** Jeff Moore

*(2016 Editor's Note: What an appropriate flashback! The Children's Aquarium in Fair Park is celebrating its 80th year…and has contributed four articles to this issue! - PJM)*

The original Dallas Aquarium was built in 1935-36 and opened to the public June 6, 1936 in conjunction with the Texas Centennial. Basically, the general construction of the Dallas Aquarium is patterned after the Shedd Aquarium . . . chilled and natural fresh water . . . having separate reservoirs. Water was pumped from the reservoir to the tanks on the roof. From there it flowed by gravity to the display tanks. From the display tanks it flowed by gravity to the filters, then back to the reservoirs. A short time later a well was dug directly behind the aquarium, and since then the natural fresh water, is used once, then dispersed to the lagoon across the street from the Aquarium.

On March 15, 1963, ground was broken for construction of the marine addition to the existing building. The left end of the building was torn down and the new construction was joined to the present building. After much research, it was decided to use synthetic sea water rather than hauling water from the Gulf off the coast of Texas. The formula which the Cleveland Aquarium brought over from Germany was used to prepare artificial sea water.

**Note on Coelacanth at U.C.L.A.**

Although not fit for an aquarium display, the ichthyology department at U.C.L.A. recently received a four foot specimen of Latameria from South Africa. For those in the vicinity, it is well worth the trip to U.C.L.A. to see this unusual creature. This is the first specimen in the U.S.

**Quinaldine: Another "Old" Anesthetic**

Although appearing in print in 1958, Quinaldine has only in the last two years become widely used. It is a very good collecting chemical although not the best chemical available for transporting.

# IPAD SIGNAGE FOR "DRUMMIES"

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The Apple iPad is an excellent platform for displaying signage and is much in use by aquariums, zoos, and museums. If properly protected, the iPad holds up well to continuous use by the public and can display identification graphics, videos, etc.

The Children's Aquarium at Fair Park (CAFP) uses the iPad as an animal identification aid for the public, and it is very popular – even two-year-olds figure out how to manipulate the screen content. Like many smaller facilities, the CAFP does not have staff dedicated to audio/video equipment and so it's up to the animal staff to figure out electronic graphics, etc. After a lot of trial and error, we've developed a workable system that is very easy to set up and to modify.

First, our iPad is not on line. We needed a program that would work with local content. After trying several options, the program **Kiosk Pro** provided the functionality we needed. This program is in use at many other institutions. There is a free version, but it only works with on-line content so we upgraded to the Kiosk Pro Basic level for ~$20 *(per device).*

The process to produce content and load it onto the tablet is fairly straight forward. Kiosk Pro works very well with presentations written in HTML, but not being fluent in that language, we write our content in **Microsoft Power Point**. This program allows the user to lay out the graphics just about any way they want and can incorporate hyperlinks that take the user to other slides within the presentation. After being satisfied with the way the presentation works in Power Point, it is saved as a pdf document. (The pdf works very well with Kiosk Pro while Power Point files surprisingly don't work as designed). To proceed, use iTunes to load the pdf file onto the tablet and associate it with the Kiosk Pro app. Then, start Kiosk Pro on the tablet, choose local content and elect to have your presentation as the home program. Simply run the program and your presentation will load. Kiosk Pro also has some other cool options. Links to other files such as videos can be accessed by initiating the navigation bar (see Kiosk Pro help for instructions). At this point it is very important to activate **Guided Access** on the tablet to insure that the users cannot leave the presentation. Guided Access needs to be activated on the iPad by selecting it under the "General" then "Access" tab of the "Settings" menu. Three taps on the tablet's home button will activate Guided Access. Also, turn off **Multitasking Gestures** found on the General tab under Settings.

Finally, the tablet needs to be protected from misuse and theft. There are plenty of products out there that can do this. We are using stands sold by **MacLocks**. With these products, the tablet charger can be hidden in the stand, and the home button can be covered over. The entire set up with an iPad 2 and stand cost us less than $500.

And that's all there is to it. Using this simple process, we have had our iPad in use for almost two years providing easily updated content for public graphics.

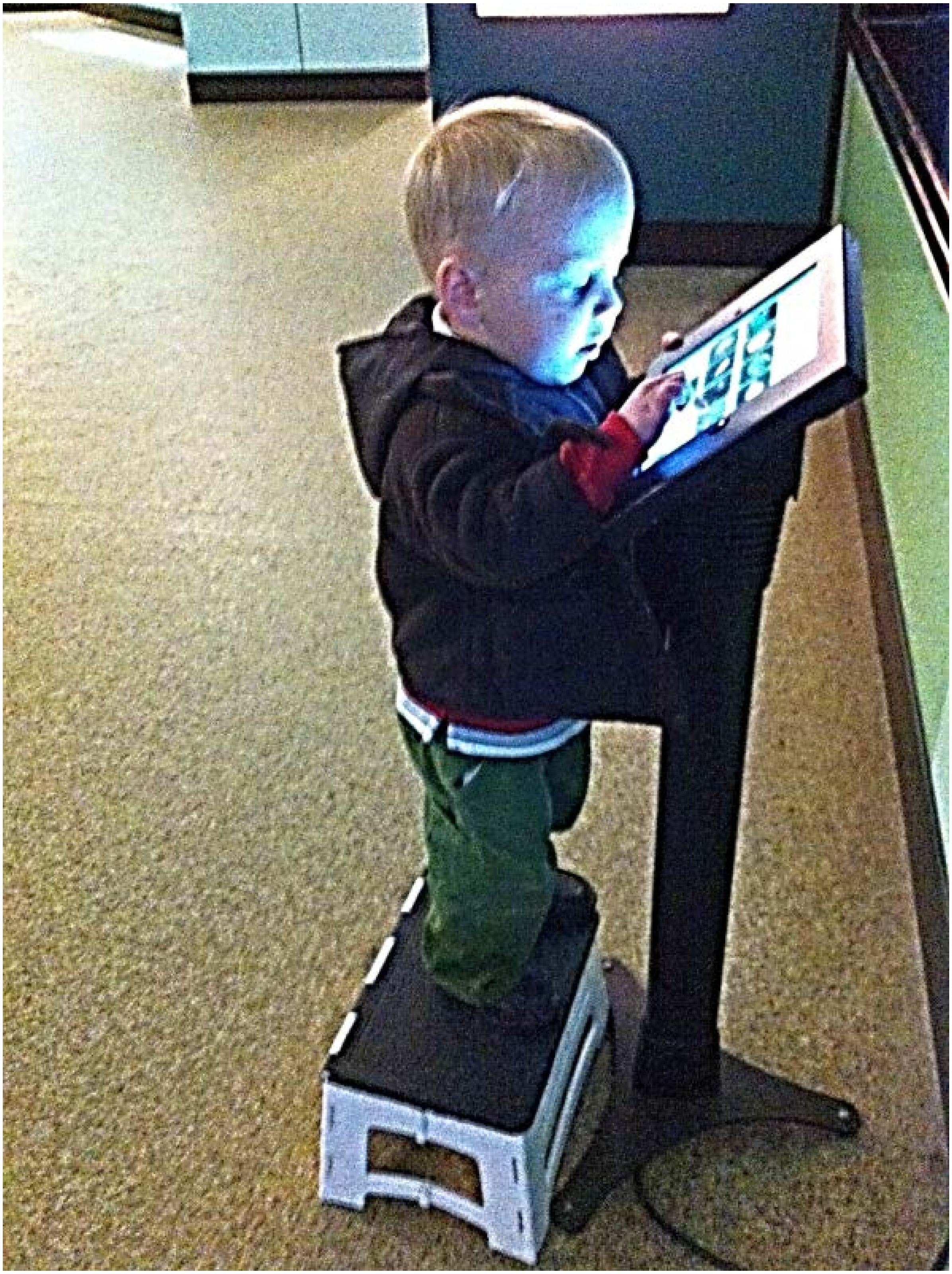

Figure 1: One of our younger visitors accessing the iPad ID system at the Children's Aquarium at Fair Park.

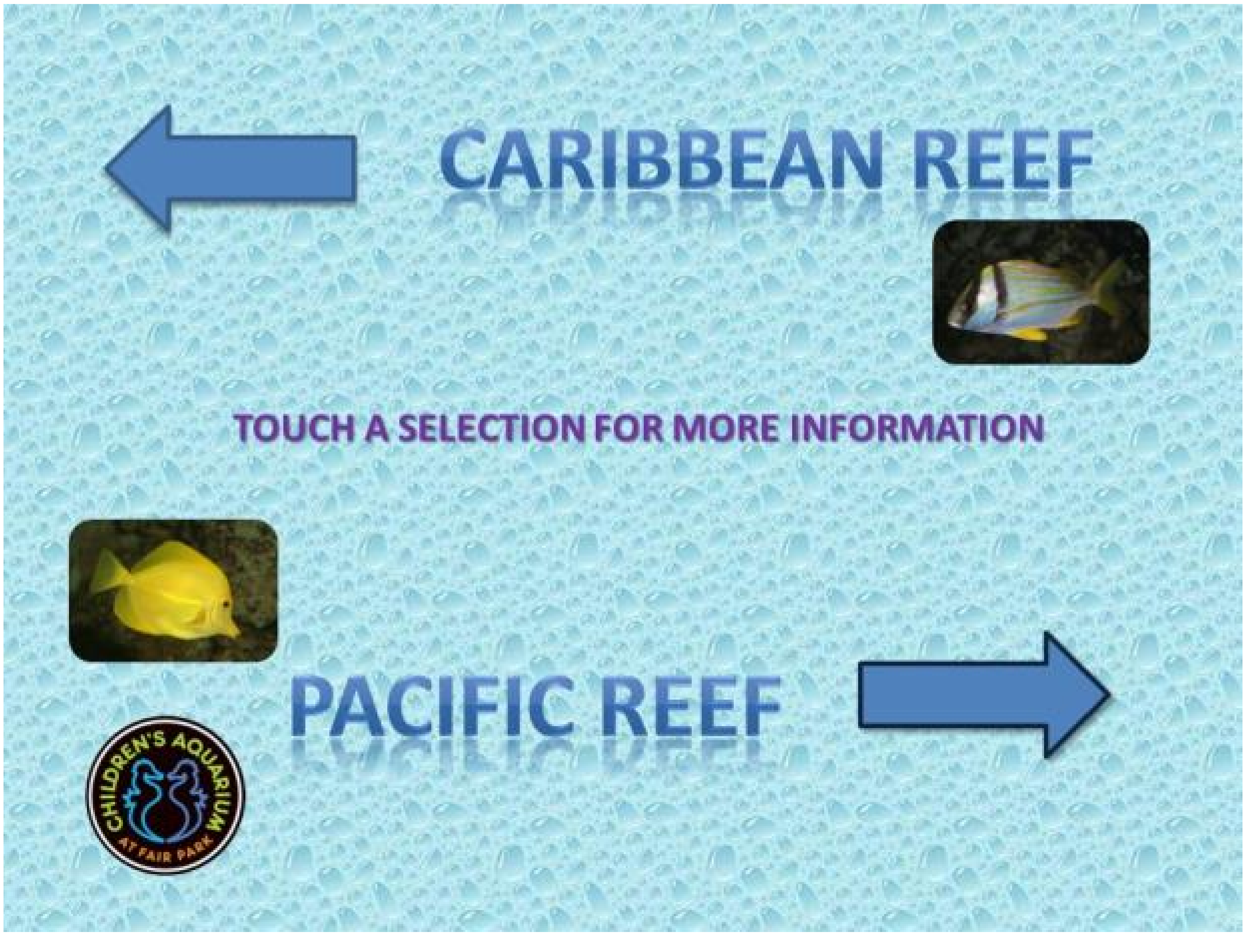


Figure 2: Home screen for identification graphics

# THE NATURAL HISTORY AND HUSBANDRY OF THE WALKING BATFISHES (LOPHIIFORMES: OGCOCEPHALIDAE)

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*"The ocean, she is strange and wondrous, and filled with animals that disturb even a Frenchman"* -Dr. Milton Love

## Introduction

Batfishes (Ogcocephalidae) are members of the order Lophiiformes which is comprised of some 367 extant species and also includes the familiar frogfishes and deep-sea anglerfishes (Antennariidae & Ceratioidei), monkfishes (Lophiidae), and sea toads (Chaunacoidei). With the exception of some of the commercially valuable monkfishes there is a distinct paucity of published information on the natural history and biology of most of these species. As little as we know about the anglerfishes and monkfishes, many of whom inhabit the deep sea, we know even less about the (comparatively) shallow water batfishes! Most ogcocephalids are critically understudied, and most of the primary literature concerning these fascinating creatures is dominated by species descriptions and checklists of fishes from particular locales. Much remains to be learned about their diet, behavior, reproduction, and ecology, an area where public aquaria are poised to make significant ichthyological contributions, yet these wonderbeasts remain a scarcity in our living collections because of their perceived delicate nature and high mortality following collection and transport. This investigation aims to present the scattershot summation of knowledge on the biology of these species as it relates to captive husbandry alongside a discussion of practices continuously refined at the Dallas Aquarium/Children's Aquarium at Fair Park since their initial inception by Schleser and Alvarado in 1992.

Both seasoned aquarists and the public alike have marveled at these fantastically bizarre fishes, and they have a long history of finding their way into captivity. The first documented report of these animals being kept in captivity was a specimen of *O. radiatus* (=*cubifrons*) at the New York Aquarium (NY Zoological Society, 1913). Accounts of various species of *Ogcocephalus* continue until the 1990's when Dr. David Schleser began empirical investigations in collaboration with veterinarians and parasitologists (see Schleser and Alvarado, 1992; Crites and Overstreet, 1997) to eliminate the underlying causes of stress precipitating mortality. Until these practices for aggressive de-worming, feeding, and management of rostral abrasion and infection were developed it was exceedingly uncommon for these fishes to survive more than a week or so in captivity; a month or two in the aquarium was nothing short of a miracle. Today longevity has been documented in excess of 12 years and it is now common for public aquaria to count their average *Ogcocephalus* life span in years instead of months. Ten species in five genera have been kept in captivity, to varying degrees of success, it is hoped this work will make batfish husbandry more successful, and encourage aquaria to work with new, exciting species.

The family Ogcocephalidae is (at present) comprised of 78 species arranged into 10 genera (Froese and Pauly, 2015), and is very much understudied. As such many species are poorly known and some have only been encountered once! As the complete taxonomy of this group is beyond the scope of this report we will refrain from a tedious recapitulation of those species here, and instead direct the reader to the pertinent literature. Bradbury (2003) provides a complete and relatively current checklist of the family Ogcocephalidae. Keys to all the species of genus *Ogcocephalus* are also provided in Bradbury (1980), keys to the batfish species of the Gulf of Mexico are provided in McEachran and Felchhelm (1998), and keys to the species of the Galápagos are provided in Bradbury *et al.* (1998). The Japanese batfishes are summarized in Masuda *et al.* (1987), the ogcocephalids of New Zealand and adjacent waters are compiled in Ho *et al.* (2013) and Marshall (1965). Also see Amaoka and Toyoshima (1981) for an overview of the genus *Dibranchus*, Ho *et al.* (2009) and Benjamin *et al.* (2013) for accounts of batfishes from the Indian Ocean, and Tinker (1982) for notes on Hawaiian species. Also noteworthy is the species description of *Ogcocephalus darwini*, an interesting batfish from the Galápagos Islands by none other than the preeminent ichthyologist Carl Hubbs (1958).

The extant genera are reviewed below in a highly simplified summary, it is not relevant here but interesting to note that an eleventh genus, *Tarkus*, is extinct and known only from the fossil record (Carnevale and Pietsch, 2011). Figure 1 shows the most recent phylogeny of the family, and a select few species that have been kept in aquaria are discussed in greater detail following that.

Genus *Halieutaea*

Nine species, most Australasian in distribution, except *H. retifera* which ranges throughout the Eastern pacific to Hawaii (Froese and Pauly, 2015). Rounded, no rostrum, most species reach sizes between 10-30cm TL (Froese and Pauly, 2015). *H. stellata*, the minipizza batfish, has been kept in captivity in Japan.

Genus *Malthopsis*

Thirteen species Ranging from East Africa to Hawaii including occurrences around Australia, Asia, the Indian Ocean, Hawaii, and Eastern Africa; one species *M. gnoma*, found in the Caribbean(Froese and Pauly, 2015). Most species are diminutive, reaching 6-13cm TL (Froese and Pauly, 2015), disks are triangular in shape with no fleshy rostrum. At least one species, *M. annulifera*, possibly two have been kept in captivity.

Genus *Dibranchus*

Fourteen species distributed worldwide (except the Mediterranean) one species, *D. atlanticus*, occurs widely throughout the Atlantic and Gulf of Mexico (Froese and Pauly, 2015). *D. atlanticus* commonly 11-15cm TL (McEachran and Felchhelm, 1998), but recorded up to 39.5cm (Froese and Pauly, 2015) . In general *Dibranchus* species reach maximum sizes between 11-39cm TL (Froese and Pauly, 2015).

Genus *Halicmetus*

Four species, all Indo-Pacific, ranging from 8-9cm TL (Froese and Pauly, 2015).

Genus *Solocisquama*

Three species, two in the eastern pacific, one species, *S. stellulata* is widespread from South Africa to Hawaii (Froese and Pauly, 2015). Size ranges from 7-13cm TL (Froese and Pauly, 2015).

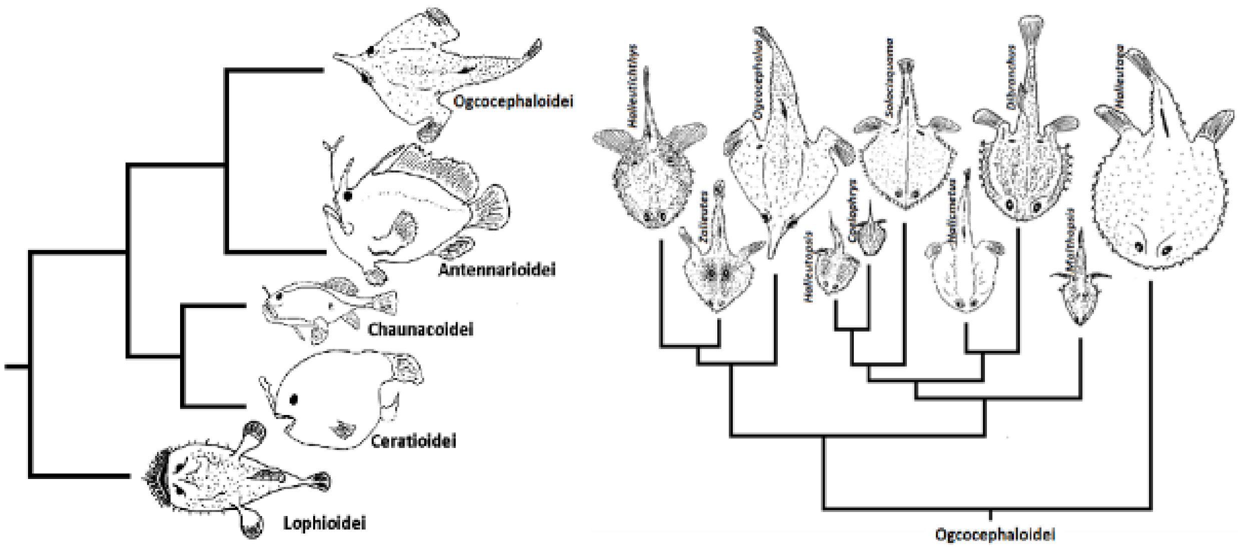


Figure 1: The most recent published phylogeny of the Ogcocephalidae, the base of which (left) is approximately 90MYBP near the origin of the Tetraodontiformes; the Ogcocephalidae diverged from the Antennaridae approximately 70MYBP and the majority of the batfishes have speciated (right) in the last 40 million years. Line drawings original (adapted from various photos), trees following DeRouen *et al*. (2015).

Genus *Coelophrys*

Seven species, all Indo-Pacific; rather small, maximum known sizes between 4-8cm TL (Froese and Pauly, 2015). *Coelophrys* are the only batfishes who are not dorsoventrally compressed, but are instead box-shaped (Nelson, 1994).

Genus *Halieutopsis*

Ten species; most occur in the Indian Ocean through the Indo-Pacific/Asia/Australia, though one species known from the Galápagos; maximum sizes known 6-8cm TL (Froese and Pauly, 2015).

Genus *Ogcocephalus*

The quintessential walking batfishes, at least to those in the Americas. Thirteen species, all found throughout the Gulf of Mexico, Caribbean, and Eastern South America except two species, *O. darwini* from the Galápagos and *O. porrectus* found in coastal Peru (Froese and Pauly, 2015). *Ogcocephalus spp.* tend to be among the larger batfishes, reaching maximum sizes between 30-38cm TL (Froese and Pauly, 2015). Many of the species characterized by a large fleshy rostral process and a triangular disk morphology with highly developed pectoral fin musculature lending the appearance of large 'legs' upon which they walk. Numerous species have been kept in captivity, with varying degrees of success.

Genus *Zalieutes*

Two species, flanking Central America; *Z. mcgintyi* from the Caribbean/Gulf of Mexico, *Z. elater* from the Sea of Cortez to South America (Froese and Pauly, 2015).

Genus *Halieutichthys*

The pancake batfishes. Four species, all known from the Caribbean and the Gulf of Mexico, maximum sizes 7-10cm TL (Froese and Pauly, 2015). The most well known species, *H. aculeatus* has been kept in captivity, and occasionally finds its way into the hobbyist trade.

**Species kept in Public Aquaria**

Below are species accounts of batfishes most likely to be available to North American public aquaria, though comments on South American and some Asian species are included as they have been part of living collections on those continents and in Europe. This list is dominated by the Gulf of Mexico and Caribbean batfishes solely because the authors have the most experience with the fauna of that region. It is our hope that the observations and data herein reported will find use in practicing husbandry for lesser-known species and establishing best practices for those never before kept in captivity.

***Ogcocephalus cubifrons* (Richardson): Polka-Dot Batfish (=*Ogcocephalus radiatus*)**

Coastal distribution more confined than some of its congeners: Atlantic coast from North Carolina, Florida Keys, north into the eastern Gulf of Mexico to Mobile Bay, Alabama; found in Cuba and the Northern Yucatan, but absent from Hispaniola or Puerto Rico (Froese and Pauly, 2015); found in the Bahamas but rare in comparison to *O. nasutus* (Böhlke and Chaplin, 1968). Morphology of rostrum is allometric and varies considerably with animal size (Bradbury, 1980). The authors have also observed this, and juveniles (as seen in Fig. 2) may bear long rostra causing their misidentification as *O. corniger*. Shallow water species, found from 68m to the shoreline (McEachran and Felchhelm, 1998). Maximum sizes reported are 23cm SL (McEachran and Felchhelm, 1998), though as *O. radiatus* the species has been reported to nearly 38cm (Böhlke and Chaplin, 1968). The taxonomic status of *O. radiatus* has long been confused, and was determined to be a synonym of *O. cubifrons* by Bradbury (1980) though the name still appears in many references and is used by collectors, despite being a *nomen dubium* in the literature for which no holotype exists.

<u>Husbandry</u>: Size collected for the aquarium trade are commonly 16±4cm TL (Christie, unpublished data) and adults typically arrive from the wild with a considerable parasite burden. They may be enticed to feed on live *Paleomonetes* feeder shrimps or juvenile *Macrobrachium*. *O. cubifrons* seems more reticent to accept feeder fishes than other large batfishes such as *O. nasutus*, though these may also be offered in the initial effort to get the animal eating. Juveniles are also occasionally encountered in the aquarium trade, and in some cases have less trouble adapting to captive life which may be due to a greatly diminished parasite burden (Christie, unpublished data). These juveniles are commonly misidentified as *O. corniger* due to their longer rostrums, but much like so many awkward adolescent *Homo sapiens* they soon grow into their disproportionate features. This species is generally peaceful with conspecifics at stocking

densities of about 1-2 animals per square meter of substrate. *O. cubifrons* is also compatible with congeners including *O. corniger* and *O. nasutus*, though lower stocking densities are recommended with *O. nasutus* to lessen competition for food. Longevity at least six years (and counting) from specimens held in Dallas by the authors, natural lifespan likely well in excess of ten years. Does well at salinities of 28-35‰ and temperatures from 26-29$^{o}$C (79-84$^{o}$F), thermal maximum is 32$^{o}$C (90$^{o}$F).

Figure 2: Specimens of *O. cubifrons* (=*radiatus*). At left adults on exhibit. Middle is a juvenile specimen, note the prominent rostrum and bright yellow fins. This allometric rostrum often leads wholesalers to erroneously classify juvenile *O. cubifrons* as *O. corniger* (compare to Fig. 5). At right a dorsal view of an adult specimen. Photos B. Christie.

***Ogcocephalus pantostictus* (Bradbury): Spotted Batfish**

This species has a more constrained range than most other Western Atlantic *Ogcocephalus spp.*, being primarily found only in the Northwestern Gulf of Mexico from Mobile Bay, Alabama to Tampico, México (McEachran and Felchhelm, 1998). Occurs in waters 9-31m, maximum size is 31cm TL (McEachran and Felchhelm, 1998). Easily confused with *O. cubifrons/O. radiatus*, though *O. pantosticus* is more commonly encountered west of the Mississippi River delta off coastal Louisiana, Texas, and into México. May be distinguished from *O. cubifrons* by having 9 or more subopercular lateral line scales (8 in *O. cubifrons*) and a wider mouth (Bradbury, 1980). The species also has more a more pronounced ocellated pattern, extending even onto the pectoral, dorsal, and caudal fins (McEachran and Felchhelm, 1998).

Husbandry: Spotted batfish are much less frequently encountered in public aquaria than their congeners, but several specimens have been kept. They may be enticed to feed with live feeder shrimps like most other species. Longevity in captivity unknown, but has been kept up to 8 months; likely lives much longer under ideal conditions. Does well at salinities of 24-35‰ (though will tolerate brief exposure to hyposaline waters better than other spp.) and temperatures from 25-29$^{o}$C (77-84$^{o}$F).

***Ogcocephalus nasutus* (Cuvier): Shortnose Batfish**

Found throughout the Gulf of Mexico and the entire Caribbean, Atlantic coast of the United States to North Carolina, south along the coast of South America beyond Rio de Janiero (Froese and Pauly, 2015). Is easily confused with several species, its South American Range overlaps with *O. vespertilio* and *O. notatus*, its Caribbean distribution creates overlap with *O. cubifrons/O. radiatus*. Bradbury (1980) posits that the populations of *O. nasutus* from the Caribbean are allopatric from the South American populations as evidenced by the relatively short rostra in Northern populations and more elongate rostra from southern specimens. *O. nasutus* may be distinguished from *O. cubifrons* by a lack of spots on the pectoral fins and from *O. vespertilio* by having a shorter rostrum and smaller diameter between the orbits (Bradbury, 1980). Maximum size 38cm (McEachran and Felchhelm, 1998) but adults more typically encountered from 2-16cm TL (NOAA, 2003). Unlike many species *O. nasutus* can regularly be found up to the shoreline (McEachran and Felchhelm, 1998) and even in shallow Bahamian tidal creeks (Rypel *et al.* 2007). Böhlke and Chaplin (1968) note that Bahamian specimens are most common from 35fsw or less.

Husbandry: *O. nasutus* is typically one of the largest ogcocephalid species commonly growing to 20-25cm TL in captivity and being rather heavy-bodied. Specimens are usually lighter in color than *O. cubifrons*, and males will display a bright red underbelly extending up the ventral side of the rostrum and lips (more so than other Caribbean *Ogcocephalus spp.* which are more orange in color). This species typically has a very heavy endoparasite burden upon arrival from the wild and requires aggressive de-worming to thrive. Accepts live *Paleomonetes* and juvenile *Macrobrachium* feeder shrimps as well as live goldfishes, killifish, or other small teleosts within a few days of deworming, can be readily conditioned to feed from a stick on superba krill and various seafood. Specimens have lived in captivity to at least 12 years of age (Christie, 2013), and have reproduced at least once in closed-system aquaria. Needs a minimum of one square meter substrate area per adult animal kept to avoid behavioral issues. Böhlke and Chaplin (1968) note that captive specimens are most active in a darkened tank, though this has not been observed by the present authors and may be an artifact of the short-term captivity of a laboratory setting versus permanent acclimatization to life in the aquarium. Does well at salinities of 28-35‰ and temperatures from 26-29$^{o}$C (79-84$^{o}$F), will tolerate temperatures to 33$^{o}$C (91$^{o}$F), but such extremes are not advised.

***Ogcocephalus vespertilio* (Linnaeus): Seadevil, Brazilian Batfish, Bacacuá, Cachimbo**

The most common species encountered in the Western Atlantic along the coast of South America, *O. vespertilio* is distributed from the mouth of the Amazon river south, along the coast of Uruguay to the mouth of the La Plata river (Bradbury, 1980). Remarkably, one specimen was encountered in pure fresh water in a “mud hole some 800 or 900 miles up the Amazon river” by DeSoto (1922)! North of the Amazon basin *O. notatus* replaces *O. vespertilio* as the dominant batfish species encountered (Bradbury, 1980). This species occurs inshore (Bradbury, 1980) and its propensity for inhabiting shallow waters may explain the disproportionately large number of references to the species in the (scant) primary literature on the Ogcocephalidae in general. Amazon natives have been known to use *O. vespertilio* as part of indigenous medicine to treat asthma (Alves and Alves, 2011). *O. vespertilio* is easily identified by its great rostrum (Fig. 3), longer than any of its congeners, and by having 13-15 pectoral fin rays compared to 10-12 in the only other two long-rostrum species, *O. corniger* and *O. pumilus* (Bradbury, 1980).

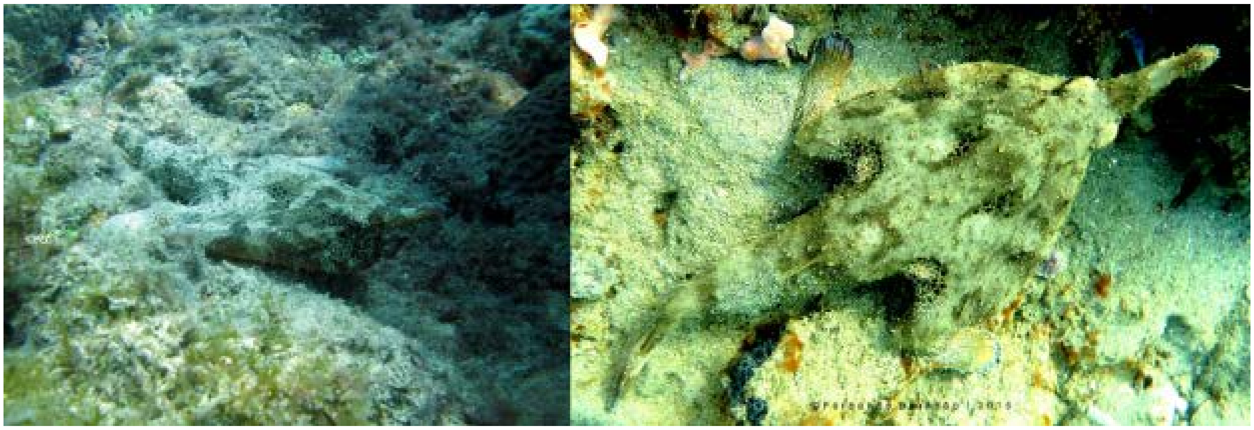

Figure 3: Specimens of O. vespertilio, at left from Abrolhos, Brazil photo © Caio Ribeiro Pimententel used under Creative Commons license CC BY 3.0 (http://creativecommons.org/licenses/by/3.0/) and at right from Maceio, Brazil photo ©Fernando Aldos Bulhões Brandão used under Creative Commons License CC BY-NC 3.0 (http://creativecommons.org/licenses/by-nc/3.0/).

Husbandry: *O. vespertilio* is a common part of the Brazilian ornamental fish trade (Monteiro-Neto *et al.* 2003) and is the most popular batfish among European aquaria. The species grows quite large, reaching lengths up to 30.5cm TL (Froese and Pauly, 2015), though anomalous specimens have been reported up to 34cm (DeSoto, 1922). Specimens acquired for the aquarium trade in Europe are typically 12-18cm (DeJong, pers. comm.) and feeding s similar to other Caribbean *Ogcocephalus*. The species is known to make interesting use of habitat in the wild, preferring to hide in crevices or amongst rocks during the day and feeding on benthic invertebrates (crustaceans, bivalves, polychaetes, brittle stars and urchins at night and in the early morning (Gibran and Castro (2005).

Adult specimens can be kept together if sufficient space allotted (approximately 1 square meter per animal); even when housed alone stereotypical behavior such as swimming against the walls/glass (as if trying to swim through it) is typical if the enclosure is too small. Such behaviors are especially problematic to such large-rostrum species in captivity as rubbing lesions quickly lead to abrasion, infection, and death. Does well at salinities of 28-35‰ (will tolerate salinities as low as 20‰) and temperatures from 26-30$^{o}$C (79-86$^{o}$F).

***Halieutichthys aculeatus*** **(Mitchill): Pancake Batfish**

Coastal distribution throughout the Gulf of Mexico, Florida, Atlantic Coast to North Carolina, south through Greater Antilles but absent from Lesser Antilles; also present around Bermuda (Froese and Pauly, 2015). Synonyms: *Halieutichthys reticulatus*. A more diminutive species found offshore (Fig.4), McEachran and Felchhelm (1998) list it as inhabiting depths between 31- 421m, but one of the authors (BLC) has collected specimens off Galveston, Texas in as little as 12-19m of water. Maximum size 100mm TL (McEachran and Felchhelm 1998), common adult size 50-75mm TL. May be differentiated from the Atlantic batfish, *Dibranchus atlanticus,* by having a conspicuous naked ventral surface *sans* tubercles or bucklers.

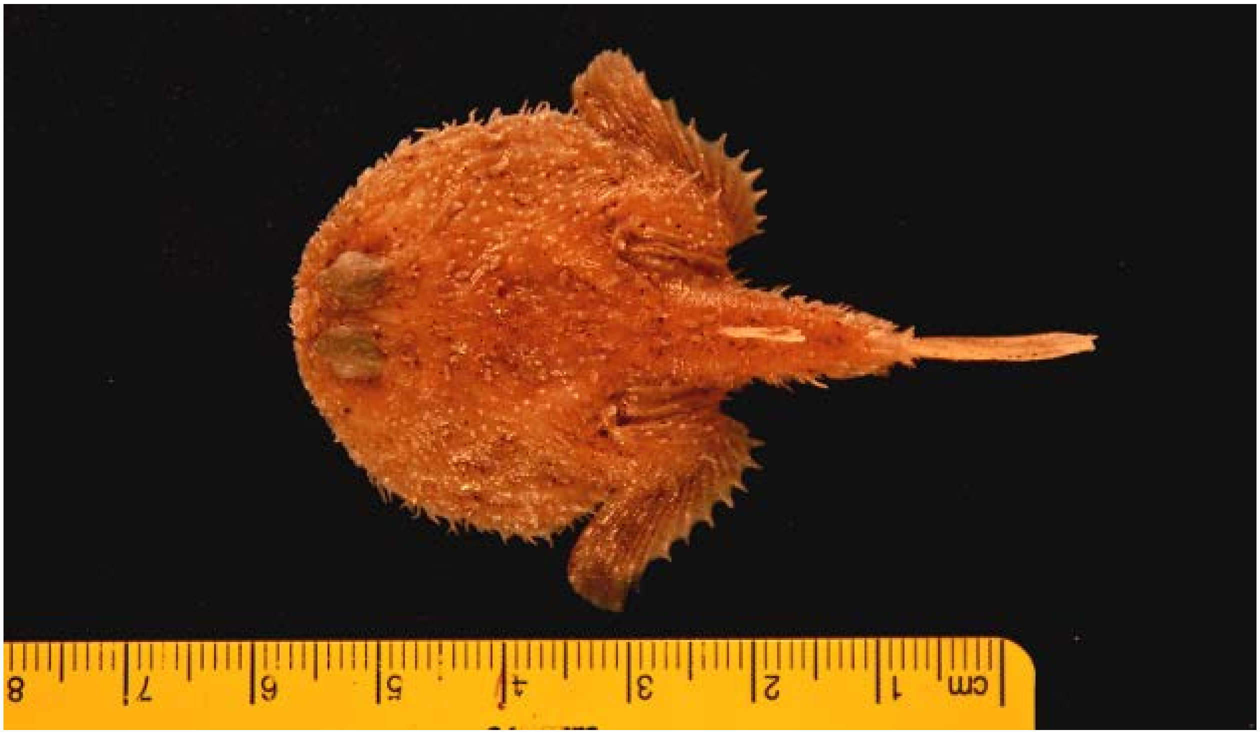

Figure 4: *Halieutichthys aculeatus* from offshore in the Gulf of Mexico. Photo B. Christie.

Husbandry: Specimens collected generally adapt well to captivity but given their size it can be challenging to administer antihelminthic medications. Will often readily take live enriched *Artemia*, though live *Americamysis* and small juvenile *Paleomonetes* shrimps are a better dietary option. Following deworming the smaller specimens may initially benefit from being placed in a small volume of water with a high density of prey items for several hours a day until they begin feeding regularly. Difficult to visualize action of the esca and illicium but upright, alert posture is key characteristic of good health. Lack of a pronounced fleshy rostral process in this species will contraindicate the anterior rubbing lesions typical of so many of the Ogcocephalidae, but be aware of rubbing lesions which may present on the margin of the disc from contact with the tank walls. Generally tolerant of being housed with groups of conspecifics at densities less than 5-7 animals per square meter of substrate, feeding becomes challenging with denser aggregations in captivity. Does well at salinities of 28-35‰ (feeding becomes irregular <25‰) and temperatures from 25-28$^{o}$C (77-82$^{o}$F). Will tolerate temperatures to 30$^{o}$C (86$^{o}$F).

### *Ogcocephalus darwini* (Hubbs): Galápagos Batfish

Once considered endemic to the Galápagos islands, though specimens have more recently been documented from the coast of Peru (Bradbury *et al.*, 1999). The species occurs at relatively shallow depths from 3-76m (Froese and Pauly, 2015) though specimens have been observed by submersible up to 120m (Bradbury *et al.*, 1999). There are only two species of *Ogcocephalus* in the Pacific (Bradbury, 1980). This species can easy be distinguished from *O. porrectus* by having a pair of dark stripes over the dorsal disk extending onto the lateral sides of the tail

(Bradbury, 1980), and it is unique among the batfishes in having a smooth integument covered by very fine spinules instead of bucklers as in other batfishes (Hubbs, 1958).

Husbandry: To the knowledge of the authors this species has yet to be kept at any aquarium. It is included here because it is arguably the most well-known batfish species amongst the general public, having been included in any number of lists of "ugliest", "weirdest", or "strangest" fishes that tend to propagate online. The reputation is not ill-deserved, as *O. darwini* is a strikingly bizarre animal, even among the ogcocephalids not just for its morphology and prominent rostrum but its coloration which tends to be a very pale white-ish grey adorned with jet-black stripes, and males present with a blood red underbelly whose color extends onto the large "lips" and rostrum. It has not been kept in captivity to date, but hopefully will one day inspire wonder at an aquarium; to that end we offer the following husbandry suggestions to the first facility to attempt captive care. Water temperature for the species should probably be roughly 21-25$^{o}$C (71-73$^{o}$F), based on their shallow distribution and average Galápagos sea surface temperature data from NOAA, though the species probably tolerates cooler temperatures as regular ENSO/La Niña events in the eastern Pacific often depress water temperatures dramatically. Salinity tolerances are probably close to that of natural seawater (NSW), and diet, like most of the other *Ogcocephalus spp*. is probably variable but largely comprised of shrimps, polychaetes and small molluscs. Gastrointestinal parasite fauna is as of yet unknown but it is likely the species will need to be aggressively dewormed as with their Caribbean congeners; it is worth noting that the species is known to harbor leeches (Manter, 2015). It is also worth noting that if any facility does acquire specimen(s) of *O. darwini* any worms recovered during the quarantine process should be carefully preserved (see Hoffman, 1999 or similar resource for techniques), ideally in ethanol as the parasites will likely be species new to science.

***Zalieutes elater*** **(Jordan and Gilbert): Roundel Batfish, Murciélago Biocelado**

*Zalieutes elater* is the batfish species most likely to be encountered in the Sea of Cortez, though its range extends through coastal Central America and into South America where it occurs at depths from 18-113m (Froese and Pauly, 2015). Roundel batfishes are easily identified by their two prominent dorsal eyespots (black and yellow) at midbody.

Husbandry: Roundel batfish are a rarity in captivity, but *Zalieutes* have been displayed in public aquaria (McCosker, 2007). In years past when much of the American marine ornamental fish trade originated from Puerto Peñasco and other northern ports along the Sea of Cortez into California and Arizona these fishes would occasionally find their way into aquaria, though most would not survive long. Like most other batfishes live feeder shrimps may be used to entice feeding. More work with this species is needed to conclusively state its captive care. Salinity should probably be close to that of natural sea water and temperature ranges of 26-29$^{o}$C (79-84$^{o}$F) are likely appropriate though precise ranges are unknown.

***Ogcocephalus parvus*** **(Longley and Hildebrand): Roughback Batfish**

Occurs from North Carolina to Brazil in the Atlantic, throughout the Caribbean, and in the Gulf of Mexico to Mobile Bay (McEachran and Felchhelm, 1998). Geographic distribution overlaps with *O. cubifrons* throughout the northern half of its range, but *O. parvus* typically occurs in deeper waters (Bradbury, 1980) and thus the two species may be more accurately described as parapatric than sympatric. Roughback batfish are typically smaller than many other

Caribbean/GOM Ogcocephalus spp. with a maximum known size of 10cm TL (McEachran and Felchhelm, 1998). It may be distinguished from its congeners by having more prominent bucklers giving the dorsal surface a “craggy” appearance, with a rostrum that is usually pointed upwards away from the body (McEachran and Felchhelm, 1998), as well as a smaller mouth relative to the body (Bradbury, 1980), often with bright orange coloration on the pectoral and caudal fins.

Husbandry: This species can be kept in smaller enclosures owing to its size, and single specimens have been successfully kept in standard 29 gallon (110 liter) glass tanks (Anderson, pers. comm.). This size tank has a substrate surface area of roughly 0.25 square meters, and this serves as a good rule of thumb for space needed per specimen, though care should be taken to avoid rostral damage in glass enclosures. Specimens may be enticed to feed using small live feeder shrimps, live mysids, and even bristleworms sieved from the substrate of a reef tank. Afterwards roughback batfish will readily accept all manner of seafoods including krill and chopped fish and clam. Longevity in captivity has yet to be well established as this species is less commonly encountered, but specimens have been kept for at least 1 year and likely live much longer. Will tolerate slightly lower salinity than other species, range about 25-35‰, and they do well at temperature ranges of 26-29$^{o}$C (79-84$^{o}$F)

***Ogcocephalus corniger*** **(Bradbury): Longnose Batfish**

Ranges from North Carolina to the greater Antilles, including the Bahamas, into the Gulf of Mexico to Louisiana, and around the Yucatan peninsula in waters from 29-230m (McEachran and Felchhelm, 1998). Was taxonomically separated from *O. vespertilio* by Bradbury in 1980 based on several morphological characteristics. Maximum recorded size is 23cm TL (McEachran and Felchhelm, 1998), and the species is characterized by its extremely long rostrum, in fact the etymology of the name *cubifrons* is “horn-bearer” (Bradbury, 1980). The pectoral fins are also typically brightly colored in this species, having a black band distally, yellow medially, and brown proximally, though this cannot be used to determine species ID as other species such as *O. cubifrons* are often similar, especially as juveniles.

Figure 5: Adult *O. corniger* on display and locked in a staring contest with a scorpionfish (photo J.W.Foster), subadult specimen (middle), and juvenile surrounded by *Artemia* (right) photos B. Christie.

Husbandry: One of the more common species acquired by aquarium wholesalers and one of the few species that is available to aquarium hobbyists as a result, though few specimens last more than a few months in the home aquarium. Often these are collected as juveniles, and are very difficult to differentiate from juvenile *O. cubifrons* (see Fig. 5). Very small juveniles need large amounts of food to grow and the authors have found that placing them in a small container with high concentrations of live foods can aid in getting new specimens to feed (Fig. 5). Live *Artemia* enriched with HUFA supplements can be used, though when available live *Americamysis bahia* gut-loaded with *Artemia* nauplii are more nutritious. Live freshwater *Tubifex* worms also work well to entice juvenile *O. corniger* to eat but do not live long in salt water and may foul the water if excess are not siphoned out promptly after feeding. Larger specimens, like most batfishes, may be enticed to feed with feeder shrimps such as *Paleomonetes* or small fishes (the authors have used *Fundulus* and *Cyprinodon* fry). Peppermint shrimps, *Lysmata wurdemanni*, have also been used as feeders to good effect when readily available. After feeding for a short time on live foods krill and other chopped seafoods are usually readily accepted. As the rostrum in this species is very large extra care must be taken to prevent abrasion leading to infection. This species is lethargic and tends not to feed well at lower salinities (<28‰) so salinity should b kept near that of full-strength seawater, ideally 30-35‰. Temperature should be 26-29$^{o}$C (79-84$^{o}$F); thermal maximum is 32$^{o}$C (90$^{o}$F). The species becomes anorectic and will lay on the bottom at warmer temperatures. Has been kept with the congeners *O. cubifrons* and *O. nasutus* and a variety of other species including lookdowns, frogfishes, small scorpionfish, toadfish, and even with the garden eels *Gorgasia* and *Heteroconger*. Longevity in captivity has been documented to at least eight years from a specimen kept at the Oregon Coast Aquarium and Disney (Dickson pers. comm.), and life span likely exceeds a decade.

***Halieutaea stellata* (Vahl): Minipizza Batfish, Starry Handfish, Red Batfish, Akagutsu**

Occurs around Japan and throughout the South China Sea and Indonesia (Masuda *et al.*, 1987) in the Torres straight and northeastern Australia and east to the Phillipines (Froese and Pauly, 2015). Masuda *et al.* (1987) also list the species ranging into the Indian Ocean but Ho *et al.* (2013) remark that the Indian Ocean specimen is of a different, undescribed species of *Halieutaea*. This species is one of the larger ogcocephalids reaching a maximum size between 22cm SL (Masuda *et al.*, 1987) and 30cm SL (Ho *et al.*, 2013; Froese and Pauly, 2015). It has a very wide, round body and the bright red/orange dorsal side and tail are armored with numerous bony tubercules and spinules, the ventral surface may be white or pink (Ho *et al.*, 2013; Masuda *et al.*, 1987).

Husbandry: Akagutsu have been kept in several Japanese public aquaria (Fig. 6) and make for a striking display. The average reported longevity at the Numazu Deepsea Aquarium is 8-10 months, where the species is kept at 8-10$^{o}$C (46-50$^{o}$F) and fed a diet of krill and mysid shrimp (Kuwamoto, pers. comm.). As one of the larger batfishes, the species should be given adequate room to avoid crowding, and is generally kept alone without other fishes (Kuwamoto, pers. comm.). This species is known to harbor a greater diversity of parasites than any other batfish species, including three nematodes, eight trematodes, and two cestodes (see Table 2); as all of these taxa are endoparasitic the importance of aggressive prophylactic deworming in this species is evident.

***Malthopsis annulifera* (Tanaka): Wanuke-Fûryû-uo, Fuuyuu**

Occurs throughout the Indo-Pacific, northeastern Australia, north to Japan (Froese and Pauly, 2015), and east to the Phillipines (Masuda *et al.*, 1987) One of five species of *Malthopsis* known to occur around the island of Japan (Masuda *et al.*, 1987), other species include *M.*

*mitrigera, M. jordani, M. lutea* , and *M. tiarella*. These fishes are rather diminutive, reaching a maximum size of 9cm TL (Froese and Pauly, 2015) with body shapes reminiscent of arrowheads. As the scientific binomial suggests the species may be characterized by having 5-12 ring-like dorsal markings (Masuda *et al.*, 1987).

Husbandry: Species has been kept in Japanese aquaria, including the Numazu Deepsea Aquarium (Fig. 6), and generally lives anywhere from 3-4 months in captivity (Kuwamoto, pers. comm.). Fuuyuu are collected from deep waters (approximately 200m) and are best kept from 8-10$^{o}$C (46-50$^{o}$F), and adapt to a diet of krill or mysids in captivity (Kuwamoto, pers. comm.).

Figure 6: Japanese batfishes, at left the diminutive *Malthopsis annulifera* and at right is *Halieutaea stellata*. Photos courtesy of and Copyright © Numazu Deepsea Aquarium, used with permission.

## Conservation Status of Batfishes

Most species of the ogcocephalidae are rarely encountered compared to other teleosts, though they do not appear, at present, to be under threat of extinction. None of the species that occur within US waters are currently listed on the Endangered Species Act, or any of the CITES appendices. The IUCN red list has evaluated eight of the 78 known species and ranked all as either data deficient or species of least concern (IUCN, 2015). Batfishes are not eaten except as native medicine among some Amazon peoples (Alves and Alves, 2011), though they are taken as by-catch. As we know almost nothing of their reproductive habits or population dynamics commercial fishing (especially Gulf of Mexico shrimp trawling) may or may not significantly impact batfish populations- there is simply insufficient data from which to draw conclusions.

## Behavior

As stated above, the Ogcocephalidae are a poorly- studied group of fishes, and this is no less true in relation to their ethology. It is interesting that a majority of the behavioral proclivities unique to this group of fishes have been observed in some form of captivity, either in the aquarium or in the laboratory. More carefully controlled observations are needed to construct a complete ethogram, but some typical behaviors are discussed here to aid the aquarist in differentiating those that are normal from those that are maladaptive.

Schleser and Alvarado (1992) describe aggressive behaviors between conspecifics such as dominant specimens spreading their fins and appearing to bow (see Fig. 7A). The authors have kept multiple specimens and even multiple species together and this behavior is occasionally observed, though never to the point where physical aggression has been noted or an animal fails to thrive as a result (several colleagues at other institutions have noted similar results). As such, it seems given enough space similarly-sized animals suffer no serious consequence from being housed together. It has also been noted that most Caribbean spp. are nocturnal or crepuscular feeders, and are most active in darkened tanks (Böhlke and Chaplin, 1968) though they seem to quickly adapt to captivity and will readily feed and hunt during daylight hours after being properly quarantined.

Though appellated 'walking' batfishes, their fascinating walking behavior is not the exclusive mode of locomotion and they are quite capable of swimming, albeit clumsily. Some of the early descriptions of behavior from Bahamian specimens note that "When batfishes do swim, they are anything but swift and graceful." (Böhlke and Chaplin, 1968); which is true of most species, but especially *Ogcocephalus* spp. Specimens with more round shaped bodies such as *Halieutaea* and *Halieutichthys* are more adept at swimming (perhaps due to lift created by their disc morphology and pectoral fins) and one can often observe them gliding just above the sand in the wild with coordinated fin movements. Most *Ogcocephalus spp*, however, more closely resemble a drowning cow when swimming, as they are quite negatively buoyant and appear uncoordinated in motion. Pietsch (2009) notes that the Antennariidae commonly use their opercular openings in "jet" propulsion when in midwater and there is no reason to suspect that other related taxa such as the ceratoids do not; this has been observed by one of the authors (BLC) on a few occasions in *O. cubifrons*. Batfishes do not appear to use their opercula in propulsion often, but when gliding downward in midwater and startled they are able to quickly change direction and jet away.

Breder (1949) undertook one of the first studies of batfish behavior in the laboratory and defined three types of walking behavior, all commonly seen in captive specimens in the aquarium. Breder (1949) noted that two distinct hopping motions were common, one he likened to a rabbit and one to a toad, though the more captivating method of walking is when pelvic fins and pectorals alternate to slowly crawl along the substrate. Specimens were also seen to freeze when startled and cover themselves with sand, requiring "considerable prodding" to move (Breder, 1949). These early Bahamian laboratory specimens were also noted as having turned a darkened color (Breder, 1949), though in the authors' experience this is quite common in response to stress such as capture or initial handling. They may be, conversely, quite inquisitive, and must sometimes be sequestered in a floating basket during maintenance to keep them away from scrub poles or power-washers in captivity. Acoustical studies have also been done, though it seems that batfishes do not produce any sounds other those associated with feeding (Moulton, 1958).

Being (very) benthic animals batfishes also have a close relationship with the substrate upon which they spend their lives and derive their prey. During the day many species will cover with sand to rest and hide (Böhlke and Chaplin, 1968), and seem to prefer small boulders or crevices to hide next to (MacEachran and Felchhelm, 1998; Walker, 2002; Gibran and Castro, 2005). The authors have observed *Halieutichthys* as using its pectoral fins to cover the body with substrate, and most *Ogcocephalus spp*. will bury or partially bury their pectoral fins in sand while attempting to lure prey. Breder (1949) noted specimens partially covering their bodies with sand prior to luring; a very interesting use of habitat as camouflage not known from many fishes.  Alternatively, batfishes may eschew substrate camouflage and raise their pectoral fins, and sometimes caudal fin and tail completely off the bottom while luring, balancing like a teeter-totter on their pelvic fins (See Fig. 7B), presumably to make a more rapid head-down strike at prey items.

The use of the esca and illicium in luring prey is one of the most fascinating aspects of batfish behavior.  The illicium is evolutionarily derived from the first dorsal spine as in the Antennariidae (MacEachran and Felchhelm, 1998) though the esca is comprised of modified glandular tissue enabling it to serve as a chemical as well as visual lure (Bradbury, 2003).  The lure is typically extended straight out or to about 30$^{o}$ left or right (Breder, 1949), and this behavior has proven critically important in husbandry as an indicator of good health (Schleser and Alvarado, 1992).  During early investigations with batfish husbandry Schleser noted that poor posture and lack of luring behavior were very much related to failure to thrive in captivity:

> "*It should be mentioned that the 'posture' of a batfish is a very good indicator of its general condition.  A healthy batfish supports the fore part of its body above the substrate with its limb-like ventral fins, while an ill animal invariably lays flat upon its ventral surface*" (Schleser and Alvarado, 1992)

Following Schleser the authors have found that observation of posture and lure activity (in addition to clarity of the corneas) to be essential to assessing the health of these animals in captivity.  Very stressed animals may also exhibit overall dark coloration and mucous production in addition to poor posture.  Once quarantined and actively hunting it is usually not too difficult a task to introduce dead foods and then condition the animals to pole-feed and (with a little patience) even swim up to the aquarist and hand-feed (Fig. 7C).

As mentioned above these animals rely on camouflage as a defense, and their extremely tough integument festooned with calcified bucklers and tubercles which also provide some disincentive to predation, but the authors have found their general disposition to be unlike any other teleost.  Batfishes (at least *Ogcocephalus spp*.) do not try to avoid nets and seem not to mind being picked up in the least!  In nature most animals with such indifference to potential danger are usually extraordinarily toxic, though this does not seem to be the case with the ogcocephalids.  Bradbury *et al.* (1998) note that *O. darwini* has been found in the vomit of sea lions, though horn sharks consume them without apparent detriment.  One of the co-authors in that study (and a distinguished ichthyologist and aquarium director) licked a live specimen (in the name of science) and "did not find it distasteful" (Bradbury et al., 1998).  One of the authors of this work (BLC) has re-created this experiment and suffered no ill effects from licking either the dorsal or ventral sides of several *O. cubifrons.*  Until more extensive studies are undertaken the question of toxicity or lack thereof in the Ogcocephalidae remains a mystery.

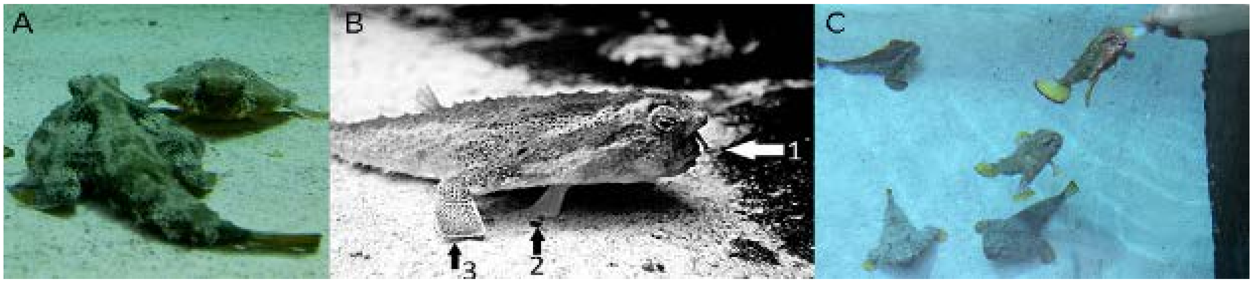


Figure 7: Behavior in batfishes (A) specimen on top right showing spread fins and "bowing" aggression behavior as originally described by Schleser and Alvarado (1992), specimen at left showing good upright posture (B) Luring behavior, note the esca and illicium fully extended (1) and pectoral fins lifted completely off the substrate (3) as it stands on its pelvics (2) while hunting (C) handfeeding a group of *O. cubifrons.* Photos B. Christie.

**Tankmates and Exhibit Design**

Batfishes need not be displayed by themselves, with careful selection of species there are a broad array of tankmates available to display with these fascinating fishes. In the earliest works published on batfish husbandry Schleser and Alvarado (1992) recommend that batfishes not be kept with each other to avoid struggles over dominance which may stress the animals and result in one being outcompeted over the other. In the authors' experience with several species of *Ogcocephalus* this concern is largely unwarranted so long as animals are closely matched in size and are given wide substrate area (0.75-1.0$m^2$ per adult specimen). The experiences of the aquarium community at large mirrored these observations, and about 60% of institutions surveyed housing batfishes had kept conspecifics or congeners together at one point without major behavioral issues. Many aquaria have exhibited other species alongside batfishes as well, including numerous invertebrates (hermit crabs, snails, sea cucumbers, starfish, *et cetera*) as well as other teleosts (lookdowns, silversides, mojarras, garden eels, scorpionfishes, toadfish, and frogfishes). In general when partnering batfishes the aquarist should look for peaceful species that are not likely to pick or nip at the largely sedentary batfishes and avoid more aggressive fishes such as damselfishes, pufferfishes, or angelfishes.

Shallow round trays with opaque, slanted walls are ideal for the holding or quarantine of batfish (Fig. 14A) to avoid rostral damage which may lead to infection and sepsis. If transparent tanks must be used visual barriers (Fig. 14B) may be employed to prevent the animals from rubbing on the glass (Schleser and Alvarado, 1992). These are described in greater detail below in the quarantine section. For exhibits the clear viewing panel is usually not problematic if sufficient substrate area is provided and the back and sides of the enclosure are opaque. Batfishes in the wild utilize habitat and structure during the day (Gibran and Castro, 2005; Walker *et al.*, 2002), and may feel most comfortable if a few small pieces of live rock or stone are included in their décor, though rockwork should not limit the amount of open space. Large specimens such as 20-25cm *Ogcocephalus* need a fair amount of open substrate and the authors have found that a stocking density of about 0.75-1$m^2$ per animal affords adequate substrate. With smaller specimens try to size an exhibit so that an animal placed in the center would have 3-5 times its body length in every direction. This amount of space will prevent it from rubbing on the walls, as well as allow it to express naturalistic hunting behaviors as it walks around in search of epibenthic prey items. Batfishes kept in smaller enclosures will often display stereotypical behaviors such as rubbing their rostra and sides against the walls, and in extreme cases have been observed incessantly swimming against the glass (as if trying to swim through it), displacing substrate and causing severe trauma to their integument leading to sepsis and death. Many Caribbean species are found in seagrass beds, and they have been displayed in aquaria with both natural and artificial sea grasses. Batfishes prefer to move between plants rather than swimming over them, so plantings should be kept sparse to allow plenty of sand space upon which to walk. A variety of substrata have proven effective, and coarser gravel such as crushed coral is generally not problematic, but finer aragonite sand seems to be a bit gentler on their fins which are in constant contact with the bottom.

**Water Quality and LSS**

Batfishes are tolerant of the same water quality conditions as most other marine fishes with no special requirements. Ammonia and nitrite should be kept low, ideally undetectable, but in any case less than 0.100mg/l. Excessive accumulation of nitrates have been known to be at the least problematic and at worst toxic to aquatic life (Carmago *et al.*, 2005); including being implicated in goiter formation in elasmobranchs (Crow, 2004, Morris *et al.*, 2011). The range of effects and stresses placed upon (phylogenetically) more advanced marine fishes by elevated nitrate concentration in captivity has yet to be fully elucidated, though management through water changes and/or denitrification to the extent that is practical should be employed as a part of husbandry for these species. The pH of water for batfishes should be kept near that of natural seawater as with most marine fishes; batfishes the authors have worked with have been maintained successfully from pH 7.80-8.40. Batfishes are, however, rather sensitive to heavy metals, perhaps unsurprisingly as they are so closely related to the Anglerfishes (Antennariidae) and the pufferfishes (Tetraodontiformes). When exposed to copper or other heavy metals they tend to have a poor posture and appear listless, and may also secrete a heavy mucous coating which stands in stark contrast to the (normally amuculent) skin. Exposure is not necessarily fatal, as some facilities have employed therapeutic copper without loss of life.

Salinity and temperature are the dominant water quality parameters which are of concern to the aquarist keeping batfishes; with a few exceptions these fishes occur in full-strength seawater in the wild (*O. vespertilio* occurs in the Amazon delta and has been recorded upriver from freshwater, and *O. pantostictus* occurs in the lower salinity areas of the Gulf of Mexico where the Mississippi river discharges). In general most of the batfishes prefer salinity close to that of natural sea water (NSW) and should be kept from 28-35‰, a few species are more tolerant of lower salinity water (see Table 1) while others become anorectic if the water is too hyposaline. Attempts to use hyposalinity as a treatment for *Cryptocaryon irritans* by the authors have not been particularly successful, as the animals refused all food after several days of exposure.

Temperature is also critically important. In the wild living many batfishes inhabit offshore waters of the continental shelf and experience a wider array of temperatures, but in captivity feeding is affected and ectoparasite outbreak (see section on disease below) is a very real consequence if water temperature falls too low in the authors' experience. Overly warm temperatures have been found by the authors to be preferable to cooler water in captivity, but excessively warm conditions (30-33$^{o}$C) have seen animals becoming irritable and hyperactive, with greater potential for damage to their rostrum or integument. There is also evidence that exposure to cold waters, even in the wild, can be catastrophic to the species; Gilmore *et al.* (1977) report on a winter kill of ogcocephalids on the gulf coast of Florida when temperatures dropped below 16$^{o}$C (61$^{o}$F) for several days.

Table 1: Salinity and temperature preferences for Ogcocephalid batfishes kept in captivity.

| Species | Temperature Range °C (°F) | Maximum °C | Salinity Range ‰ | Minimum ‰ |
|---|---|---|---|---|
| *Ogcocephalus corniger* | 26-29 (79-84) | 32 | 30-35 | 28 |
| *Ogcocephalus cubifrons* | 26-29 (79-84) | 32 | 28-35 | |
| *Ogcocephalus nasutus* | 26-29 (79-84) | 33 | 28-35 | |
| *Ogcocephalus pantostictus* | 25-29 (77-84) | | 24-35 | |
| *Ogcocephalus parvus* | 26-29 (79-84) | | 25-35 | |
| *Ogcocephalus vespertilio* | 26-30 (79-86) | | 28-35 | 20 |
| *Halieutichthys aculeatus* | 25-28 (77-82) | 30 | 28-35 | 25 |
| *Halieutaea stellata* | 8-10 (46-50) | | NSW | |
| *Malthopsis annulifera* | 8-10 (46-50) | | NSW | |

**Thermal maximums and saline minimum as observed by the authors or reported anecdotally*

Life support systems (LSS) for batfishes need not be very complicated, and a variety of techniques have been employed. In general, these animals will place a fairly low burden on biofiltration, as the amount of ammonia production per unit biomass is about 180-260mg $NH_3$-N $day^{-1}$ $kg^{-1}$ under normal conditions. Given that even a large adult 25cm specimen weighs about 250-350g the ammonia input to a closed system per unit biomass is not enormous. As their exhibits typically include a fair amount of sand or gravel additional biofiltration is often not strictly necessary, but may be beneficial. Protein skimmers are recommended as with most modern marine aquaria as they will help remove surfactants and DOC/POC ultimately keeping water quality more stable with fewer required water exchanges. Activated carbon may be employed if necessary to remove Gelbstoff (yellowing compounds) or medications, but should be rinsed thoroughly before use to prevent introduction of carbon dust to the system which has been implicated in irritation of the lateral lines of some fishes (Hemdal and Odum 2011).

## Diet and Feeding Ecology

We know precious little about the biology of batfishes in the wild, but we do know that most of the Caribbean/Gulf of Mexico species actively feed on small invertebrates, and they seem to be rather opportunistic, eating just about anything they can catch including shrimps, small crabs, small bivalves, gastropods, polychaetes, brittle stars, urchins (Gibran and Castro, 2005), and even small fishes (Böhlke and Chaplin, 1968; Rypel *et al.*, 2007). Naturalist Jack Rudloe of Gulf Specimen noted that *Ogcocephalus spp*. in Florida seem to have a particular affinity for juvenile *Busycon* whelks, and will actively seek out and devour the small gastropods after hatching (Rudloe, 1966). Given their size one would expect these fishes to only consume the smallest shelled molluscs, but a radiograph of *O. corniger* (Fig. 8) taken by the Smithsonian National Museum of Natural History shows that the gut is filled with surprisingly large shells!

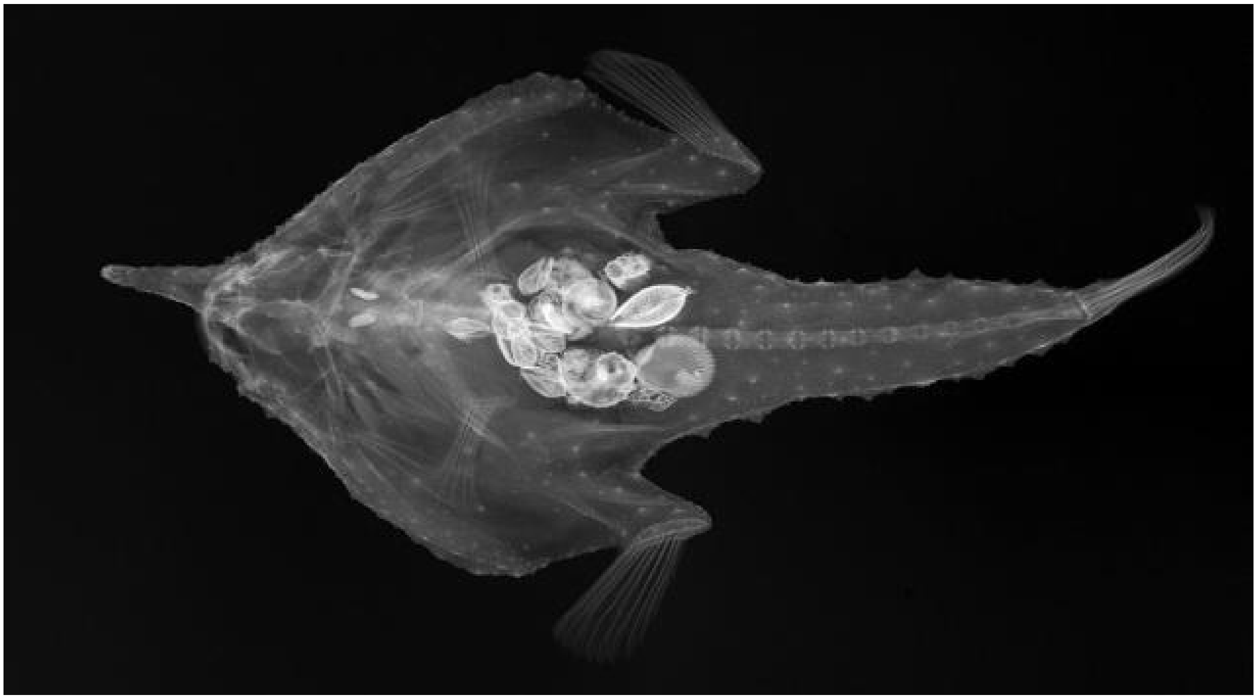

Figure 8: Radiograph of *Ogcocephalus corniger* (holotype) showing the calcareous mollusc shells as part of the gut contents. Copyright ©2009 Smithsonian National Museum of Natural History, Sandra J. Raredon, Division of Fishes, used under a Creative Commons License CC BY-NC 2.0. https://creativecommons.org/licenses/by-nc/2.0/

In captivity, it is beneficial to force-feed a high-calorie gruel immediately after arriving at the aquarium along with antihelminthic medications (see quarantine section below). Once de-wormed live feeder shrimps are generally the most useful feed item to start the animal feeding, then they can be weaned onto dead food such as superba krill, and eventually all manner of chopped seafood. Small specimens can be placed in a container with highly dense prey (see Fig. 5) for several hours per day until they begin feeding regularly and grow. The use of nontraditional foods such as live freshwater *Tubifex* worms or bristle worms sieved from the substrate of a reef tank may also be useful to entice smaller specimens into feeding. Overall, the captive diet should be diverse, as these animals are opportunists, and invertebrate protein should make up 50% or more of the total diet given their propensity for feeding on molluscs, worms, and crustaceans. Schneidewind (2006) notes that in the closely related Antennariidae specimens have been kept long-term feeding on non-marine fish protein without apparent detriment, though in the authors' opinion this should be avoided wherever possible because lack of highly unsaturated fatty acids (HUFA) has been shown to depress fecundity in fishes (Rainuzzo et al., 1997) and the full effects of such unnatural diets are unknown. Schneidewind (2006) also notes that in frogfishes excessively large meals may result in a large bolus of food in the GI tract becoming problematic due to gas production, and the same has been observed in the batfishes, underscoring the need to avoid overfeeding.

**Body Condition**

Visual assessment of batfishes on arrival permits a rapid assessment of the animal's body condition, and is important to long-term husbandry. Emaciated animals are readily apparent after flipping them over and examining the ventral side, as they will have a "sunken" appearance towards mid-body (see Fig. 9A) where well-fed specimens are fuller throughout (Fig. 9B). These animals also store adipose tissue in their tails, which will appear thick at the base when well fed (or even plump in overweight animals and breeding females, as in Fig. 9C). As these fishes are not commercially valuable and rarely encountered in the wild we have little fisheries data from which to draw conclusive assessments of "normal" weights for a given size. The sole published length-weight relationship for a batfish species is Vianna *et al.* (2004) for *O. vespertilio* (a=0.0302, b=2.61, $r^2$=0.850). These data are extrapolated over a range of sizes in Fig. 10A, and while this gives a rough estimate of size for that species, the author's find that the morphometrics of *O. vespertilio* are not congruent with those of our own long-term population of *O. cubifrons* and as such should not be used to assess the health or condition of other species.

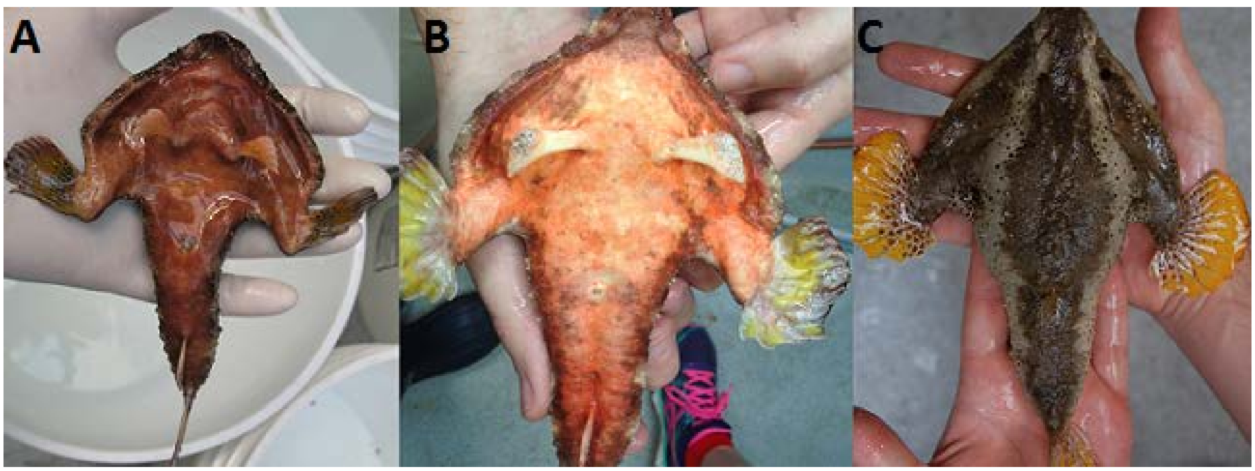


Figure 9: Body condition in *Ogcocephalus cubifrons*. (A) emaciated specimen (B) healthy adult (C) overweight specimen or possibly gravid female. Note the red ventral sides usually indicating a mature male (also note the fluorescent pink shoelaces characteristic of this subspecies of veterinarian). Photos B. Christie (A,C), L. Torres (B).

An approximate length-weight distribution from captive specimens of *O. cubifrons* can be inferred from data in Fig. 10B, and might be comparable to other large-bodied batfishes such as *O. nasutus*, though these data are extrapolated from a small sample size of a single species. A model of growth (TL and mass) over time for *O. cubifrons* is also presented in Fig. 11 from five years of data. In *O. cubifrons* measured by the authors total length has ranged from 7.5-22.8cm TL, standard length 6.5-19.5cm SL, disc width (at widest point) 3.5-12.0cm, tail width (at base) 1.0-6.5cm, and weight 74.5-337g. Some relationships can be calculated from this (limited) dataset on *O. cubifrons* to aid in interpreting values in the literature such as TL=1.237SL, and for adults one can estimate weight as 12.3±4.3g $cmSL^{-1}$ (approximately). Fuller's condition factor (K) for healthy captive adult *O. cubifrons* can also be calculated to aid in assessing general robustness, being roughly K=4.82±1.24 (range 2.53-8.01).

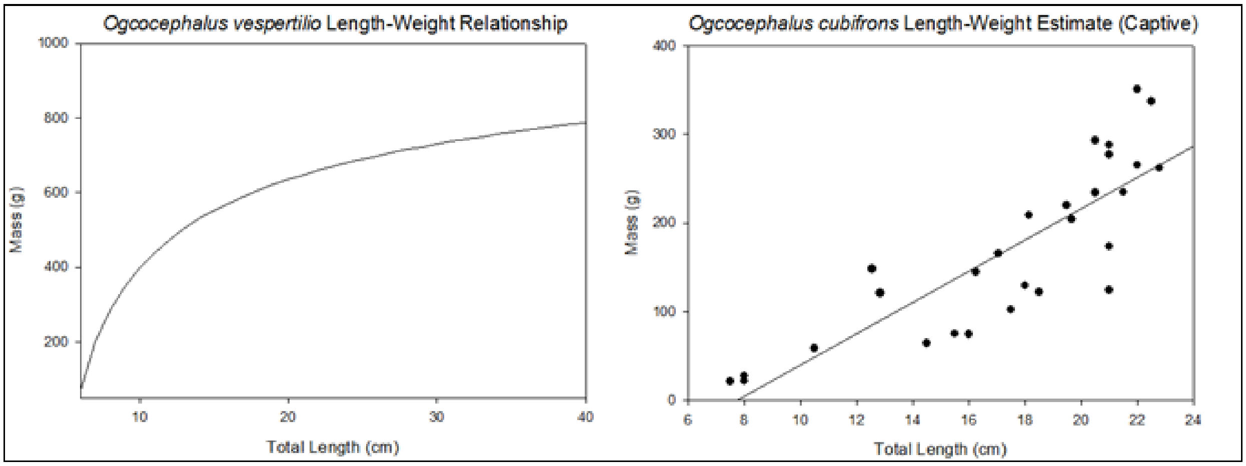


Figure 10: Length-weight relationships for *O. vespertilio* extrapolated from data in Vianna *et al.* (2004) (left) and (right) scatter plot of L-W from healthy captive specimens of *O. cubifrons*.

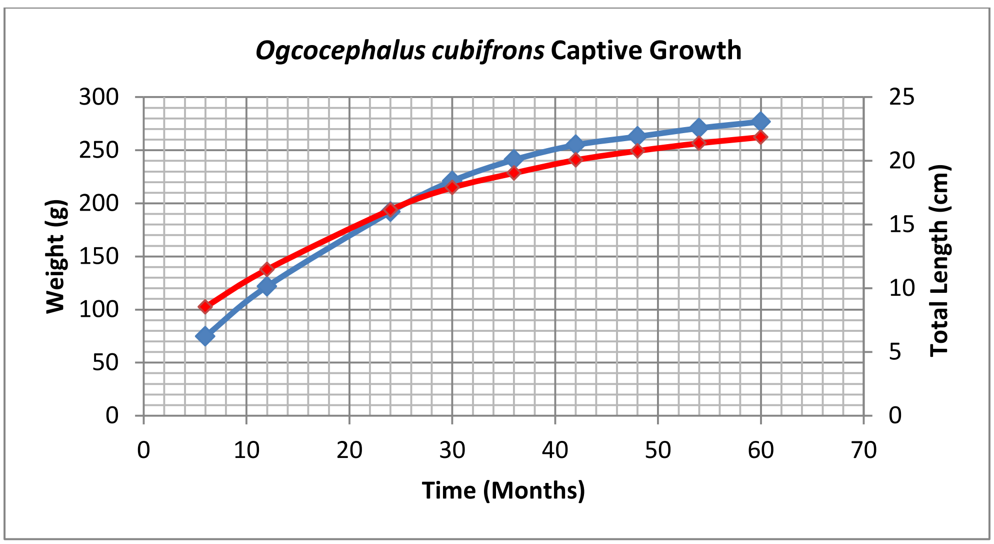


Figure 11: Approximate growth curve from morphometrics of captive individuals (n=12) of *O. cubifrons;* weight shown in blue, total length is red.

## Quarantine, Prophylaxis, Parasites, and Disease

In the experience of the authors, a stringent quarantine process involving an aggressive antihelminthic prophylaxis is essential for long-term survival of captive batfishes. Most specimens of *Ogcocephalus spp.* come from the wild with an enormous parasite burden (Fig. 12B). Data will be presented in a forthcoming paper but initial estimates show that in some cases nematode biomass is nearly 25% of the total animal mass (Christie unpublished data). Aggressive force-feeding of fenbendazole (Panacur™) at 75-100mg/kg was employed by Schleser and Alvarado (1992) in their initial investigations into causes of failure to thrive; they reported the results of such deworming as “astonishing” compared to their prior experiences. In Schleser and Alvarado’s original paper they report that de-wormed specimens were alert and fed readily 24-48h after medication, and defecated copious quantities of dead nematodes, later identified as a new species, *Cucullanus palmerei* (Crites and Overstreet, 1997).

The quarantine regimen employed by the authors based on the work of Schleser and Alvarado (1992) and refined by our experiences calls for immediate force-feeding of fenbendazole at 100mg/kg via a French tip catheter and syringe as soon as the animals complete acclimation (Fig. 14C), following a 1-5 minute freshwater dip to kill leeches, ciliates, and monogenes. After forced antihelminthics the animals are also given forced nutrition in the form of a high calorie protein-based gruel with glucose, electrolytes, and B-complex vitamins. Fenbendazole administration is repeated at least twice more every other day or until the animals stop expelling worms (usually after 4-5 rounds of treatment). Very small specimens have been successfully de-wormed by administering fenbendazole gut-loaded in *Artemia* or other feeder shrimps. Such use of *Artemia* bioencapsulation has been shown to be effective at delivering sufficient quantities of medication to diminutive fishes (Allender *et al.*, 2012).

The use of this amount of fenbendazole is extreme, and it should be noted that fenbendazole has been implicated in toxicosis in some species of sharks and rays (Myers *et al.*, 2007), as well as other lower vertebrates including reptiles (Alvarado *et al.*, 2001; Neiffer *et al.*, 2005), and birds (Howard *et al.*, 2002; Bonar *et al.*, 2003). This being said, dozens of animals in Dallas have been treated with this extreme fenbendazole regimen, and no mortality or abnormal behavior have been seen, nor have lesions characteristic of fenbendazole toxicosis (e.g. GI ulcers, crypt cell necrosis) in deceased specimens. During this time period the specimens are kept under a 2mg/l (each) bath of nitrofurazone and furazolidone to combat skin lesions, they then receive a 50mg/ 3h bath of praziquantel to combat cestodes, and 21 days of chloroquine diphopshate (10mg/l) and praziquantel (2mg/l) to counter ciliates and monogenes, respectively.

The exclusive use of fenbendazole as an antihelminthic may not be strictly necessary with these animals; the authors have experimentally treated a specimen of *O. cubifrons* with pyrantel pamoate at 10mg/kg PO and waterborne milbemycin oxime at 0.5-1.0mg/l 3h with no ill effects on the animal observed (though as this animal had already been de-wormed the efficacy against batfish-specific parasites cannot be confirmed). Other facilities have also reported successful killing of endoparasitic nematodes with levamisole baths between 1.0-1.3mg/l for 24h (Rawlings, pers. comm.). Ivermectin and mebendazole may also be useful alternatives to fenbendazole, but have yet to be tried with these fishes. Also, the authors have force fed up to 250mg of psyllium fiber (Metamucil™) with no apparent detriment, a practice listed in Carpenter (2013) as being helpful in flushing large amounts of worms from the GI tract of heavily-parasitized birds.

A total of eight individual species of nematodes have been identified from five batfish species, but these fishes play host to other parasitic taxa the aquarist should be wary of (see Table 2 for a complete listing). Tapeworms (Platyhelminthes: Cestoda), as both adults and pleurocercoid larvae are known from several species (Schleser and Alvarado, 1992; Guyalev, 1997; NHML, 2015; USNPC 2015). A variety of trematodes (Platyhelminthes: Trematoda) are also known from the batfishes (14 spp. from 6 hosts), including a broad diversity (8spp.) from *Halieutaea stellata*. Both of the abovementioned taxa can be easily controlled with oral administration of praziquantel or a high concentration/short duration bath (e.g. 50mg/l, 3h) of the same.

In addition to the GI parasitofauna several ectoparasites are known, including leeches (Hirudinea) and the fish louse Argulus varians (Branchiura). Copepods have been noted on several species at multiple institutions (Fig. 15F), and have been effectively controlled with diflurobenzuron (0.03mg/l), formalin (25mg/l), and freshwater dips. It is very likely that the copepods present on animals are not strictly parasitic, but are opportunistic, and start to feed on integument and fins of batfishes once they become moribund. These opportunistic scavengers can rapidly multiply and exacerbate the symptoms of “batfish rot” (described below), having dramatic effects on moribund animals in <24h (see Fig. 15E).

A well-known paradigm of parasitology is that the most successful parasites do not kill their hosts, and indeed, batfishes survive in the wild with their enormous parasite burdens. It seems paradoxical, then, that ogcocephalids would decline thus; though captivity is inherently different from nature, and it seems that the additional stress of collection and transport upsets this delicate balance. Quinn *et al.* (2011) state that (in general) heavy parasite burdens predispose animals to acquired immunodeficiency; and Colditz (2008) broadly lays out six major effects of gastrointestinal nematodes on vertebrate immunity including increased metabolism, reduced nutrient availability, altered nutrient utilization, change in number/types of immune cells, and hyper- or hypoactive immune response. While the exact physiological mechanisms underlying decline in batfish health after collection will require much more study to elucidate, it is reasonable to assume that the extreme parasite burden coupled with the acute stress of collection and transport exacerbate some or all of the abovementioned effects precipitating disease and death in the animal.

Schleser and Alvarado termed a curious condition seen in ogcocephalids by many public aquaria and home aquarists alike as “batfish rot” in their 1992 paper. They characterize the condition as an integumentary eroision of the rostrum which may lead to the sloughing of the entire rostral process if it becomes necrotic, the eyes cloud and swell, and secondary areas of skin erosion and fin rot follow (Schleser and Alvarado, 1992). Abrasion of the rostrum and the anterior margin of the disc is a common problem, and often leads to secondary infection and severe dermatitis as the lesions are rapidly colonized by bacteria and protozoa.

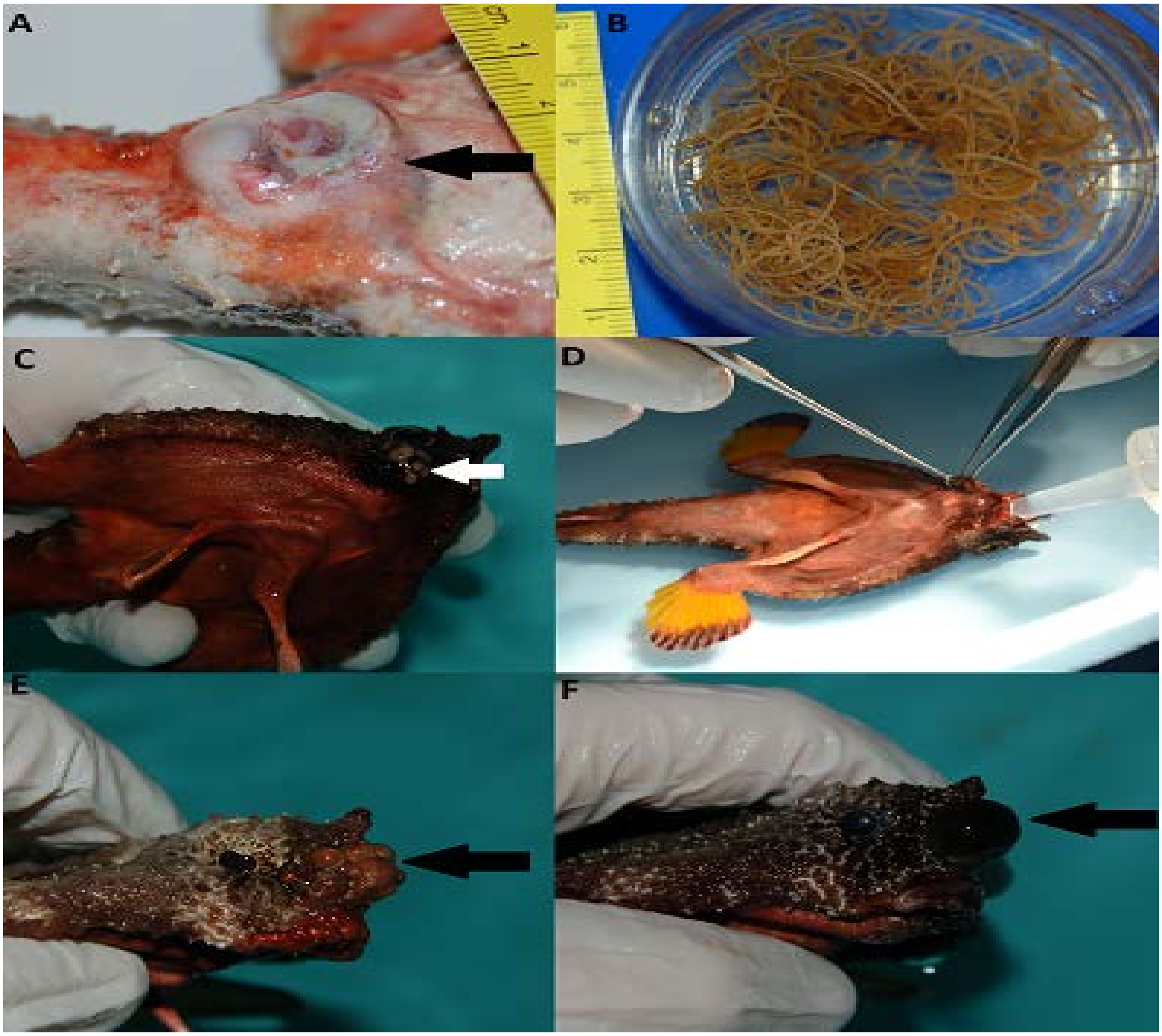


Figure 12: Medical issues common in *Ogcocephalus spp*. (A) prolapsed rectum and inflamed cloaca (B) nematodes (cf. *Hysterothylacium spp*.) expelled during quarantine from a single 90g batfish (C-D) recurring jaw lesion originating from *Mycobacterium* infection and surgical debridement of same under anesthesia with MS-222 (E) lymphocystis lesions of the lure and lips (F) non-specific swelling of the skin over the lure (up to 27cc of fluid week$^{-1}$ was drained from this lesion). Photos B.L.Christie.

Table 2: Parasites of the Ogcocephalidae.

| Parasite | Host | Group | Reference |
| --- | --- | --- | --- |
| Roundworms (Nematoda) | | | |
| *Cucullanus palmeri* | *Ogcocephalus nasutus* | Seuratoidea | Crites and Overstreet, 1997 |
| *Hysterothylacium ogcocephali* | *Ogcocephalus cubrifrons* | Ascaroidoiea | Olsen, 1952; Bruce, 1994 |
| | *Ogcocephalus radiatus* | | Olsen, 1952; Bruce, 1994 |
| | *Ogcocephalus nasutus* | | Crites and Overstreet, 1997 |
| *Hysterothylacium reliquens* | *Ogcocpehalus cubifrons* | Ascaroidoiea | Norris and Overstreet, 1975 |
| | *Ogcocephalus pantostictus* | | Norris and Overstreet, 1975 |
| *Ortoanisakis halieutaeae* | *Halieutaea stellata* | Ascaroidoiea | Bruce, 1994 |
| *Raphidascaroides halieutaeae* | *Halieutaea stellata* | Ascaroidoiea | NHML, 2015 |
| *Raphidascaroides nipponensis* | *Halieutaea stellata* | Ascaroidoiea | Moravec, 2000 |
| *"Ascaris sp." Hysterothylacium?* | *Ogcocephalus vespertilio* | Ascaroidoiea | USNPC, 2015 |
| | *Ogcocephalus radiatus* | | USNPC, 2015 |
| *Stenurus sp.* | *Ogcocpehalus nasutus* | Strongylida | Schleser and Alvarado, 1992 |
| Trematodea (Platyhelminthes: Trematoda) | | | |
| *Hemiperina nicolli* | *Dibranchus atlanticus* | Derogenidae | NHML, 2015 |
| *Derogenes varicus* | *Halieutaea stellata* | Derogenidae | NHML, 2015 |
| *Lomasoma wardi* | *Halieutaea stellata* | Fellodistomidae | NHML, 2015 |
| *Lintonium sp.* | *Ogcocephalus vespertilio* | Fellodistomidae | USNPC, 2015 |
| *Adinosoma robusta* | *Halieutaea stellata* | Hemiuridae | NHML, 2015 |
| *Aponurus halieutae* | *Halieutaea stellata* | Hemiuridae | NHML, 2015 |
| *Aponurus pyriformis* | *Ogcocephalus radiatus* | Hemiuridae | Gomez, 1996 |
| *Lecithochirium kawalea* | *Halieutaea stellata* | Hemiuridae | NHML, 2015 |
| *Lecithochirium magnus* | *Halieutaea stellata* | Hemiuridae | Bray, 1991 |
| *Separogermiductus magnus* | *Halieutaea stellata* | Hemiuridae | NHML, 2015 |
| *Sterrhurus floridensis* | *Ogcocephalus radiatus* | Hemiuridae | Manter, 2015 |
| *Sterrhurus microvatus* | *Halieutaea stellata* | Hemiuridae | NHML, 2015 |
| *Lepidapedoides nicollo* | *Halieutichthys aculeatus* | Lepocreadiidae | Bray, 1996 |
| *Clonorchis sinensis* | *Malthopsis luteus* | Opisthorchiidae | NHML, 2015 |
| Tapeworms (Platyhelminthes: Cestoda) | | | |
| *Echeneibothrium sp.* | *Halieutaea stellata* | Phyllobothridae | Gulyaev, 1997 |
| *"Scolex pleuronectis"* | *Halieutaea stellata* | *incerta sedis* | NHML, 2015 |
| Cestoda unspecified | *Ogcocephalus nasutus* | | Schleser and Alvarado, 1992 |
| Cestoda unspecified | *Ogcocephalus vespertilio* | | USNPC, 2015 |
| Ectoparasite Fauna (Crustacea & Hirudinea) | | | |
| *Argulus varians* | *Ogcocephalus nasutus* | Branchiura | Poly, 2009 |
| Copepoda spp. Unknown | *Ogcocpehlus cubifrons* | Copepoda | Christie, unpub. data |
| | *Ogcocphalus radiatus* | Copepoda | |
| | *Ogcocephalus corniger* | Copepoda | |
| *Platybdella sp.* | *Ogcocephalus darwini* | Hirudinea | Manter, 2015 |

Table 3: A Batfish Pharmacopeia: Drugs and Chemicals used with Ogcocephalid Fishes.

| Drug | Dosage | Duration | Efficacy | Tolerance | Notes |
|---|---|---|---|---|---|
| Antibiotics | | | | | |
| Ceftazidime | 20-33mg/kg IM | q3d 14d | + | + | |
| Oxolinic Acid | 2mg/l | 7-14d | + | + | |
| Oxolinic Acid | 25-100mg/l | 7d- b.i.d. 30m | + | + | |
| Oxolinic Acid | 100mg/l | 24h | + | + | |
| Nitrofurazone | 2mg/l | 5-14d | + | + | |
| Nitrofurazone | 10-25mg/l | 24-72h | + | + | |
| Furazolidone | 2mg/l | 5-14d | + | + | |
| Furazolidone | 10-25mg/l | 24-72h | + | + | |
| Kanamycin | 100mg/l | e.o.d. 5-21d | + | + | |
| Nalidixic Acid | 13mg/l | 6h | + | + | |
| Oxytetracycline | 25-50mg/l | 7-10d | + | + | |
| Enrofloxacin | 10mg/kg IM/IP | q3d/e.o.d. | + | + | |
| Chloramphenicol | 5.25mg/l | 5d | + | + | Florfenicol is safer alternative |
| | | | | | |
| Antifungals | | | | | |
| Itraconazole | 5mg/kg PO | 14d | + | + | |
| | | | | | |
| Antihelminthics/Antiparasitics | | | | | |
| Praziquantel | 2-5mg/l | 14-21d | + | + | Ectoparasite Bath |
| Praziquantel | 50mg/l | 3h | + | + | Endoparasite Dip |
| Fenbendazole | 25-100mg/kg PO | e.o.d. 3-9x | + | + | Nematode Reduction |
| Fenbendazole | PO* | 3-9x | + | + | *In gut-loaded feeder shrimp |
| Levamisole | 1.0-1.3mg/l | 4h | + | + | |
| Pyrantel Pamoate | 50mg/kg PO | once | ? | + | |
| Hyposalinity | 0‰ 1-10min | dip | + | + | |
| Hyposalinity | <15‰ | 3-5d | - | + | |
| Hyposalinity | <15‰ | 5d+ | - | - | Tolerated poorly |
| Diflurobenzuron | 0.03mg/l | 30d | + | + | |
| Chloroquine | 10-21mg/l | 21d | + | + | |
| Formalin | 25mg/l | e.o.d. 3x | + | + | |
| Formalin | 250mg/l | once | + | + | |
| Copper Sulfate | 0.15-0.25mg/l | 14-21d | +/- | - | Poorly tolerated |
| Cupramine | 0.25-0.50mg/l | 14-21d | +/- | +/- | Tolerated better than ionic Cu |
| Milbemycin Oxime | 0.5-1.0mg/l | 3h | ? | + | |
| Metronidazole | PO* | 5x | + | + | *In gut loaded feeder shrimp |
| | | | | | |
| Anesthesia/Miscellaneous | | | | | |
| MS-222 | 25-100 mg/l | | + | + | Anesthetic |
| Meloxicam | 2mg/kg IM | | + | + | NSAID antinflammatory |
| Aspirin | 1.7-3.4mg/l | 6h | + | + | NSAID antinflammatory |
| Regranex | Topical | | + | + | Growth Factor |
| Psyllium (Metamucil) | 250mg PO | | ? | + | Aid in nematode expulsion |

The authors have observed that the secondary spots of skin erosion that Schleser and Alvarado describe typically form at sites where the calcified tubercles and bucklers (modified scales) detach (Fig. 15A), erosion and infection then radiate outwards. This initial damage to the rostrum and loss of tubercles/bucklers from the leading edge of the disc are almost always precipitated by rubbing against the glass or acrylic viewing panel. Schleser and Alvarado (1992) found this could largely be prevented through the use of visual barriers when the animals must be kept in a transparent enclosure. Early reports used a solid black barrier, though the authors have found that a cross-hatch pattern of electrical tape works equally well and allows the aquarist to better observe the animals (Fig. 14B), though a shallow tray with opaque sides is ideal for quarantine/holding of these species (see Fig. 14). Once rostral abrasion and/or patches of integumentary erosion are noted medical intervention is warranted and aggressive antibiotic treatment may prevent death. A summary of the antibiotics used successfully in batfishes is presented in Table 3. The swelling of the eyes seen during such infections has been successfully reduced with injectable NSAIDs (meloxicam) in *Ogcocephalus spp.* and specimens of *Halieutichthys aculeatus* have also seen improvement following baths in Aspirin™ (1.7-3.4 mg/l up to 6h) as advocated by Herwig (1979).

**Cryptic Presentation of *Cryptocaryon irritans***

*Cryptocaryon irritans* is a ubiquitous parasite of marine fishes, where it is usually easily detected and treated. Often the tell-tale white spots allow for gross identification, and even in cases where it does not present as such a skin scraping is usually all that is needed for diagnostic confirmation. In the ogcocephalids *Cryptocaryon* presents cryptically. White spots are never apparent, and even scrapings of the skin will rarely turn up the parasite as it is concentrated on the gills, eyes, cloaca, and throughout the buccal cavity attacking the softer mucous membranes. It is our hypothesis that the skin, normally devoid of mucous, and very thick and covered with calcified and keratinized structures, does not offer a suitable environment for the parasite as in other teleosts. Careful observation of the eyes in batfishes is required to diagnose ciliate infections before it is too late, the slightest clouding of one or both eyes should prompt immediate action (Fig. 13C&D). Scrapings may be directly taken from the cornea by (very gently) using the back of a scalpel blade (see Fig. 13B) or with dental floss as described by Leitner and Christie (2006). The use of copper with these fishes is problematic; it has been used without mortality in several facilities but the animals often respire heavily, appear listless, and sometimes secrete a heavy mucous coating. The authors have found this latter symptom of heavy metal exposure to be particularly counterproductive, as the mucous coat can enclose the ciliates and shield them from the waterborne chemotherapeutics. That being said, if copper must be used EDTA-chelated copper products such as Cupramine™ seem to be better tolerated by batfishes than ionic copper formulations or citrate-chelated copper solutions. Chloroquine diphosphate and formalin are both well-tolerated by ogcocephalids and may be used to counter *Cryptocaryon* outbreaks without apparent irritation or detriment to the animal. The authors have found that outbreaks in established (i.e. already quarantined) animals are easily precipitated by cold stress (in *Ogcocephalus* and *Halieutaea*), and in many cases may be avoided by fastidiously maintaining the animals at the warmer end of their temperature ranges.

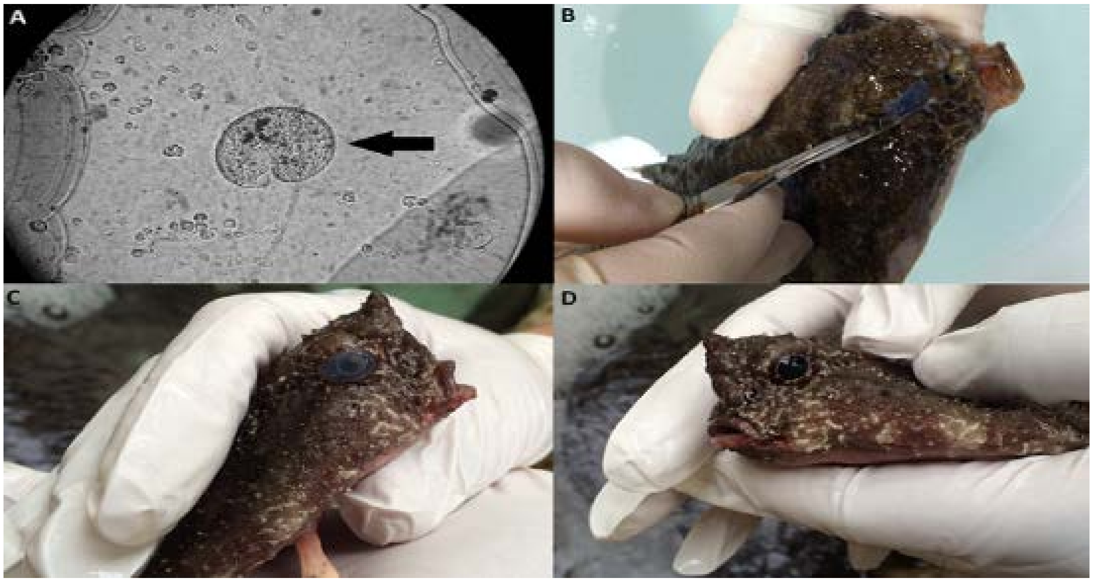

Figure 13: Cryptic presentation of *Cryptocaryon irritans*, (A) living ciliate (100x), from eye scrape (B) technique for very gently scraping cornea (C) a cloudy eye, compared to normal (D). Cloudy eyes are the only gross symptom the aquarist will see and immediate attention is required. Photos B. Christie.

**Diseases and Veterinary Care**

Apart from outbreaks of *Cryptocaryon* the most commonly seen disease presentation in captive ogcocephalids (once acclimated to captivity) is fin rot and patches of erosion on the skin. As with many other fishes, fin rot in batfishes seems to be tied to poor water quality, though such conditions are rare in public aquaria. Fin rot most commonly affects the caudal and pectoral fins, and may be severe or minor as seen in Fig. 15B&C. Minor fin rot, where the fins retain most of the webbing between the rays, can be halted with antibiotic baths in the authors' experience, and the human growth factor Regranex™ has been employed to good effect at regenerating this lost tissue in *Ogcocephalus* (Hudec, pers. comm.). Major fin rot, characterized by a total loss of the webbing leaving only partial fin rays, can usually not be reversed, but the animal's life may still be saved with antibiotic therapy, and given their mode of locomotion the loss of a caudal or pectoral fin is usually not a tremendous disadvantage. Skin lesions also occur from time to time (as seen in Fig. 15A), and may be similarly treated with antibiotics, though in these cases the aquarist should closely observe the lesion daily for opportunistic copepods and take steps to control them if present.

Lesions of the jaw and face have also been seen in ogcocephalids in captivity, including outbreaks of lymphocystis, a virus which has also been documented afflicting fishes it the wild (Lowler and Ogle, 1977). The authors have encountered several batfishes with cysts or open lesions at the corner of the mouth (see Fig. 12E). Bacterial culture and sensitivity testing is recommended to find the appropriate chemotherapeutic as such infections have, in some cases, been caused by *Vibrio spp*. which responded to antibiotics variably. These lesions have also resulted from *Mycobacterium spp.* which proved untreatable except by surgical debridement of the lesion and management of opportunistic bacterial and fungal infections (see Fig. 12C&D).

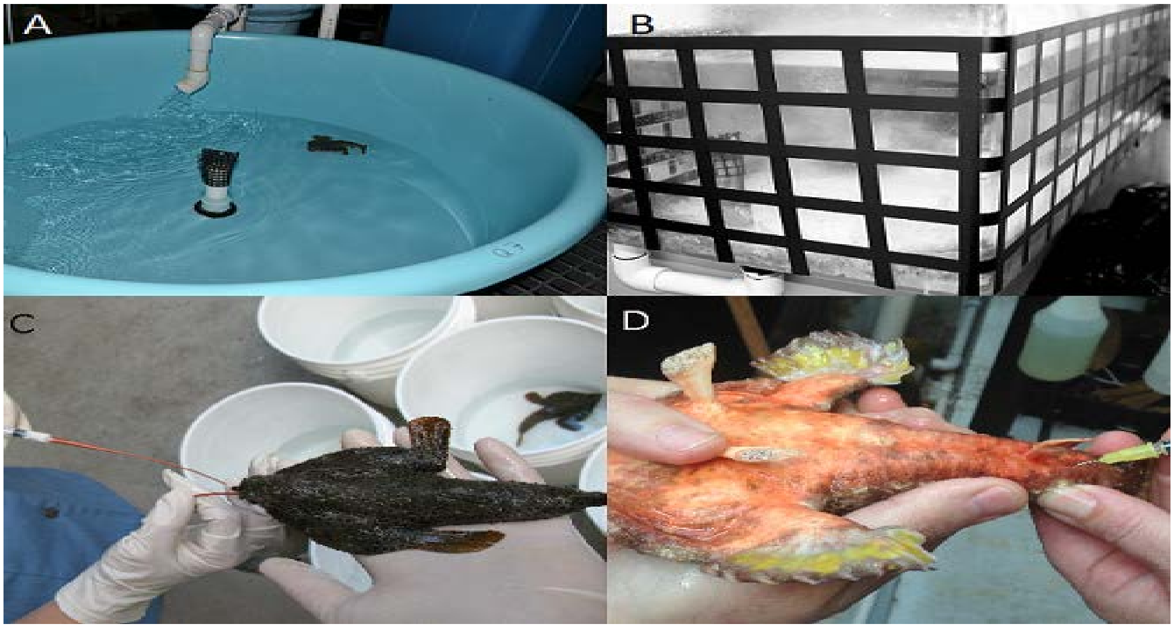


Figure 14; Quarantine of batfishes (A) ideal quarantine/holding setup, a shallow tray with opaque walls that slant outward as they rise (B) clear acrylic tank with visual barriers (C) force-feeding deworming medication with syringe and catheter (no.10 Fr.) (D) caudal venipuncture. Photos (A, B, C) B. Christie (D) L. Torres.

Swellings of the skin over the esca and illicium have also been seen (Fig. 12F), which were never linked to any specific pathogen and were deemed nonspecific inflammation from histopathology reports and responded to NSAID treatment and aspiration of the fluid-filled sac e.o.d. with a small needle and syringe. In one case of such swelling up to 27cc of fluid per week was drained from a lesion on a small 400g specimen of *O. cubifrons*!

Batfishes will occasionally be observed with a prolapsed lower intestine (see Fig. 12A). This seems to be a normal occurrence given the size of the prey they regularly consume in the wild (see diet section above). The condition can generally be avoided by abstaining from feeding large meals to captive specimens, though when this occurs it generally resolves itself within 72h and does not need medical intervention. If a portion of the rectum/lower GI is still visible after several days a veterinarian can manually reinsert the tissue and place a suture to hold it in place as it heals.

**Hematology**

Normal hematological parameters, and even the optimal site for drawing blood from ogcocephalids has yet to be determined. Certainly only the largest ogcocephalid specimens are even candidates for a blood draw (several *Ogcocephalus spp.* and perhaps *Dribranchus atlanticus* and *Halieutaea stellata*), and even then the quantity that may be safely taken is miniscule. The veterinary team at the Dallas Zoo have attempted to draw blood from three large *O.cubifrons* via caudal venipuncture with a 31 gauge insulin needle and a 27 gauge standard hypodermic needle to gain baseline data for this work (see Fig. 14D), though none produced enough for serum chemistry and only one had sufficient volume for cell counts. *Ergo*, the following data is from a single specimen, of a single species, and as such the reader is advised to interpret this data *cum grano salis* until more complete data is available: HCT=25%, WBC= 8.8k, with Heterophils 1672µl$^{-1}$(19%), Lymphocyte 7128 µl$^{-1}$(81%), and Monocytes, Eosinophils, Basophils all=0% (IDEXX Laboratories, Avian/Exotic CBC panel Accession no. D5631915).

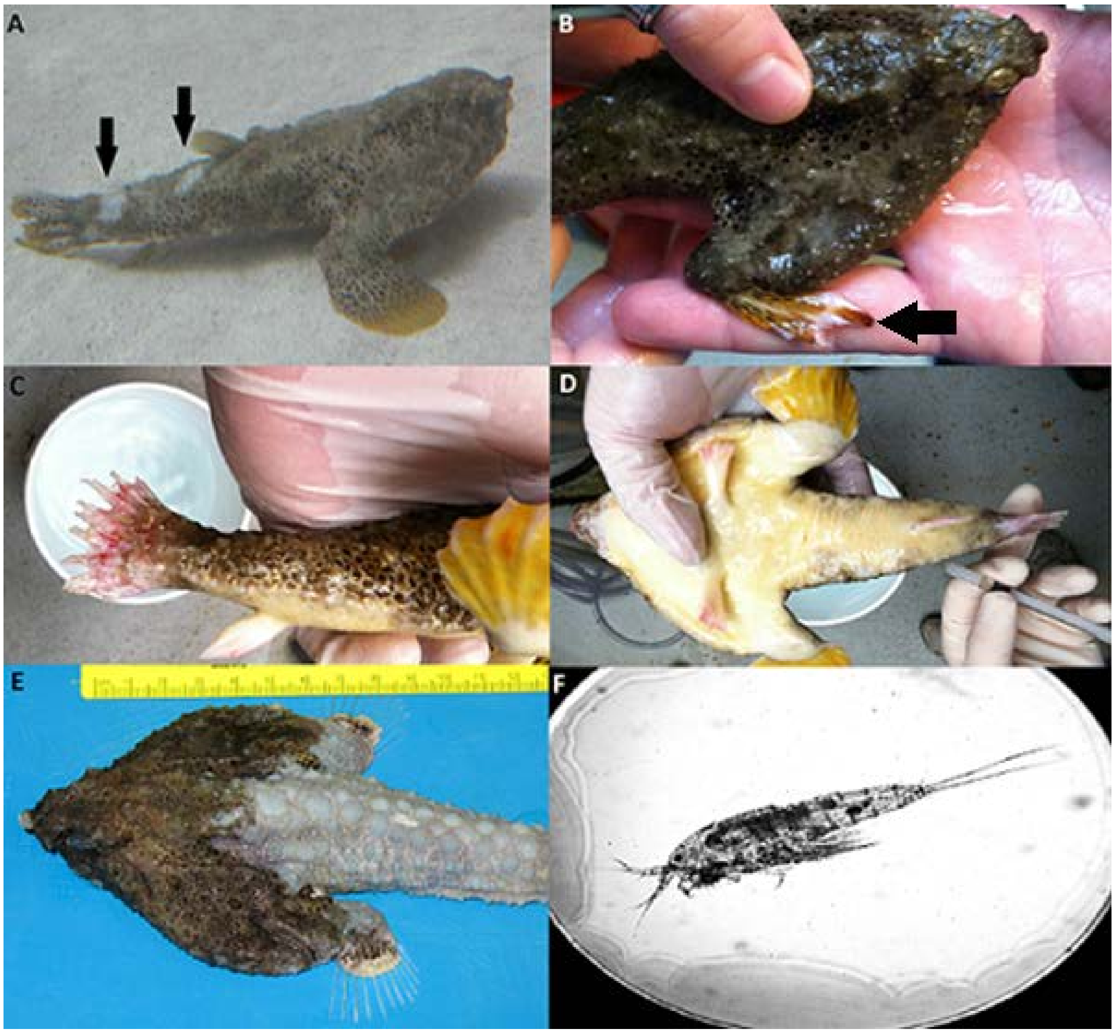


Figure 15: Additional common medical issues in batfishes (A) skin erosion, which is easily addressed with antibiotic baths (B) minor fin rot, may be addressed with antibiotics and/or growth factors (C) severe fin rot, fins this badly damaged rarely regenerate, though the animal may be saved (D) IM injection site for batfishes (E) damage caused in <24 hours by unchecked opportunistic copepods (F) on an already moribund specimen. Photos (A, B) P.Z. Montoya (C-F) B. Christie.

## Reproduction

We know precious little of the reproductive habits of batfishes. When Charles Breder of the American Museum of National History compiled his 1961 masterwork cataloging fish reproduction, the family Ogcocephalidae merited but a single sentence:

> "*Nothing is known of the reproductive habits of these fishes except that they possess scroll like ovaries similar to Histrio, according to Rasquin.*"

One would think that in the following half century a greater amount of knowledge would have been amassed on the reproductive biology of batfishes, but we still know surprisingly little. Only four known captive spawning events have been observed to the knowledge of the authors, one in *O. nasutus* and three in *O. cubifrons,* both at the Fair Park aquarium in Dallas. These events confirmed that the batfishes do indeed produce egg rafts (veils) similar to the frogfishes, though the eggs seem much larger (see below). Consequentially fecundity is likely much lower for these fishes than for antennarid fishes such as *Histrio* which may produce hundreds of thousands of eggs (Christie, 2006).

Richards and Bradbury (1999) give the only known account of ogcocephalid postlarvae describing the morphology of *H. aculeatus* and *O. parvus* from wild-collected specimens (Fig. 16). Even as larval fishes these two species appear well-armored and interestingly enough, nearly globose instead of dorsoventrally flattened (Richards and Bradbury, 1999). That report also gives some clues to the seasonality of reproduction, noting that Caribbean *O. parvus* likely spawned January to April and GOM populations from April to August; *H. aculeatus* spawned May to July in Florida (Richards and Bradbury, 1999). The bright red ventral and lip coloration of sexually mature males was also noted by Richards and Bradbury (1999) and has been observed in captivity by the authors for multiple batfishes.

*Egg production in captive O. cubifrons*

Three complete (and one partial) mucoid egg rafts were produced by *O. cubifrons* between May and June of 2015, all laid between five and 29 days of each other (with the exclusion of the partial raft). No unusual behavior of the batfish was observed by the authors prior to spawning, including signs of courting, decrease in diet consumption, or physical changes (coloration/size). All rafts were also released overnight while tankmen were not present, thus it cannot be determined which female(s) produced the eggs. Following each spawn, the rafts were found floating near the surface of the water column; the water flow pushed them around gently but they remained intact and never came in contact with the exhibit's sandy bottom.

The sturdy jelly framework around the eggs did not appear to have a defined shape to it (Fig. 16), unlike the scrolled rafts of *Histrio histrio* or ballon-shaped rafts of *Antennarius nummifer*, other members of the order Lophiiformes whose spawning habits are better-known. Each raft contained a couple thousand eggs, each of which contained one or two oil droplets (more specific data on fecundity and egg size to be presented in a forthcoming paper). The authors were able to confirm development within the eggs, but none appeared to hatch. Within a few days, the mucoid structure had either mostly or completely deteriorated, in correlation with the egg masses produced by both *H. histrio* and *A. nummifer* (Ray 1961; Mosher 1954). Water quality was recorded regularly during reproduction, starting the day before first sighting of an egg raft and concluding the day the last raft degraded, and parameters were normal ($NH_3$=0mg/l, $NO_2$=0.014mg/l, pH=8.25, and salinity=32.5‰). It may also be worth noting that two of the egg-laying events were preceded 12-48 hours by heavy tank maintenance which involved turning off LSS pumps for 10-15 minutes with heaving scrubbing of algae which clouded the tank water significantly. It is possible that this agitation may have encouraged spawning.

Figure 16: Egg raft of *O. cubifrons* (Left, Middle) and morphology of larval batfishes (Right) from Richards and Bradbury (1999). Left two photos L. Torres & B. Christie, illustrations from NOAA (public domain).

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# EFFECTS OF AQUARIUM-BATH PHARMACEUTICALS ON SPECTROPHOTOMETRIC WATER-QUALITY TESTS

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## Introduction

The aquarium industry uses various therapeutic bath treatments both prophylactically and in response to disease. Some treatments must run for extended times and therefore overlap with scheduled and responsive water-quality testing. The industry relies on various analyses to quantify several husbandry-relevant substances (ammonia, nitrite, etc.) in the system water they care for. Many facilities take advantage of spectrophotometric tests to get precise, accurate values of these substances. These tests usually require mixing a sample of water with a reagent (or reagents) designed to interact with a specific substrate and measurably change the light-absorbance properties of the sample itself (Hach, 2014).

It is not uncommon to have systems undergoing therapeutic bath treatments while simultaneously struggling with water-quality issues; this can be especially true during quarantine and medical isolations. These treatments sometimes impart visible colors or turbidity to the aquarium water or change its chemical properties, potentially affecting water chemistry test values. Absorbance curves (without reagent) have been validated for chloroquine, metronidazole, and trimethoprim with sulfathiazole sodium, in seawater with a UV-Vis spectrophotometer (Hach® DR5000) (Marrero *et al.*, 2015).

Each spectrophotometric test procedure (Hach, 2014) includes a list of substances known to cause interference with that specific test, however these lists are not exhaustive. Heinen *et al.* (1995) reported formalin added at 100 mg/L (as 37% formaldehyde) induced a false-negative interference with the salicylate ammonia test (Hach® method 8155) in freshwater samples, but formaldehyde is absent from the Hach® interference list for that test (Hach, 2014).

This study presents preliminary findings on whether therapeutic concentrations of several drugs commonly used in aquarium bath treatments cause significant effects on spectrophotometric tests targeting ammonia, nitrite, nitrate, and copper concentrations.

## Methods

For each treatment studied, 1L of freshwater (0‰) and 1L of artificial seawater (30‰, Instant Ocean™) each had a target concentration of one test substrate (i.e. ammonia, nitrite, nitrate, or copper) added from stock solutions at 25°C. Three samples were pulled from each 1L batch to test the control level of target substrate, then a bath-treatment drug at a high end target concentration (also from stock solution) was added to each batch. Three more samples were pulled from each treated 1L batch for testing, then the batches were discarded before the next set of substrate tests was prepared. All glassware used was acid washed with 6.1 M HCl acid and water and rinsed, and disposable plastic components were replaced between uses to reduce the chance of cross contamination of treatments and substrates.

The substrate stock solutions were each made with 1L of reverse-osmosis water and one of the following substrates (as anhydrous salts): ammonium chloride, sodium nitrite, sodium nitrate, or a combination of copper sulfate and citric acid. Target concentrations of substrate in the 1L batches were 0.20 mg/L ammonia (as $NH_3$), 0.200 mg/L nitrite (as $NO_2^-$), 20 mg/L nitrate (as $NO_3^-$), and 0.50 mg/L copper ($Cu^{2+}$). These concentrations were chosen (rather arbitrarily) as control values because they are well within the measurable ranges of the respective methods used to test for them. A controlled initial concentration of substrate was necessary to determine any positive (exaggerating) or negative (masking) effects from the treatments.

Each treatment stock solution was made with 1L of reverse-osmosis water and one of the tested drugs. Target concentrations of each treatment (except those described below) added to the 1L batches were chosen on the basis of the highest recommended prolonged immersion bath concentrations listed in Noga (2010). The few exceptions to this were nitrofurazone, which was tested at 10 mg/L rather than the listed 2 mg/L because the higher concentration is frequently used as a medically responsive measure; and Amquel™ and AmquelPlus™, which were added at 3 mg/L each, which is the manufacturer's recommended treatment response to the target levels of ammonia and nitrite used for the study (Kordan, LLC).

Samples were processed according to the substrate that was added and tested for. Hach® guidelines and reagents were used; method 8155 (Nitrogen, ammonia salycilate) for ammonia, method 8507 (NitriVer3™) for nitrite, method 10206 (TNT836™) for nitrate, and method 8506 (CuVer2™) for copper (Hach, 2014). Each of the 16 treatments was tested against each of the four substrates in freshwater and seawater, and each batch was sampled six times (three untreated, three treated). Batches were mixed on a stir plate for a minimum of 60 seconds to promote homogeneity before being sampled. Each sample was read three times on a Hach® DR 2800 Spectrophotometer. Values of nitrogenous compounds read on the spectrophotometer were displayed and recorded as $NH_3$ for ammonia, $NO_2^-$ for nitrite and $NO_3^-$ for nitrate.

Two-tailed heteroscedastic T-tests and average percent differences were determined on untreated against treated samples.

## Results and Discussion

Interferences found in these spectrophotometric tests resulted from some alteration of the absorbance properties of the samples. Such interferences may have been caused by the treatment interacting with the test reagents (altering the activity of the reagents), the treatment interacting with the substrate (altering the activity of the substrate), the treatment affecting the absorbance of the sample due to innate properties (altering color and/or turbidity), or the treatment introducing extra substrate into the sample (as remnants of manufacturing, or through degradation in water). None of these interferences are exclusive of the others, making combinations of these reasons possible within any single sample.

T-tests run on untreated samples against treated samples showed statistically significant differences ($\alpha < 0.05$) between a batch's samples before and after addition of a treatment (Table 1) in a majority (65%) of the tests run. This chart may appear startling in the sheer number of significantly differing results; however, this may be due in part to the low total number of data points available for each t-test (nine untreated and nine treated sample values each), and in part to the high accuracy and low variance of the tests themselves. For example, while the t-test result for difference after the addition of Amquel™ in a nitrite test on seawater was statistically significant, the percent difference (Table 2) for that particular test was <1% (absolute value) of the average readings between the two. A greater number of data points may have helped to better round out the data sets, increase standard deviation, and reduce the number of low t-test results and corresponding alpha values, but would also drive up experiment cost and time commitment (and ain't nobody got time fo' dat).

Table 1: T-Test alpha values comparing treated against untreated batch-sample readings. The red highlighted values fall below the 0.05 level of significance, indicating those tests were significantly different after the listed treatment was added. Seawater nitrate test interference results may be compounded by the innate interference of some component of seawater. Hach® method 10206 for nitrate is approved "For wastewater, drinking water, surface water, and process water" (Hach, 2014), but not seawater.

| T-Test alphas (<0.05 = Statistically significant) | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | FW $NH_3$ | SW $NH_3$ | FW $NO_2$ | SW $NO_2$ | FW $NO_3$ | SW $NO_3$ | FW $Cu^{2+}$ | SW $Cu^{2+}$ |
| Nitrofurazone (10 mg/L) | 2.97E-05 | 0.164827 | 0.000232 | 1.96E-06 | 3.33E-07 | 9.56E-09 | 1 | 3.93E-05 |
| Furazolidone (10 mg/L) | 0.187298 | 0.190645 | 0.000695 | 0.005472 | 0.923858 | 0.057504 | 0.005942 | 0.001061 |
| Kanamycin (100 mg/L) | 0.001912 | 8.29E-10 | 3.23E-05 | 0.004845 | 0.406067 | 0.518897 | 0.205035 | 0.267016 |
| Oxolinic acid (200 mg/L) | 0.002411 | 0.128445 | 1.04E-10 | 1.15E-09 | 4.12E-10 | 1.1E-10 | 1.29E-13 | 7.92E-15 |
| Nalidixic acid (13 mg/L) | 0.012109 | 0.071447 | 0.000401 | 0.495575 | 0.969675 | 0.122565 | 2.38E-05 | 2.79E-05 |
| Oxytetracycline (100 mg/L) | 0.028477 | 0.000432 | 2.95E-19 | 5.55E-18 | 1.86E-11 | 3.04E-10 | 0.018651 | 0.000736 |
| Praziquantel (2 mg/L) | 0.005471 | 0.950294 | 0.014457 | 0.207828 | 0.101578 | 0.02668 | 0.001557 | 0.229373 |
| Formalin (25 mg/L) | 5.16E-15 | 1.38E-10 | 0.0198 | 0.216895 | 0.000866 | 3.85E-05 | 0.035693 | 2.94E-05 |
| Malachite green (0.1 mg/L) | 3.24E-08 | 0.00073 | 0.019537 | 6.11E-12 | 0.791375 | 3.47E-07 | 0.346594 | 0.010471 |
| Methylene blue (3 mg/L) | 8.54E-18 | 1.87E-24 | 4.41E-05 | 7.58E-21 | 0.278481 | 0.00185 | 0.652544 | 0.09067 |
| Cupramine (0.5 mg/L) | 0.080516 | 0.058844 | 1 | 3.91E-06 | 0.10962 | 0.910586 | 8.68E-29 | 9.31E-29 |
| Chloroquine (10 mg/L) | 2.81E-07 | 0.226857 | 0.429884 | 0.000271 | 0.00041 | 7.94E-10 | 0.035265 | 0.000979 |
| Trichlorfon (0.5 mg/L) | 0.136726 | 0.002556 | 2.26E-05 | 2.16E-07 | 0.064181 | 0.074269 | 0.080516 | 0.003833 |
| Metronidazole (25 mgL) | 0.000621 | 0.005406 | 0.000926 | 0.435502 | 0.035669 | 0.118638 | 4.37E-05 | 0.018651 |
| Amquel (3 mg/L) | 1.84E-10 | 0.00078 | 0.001694 | 0.040173 | 0.968832 | 0.855593 | 2.38E-05 | 1 |
| Amquel Plus (3 mg/L) | 4.71E-10 | 0.320209 | 4.09E-21 | 8.81E-14 | 0.152357 | 0.191221 | 0.000137 | 0.260242 |

Table 2 outlines the percent difference found between average readings taken from before and after addition of a batch treatment and may offer more readily applicable information to the aquarium industry. Differences <10% (For example: 0.01 mg/L ammonia one way or another) would not likely affect husbandry decisions made on the basis of test outcomes, and differences between 10 and 25% may not be relevant, depending on the situation. However, most aquarists would want to know whether their test results will be affected by interference ≥25%.

Table 2: Difference between the averages of the untreated against treated batch-sample readings. Green highlighted results are those with <10% (absolute value) difference in test readings after dosing with the listed treatment, and, as such, are probably of little concern to the aquarium industry. Those highlighted yellow are between 10 and 25% difference and may warrant a little more consideration. Those highlighted red are >25% (up to nearly 400%) and should be given serious attention during water-chemistry testing. Owing to the nature of the data, negative numbers indicate false positive readings (exaggeration) and positive numbers indicate false negative readings (masking effect). As noted previously, seawater nitrate test interference results may be compounded by the innate interference of some component of seawater because Hach® method 10206 for nitrate is not approved for seawater.

| Percent Mean Difference (+ = false absence, - = false presence) | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | FW $NH_3$ | SW $NH_3$ | FW $NO_2$ | SW $NO_2$ | FW $NO_3$ | SW $NO_3$ | FW $Cu^{2+}$ | SW $Cu^{2+}$ |
| Nitrofurazone (10 mg/L) | 7.786885 | 8.333333 | -1.83908 | 3.925845 | -30.7442 | -216.016 | 0 | -3.64683 |
| Furazolidone (10 mg/L) | -13.5135 | -12.8713 | 5.254042 | 2.9615 | 0.054348 | -7.88263 | 2.231237 | 1.541426 |
| Kanamycin (100 mg/L) | -187.912 | -152.857 | -3.87397 | -2.64576 | 1.605759 | 1.382269 | 0.722892 | 0.699301 |
| Oxolinic acid (200 mg/L) | 15.84158 | 11.86441 | -15.8889 | -66.0377 | -56.8309 | -347.927 | -50.348 | -27.027 |
| Nalidixic acid (13 mg/L) | 3.448276 | 11.38211 | -4.37094 | -0.1612 | 0.050736 | -5.1259 | 2.087683 | 2.291667 |
| Oxytetracycline (100 mg/L) | -10.2941 | -16.1765 | 37.51419 | 33.10271 | -28.5714 | -118.147 | -1.27551 | -1.7199 |
| Praziquantel (2 mg/L) | -18.5393 | -1.03093 | -1.67564 | -0.77484 | -1.31712 | -21.842 | -2.98507 | 0.91954 |
| Formalin (25 mg/L) | 88.13559 | 62.16216 | -1.34003 | 1.452394 | -2.28238 | -12.7707 | -1.20773 | 2.790698 |
| Malachite green (0.1 mg/L) | 15.57789 | 22.58065 | -1.32485 | 6.032819 | -0.31646 | -11.9987 | 0.236407 | 1.382488 |
| Methylene blue (3 mg/L) | -281.538 | -385.034 | -14.3472 | -17.2967 | -0.98684 | -15.9951 | -0.21231 | 0.627615 |
| Cupramine (0.5 mg/L) | -1.53846 | 4.608295 | 0 | 0.983284 | 1.669275 | -0.37696 | -101.577 | -96.9762 |
| Chloroquine (10 mg/L) | 7.981221 | 3.921569 | -0.52826 | 1.240695 | -5.31067 | -26.8913 | 0.956938 | 2.06422 |
| Trichlorfon (0.5 mg/L) | 3.240741 | 13.04348 | -3.29793 | -4.21622 | 1.736842 | -8.88959 | -0.71429 | 1.601831 |
| Metronidazole (25 mgL) | 4.225352 | 11.53846 | -3.23584 | -0.20576 | 2.793885 | -16.1161 | 1.619433 | 1.008065 |
| Amquel (3 mg/L) | 15.38462 | 9.210526 | 0.874126 | -0.94173 | 0.053591 | -0.49725 | 2.304147 | 0 |
| Amquel Plus (3 mg/L) | 14.87179 | 10 | 61.52113 | 49.49777 | 3.065917 | 5.901116 | 2.298851 | 1.128668 |

Kanamycin (100 mg/L) and methylene blue (3 mg/L) created strong positive interferences with ammonia tests (150 to 188% and 280 to 385% increases respectively). Formalin (25 mg/L), as previously described (Heinen *et al.*,1995), also created a strong negative interference (masking 60 to 90% of the pretreatment value) with ammonia tests. Interestingly, methylene blue caused a greater positive interference in seawater than in freshwater, by almost 100% of the actual ammonia (as $NH_3$) present in the sample (Figure 1). While Amquel™ and AmquelPlus™ (each at 3 mg/L) are advertised as binding to and inactivating ammonia (Kordon, LLC), the salicylate method appears to mostly free the ammonia ion from the aminomethanesulfonate molecule during analysis, giving fairly accurate readings (also as advertised) to within ≈15%.

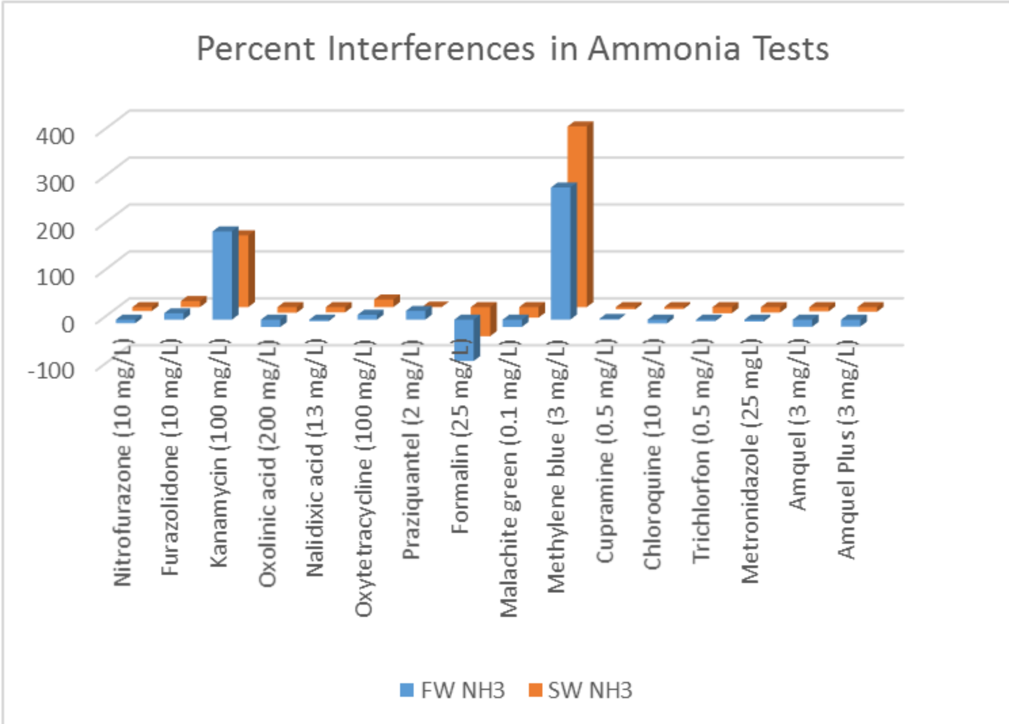


Figure 1: Interference levels in ammonia tests (as $NH_3$) by percent difference. Negative interference reaches a minimum of -100%.

When untreated seawater sample readings are compared against untreated freshwater sample readings across all ammonia batches (Table 3), it is interesting to note that ammonia in seawater was read at an average amount ≈30% lower than the same concentration of ammonia tested in freshwater with the Hach® method 8155 (salicylate method). This implies that the chemistry of artificial seawater acted to interfere with the ammonia test used. Kingsley *et al.* (2015) determined the magnesium and calcium ions of natural seawater are high enough to match their listed interference levels for Hach® method 8155, "which makes use of these reagents for low level detection of total ammonia in natural seawater extremely difficult if not impossible." (They go on to describe the application of a complexing reagent that was used to improve the performance of the Hach® salicylate method in natural seawater.) The levels of magnesium and calcium in artificial seawater may also reach similar levels (Eric Kingsley, personal communication, January 21, 2015) and help explain the results of this comparison.

Table 3: T-Test alpha values and difference of the averages of untreated seawater against untreated freshwater sample readings for the four series of substrates tested.

| T-Test alphas (<0.05 = Statistically significant) | | | | |
|---|---|---|---|---|
| | NH3 | NO2 | NO3 | Cu |
| Freshwater vs. Seawater | 1.54E-04 | 2.25E-03 | 2.11E-22 | 0.26 |
| Percent Mean Difference | | | | |
| | NH3 | NO2 | NO3 | Cu |
| Freshwater vs. Seawater | 28.49 | -7.79 | 67.32 | -3.17 |

Oxolinic acid (200 mg/L) created a strong positive interference with nitrite tests in seawater (66% increase) and a milder positive interference with nitrite tests in freshwater (16% increase) (Figure 2). It also produced a fine particulate that remained in suspension in the seawater tests for nitrite, which was not observed in the freshwater tests. Methylene blue created weak positive interferences (14 to 17% increase) in the tests for nitrite. Oxytetracycline (100 mg/L) created strong negative interferences (33 to 38% decrease), masking approximately one-third of the nitrite in the samples. AmquelPlus™ created strong negative interferences in nitrite tests (≈50 to 60% decrease), an effect not found in the same tests run with Amquel™ (ah, the difference a "Plus" can make).

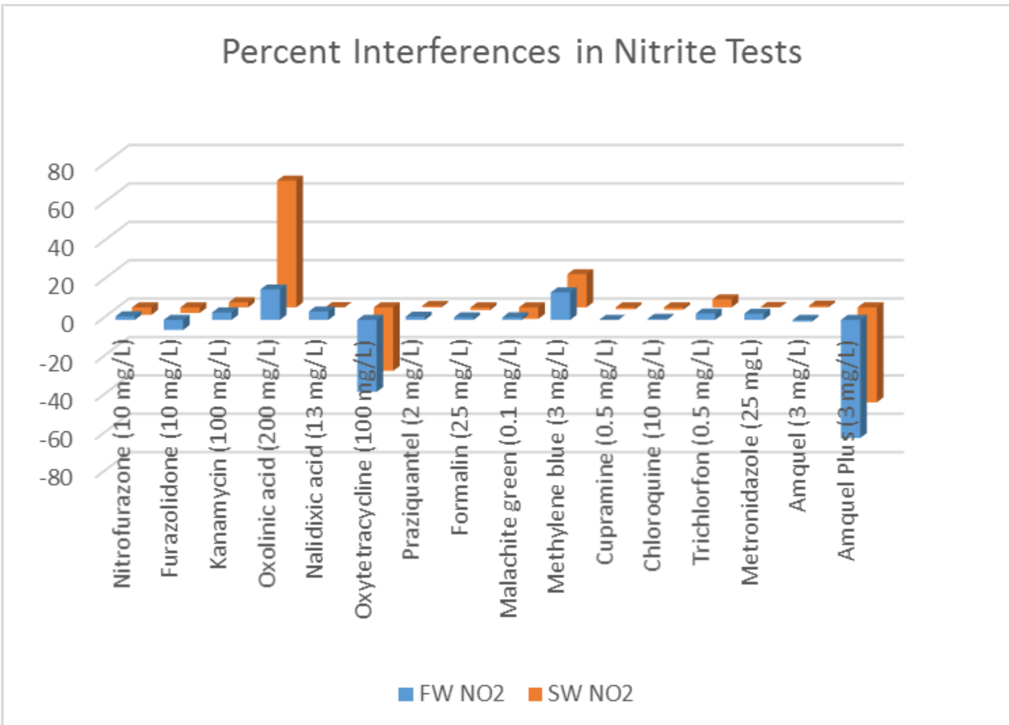


Figure 2: Interference levels in nitrite tests (as $NO_2^-$) by percent difference.

Nitrofurazone (10 mg/L), oxolinic acid and oxytetracycline created strong positive interference with nitrate tests (≈31 to 216%, 58 to 350% and 29 to 120% increases respectively); and chloroquine (10 mg/L) created strong positive interference with nitrate tests (27% increase) in seawater, but not freshwater (Figure 3). However, all nitrate test results for seawater in this study are potentially faulty, because we discovered during the experiment that Hach® method 10206 for nitrate is approved "For wastewater, drinking water, surface water, and process water" (Hach, 2014), but not seawater. When comparing untreated seawater against untreated freshwater sample readings across all nitrate batches (Table 3), this becomes apparent because the nitrate values in the seawater batch samples differ (to the negative) by ≈70% on average from the values in the freshwater batch samples. While seawater test results with 10206 method may be innately faulty due to some component of seawater, the nitrate test interference in seawater data gathered for this study is still included in Tables 1 and 2 (highlighted orange) to show what treatment chemicals act to further affect spectrophotometric readings of this test. This will be useful information should future studies discover an improved method/analysis for this test in seawater.

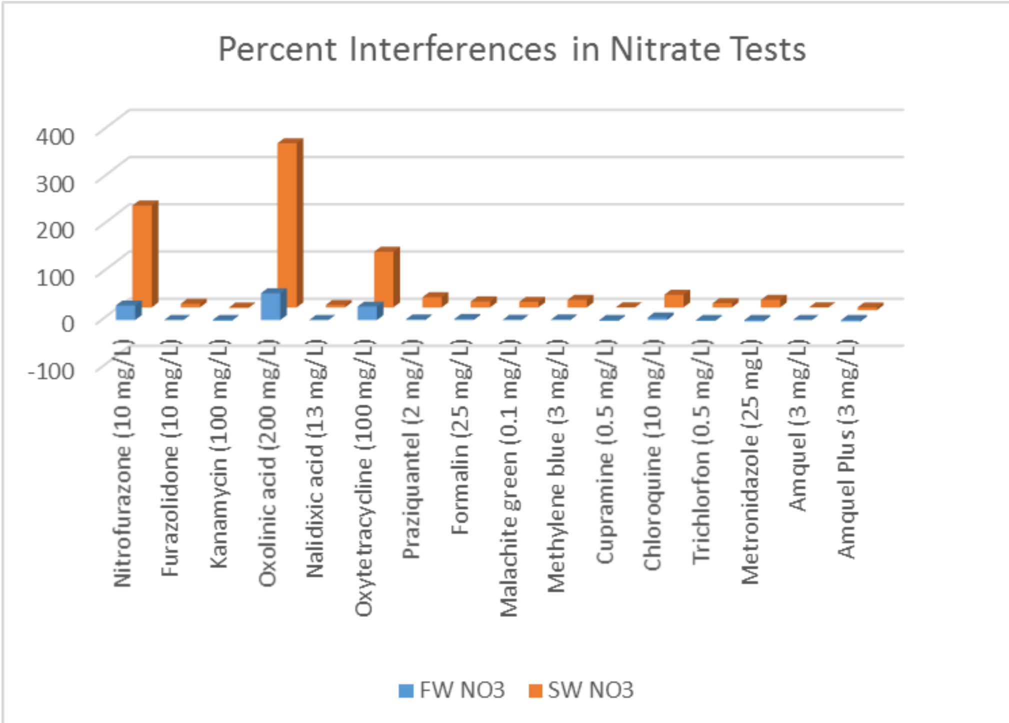


Figure 3: Interference levels in nitrate tests (as $NO_3^-$) by percent difference. Note that readings for the seawater in this test are potentially suspect.

Hach® method 8506 (for copper) was the least affected test in this study with only oxolinic acid and Cupramine™ (0.50 mg/L) producing strong positive interferences (27 to 50% and ≈100% increases respectively). Oxolinic acid also produced a slight visible haze to the samples, but less intense than the particulate suspension it produced in the nitrite tests. Cupramine™ interference was predicted because it is a copper-based treatment; it positively "interfered" with the copper-treated samples by 100% (exactly what one would expect to see in a sample with 0.50 mg/L of copper already present).

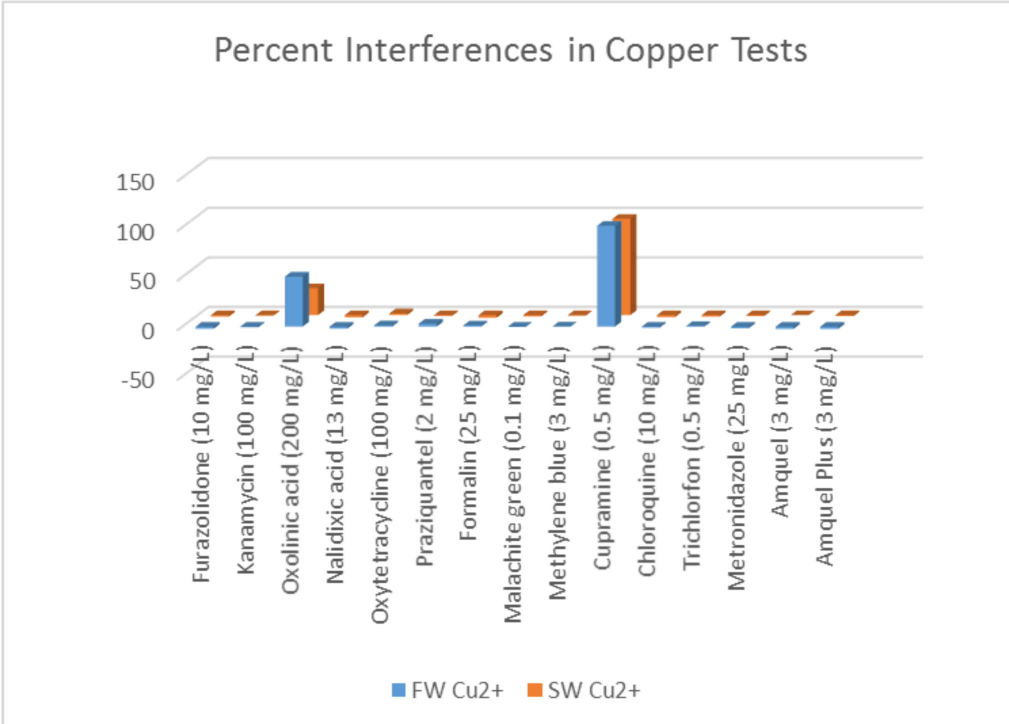


Figure 4: Interference levels in copper tests ($Cu_2^+$) by percent difference.

Furazolidone (10 mg/L), nalidixic acid (13 mg/L), praziquantel (2 mg/L), malachite green (0.1 mg/L), trichlorfon (0.5 mg/L), metronidazole (25 mg/L), and Amquel™ (3 mg/L) created no strong interferences with any of the tests run. Aside from the predicted copper interference, Cupramine™ (0.50 mg/L) created no strong interferences. With one exception in the already questionable column of nitrate testing in seawater, chloroquine (10 mg/L) also created no strong interferences.

**Conclusion:**

The ability to differentiate between a potential interference in water-chemistry tests and actual changes in water parameters is important for public aquarists and the animals under their care whose survival often rests the values derived from those tests. Knowing the limits of the tests used and accurately interpreting their results can only improve husbandry practices.

Table 2 can be used as a rough guide for aquarists to predict the possible effects of some bath therapeutics on spectrophotometric water-chemistry tests; however, this chart may only be accurate to within the parameters of the samples used. No two aquarium systems have the same set of parameters. Variable temperature, pH, aeration rates (Samuelson, 1987) hardness, light exposure, concentrations of both substrate and treatment (several treatments list various concentrations used over different lengths of time for application and sensitivity), degradation of the treatment chemical over time (Knight *et al.*, 2014, Marrero *et al*, 2015), interactions between multiple concurrent treatments and substrates, chemical-absorbing and adsorbing properties of the aquarium substrate (gravel/sand substrate, not test substrate)(Christie, 2016), and other factors might each have different effects on the degree of those interferences reported here.

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# *Mylobatis goodei* CONDITIONING FOR RESEARCH AND VETERINARY TREATMENTS

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## Introduction

The Southern Eagle Ray (Myliobatis goodei), commonly known as sea eagle in our country, is a cartilaginous fish that belongs to batoids elasmobranches. This species habits coastal waters from South Carolina in the US (35°N) to Santa Cruz, Argentina (44°S) (Stehmann, 2009). Wide salinity ranges are tolerated with the ray being reported both estuarine and marine water (Refi 1975; Lopez Cazorla, 1987). They have been captured in Argentina from 10 to 180 meters deep (Cousseau et al., 1997). The maximum size registered for *M. goodei* is 110 cm wide and 130 cm disk total length (Ruocco, 2012). Their diet is mainly based on mollusks and bivalves.

The available literature about Southern Eagle Ray consists of a taxonomic description published by Refi (1975), a biological study (Ruocco, 2012), and other studies on uterine contractions (Colonello, et. Al., 2013), bycatch levels (Vooren and Klippel 2005; Mendoza et al, 2003) and parasitic fauna (Brooks et al, 1981; Ivanov and Campbell, 1998). Besides there being few studies of the biology of this species, even less is known about its behavior, whether in the wild or captivity.

The Southern Eagle Ray is a specie easily adaptable to the controlled environment; Aquarium Temaikèn Foundation has a population of *M. goodei* in its collection as representatives of our native fish fauna. As part of the usual handling of organisms in the aquarium, either for clinical screening, veterinary treatment and / or research, specimens have been captured and then subjected to tonic immobility. It was observed qualitatively that once we completed these procedures, the individuals exhibited stereotyped behaviors, discoloration, erratic swimming, lack of movement for long periods of time, and in some cases, spontaneous abortions, which can be said to be consistent with a stressful situation. Given the importance of the handling of these animals in the aquarium, and giving priority to animal welfare, we therefor sought to evaluate the application of training techniques to reduce the stress of capture and tonic immobility in the Southern Eagle Ray (*M. goodei*) during the above procedures.

To summarize some fundamentals of animal behavior and training: the processes of understanding and assimilation of new behaviors in animals are based on the learning cycle (activator-behavior-consequence) where individuals receive positive reinforcement when they achieve the desired behavior and, instead, are not reinforced when a behavior is not desired or required. In both cases, learning occurs by the animal, and behaviors that are not rewarded tend to die out, while positive behaviors are rewarded and tend to recur. Each major step in the reinforcement process improves the incorporation of new and more complex behaviors. This system works because every animal constantly learns and interprets the surrounding environment and quickly assimilates the beneficial behaviors to help their survival. That is why, if the result of a behavior does not translate into something beneficial for him, the individual understands that he must not repeat that behavior and instead should replace the behavior if positive results are to accrue to him. These positive results are not necessarily food, but anything that meets its different types of needs. For example, positive reinforcement for an animal may be something they "enjoy" or something that stimulates their senses.

**Materials and methods**

Three adult female *Myliobatis goodei* identified by natural marks, and measuring about 1 m disk width, were isolated in a pool 7 meters in diameter with a water depth of 1.10 meters. Within that pool a ramp made of blue fiberglass running from the floor to 20 cm below the water level was placed (Figures 1 and 2). This room has a 12:12 photoperiod, and the following water quality conditions: T °: 14°C to 19°C (winter to summer cycle); pH: 8.05; and Salinity: 30 ppm.

The training plan was divided into 2 stages: The first stage mainly involved learning animal diet preferences and establishing their reaction times to food. In the second stage, we proceeded to work directly on the desired behaviors.

Figure 1: The fiberglass ramp and platform, seen out of water in association with the acrylic target.

**Results**

Once the individuals were introduced to the pool, we proceeded to perform desensitization to human presence, entering daily with a plastic tray with food, which was left floating nearby. In the first sessions, the aquarist stood still in the middle of the pool, throwing pieces of fish on one end and waiting until the Southern Eagle Rays were observed eating. Feeding times were recorded and evaluated. Individuals were given 10 minutes to participate in the training session, otherwise, the aquarist retreated and tried again later in the day. Eventually, an immediate reaction was obtained from the animals as the aquarist entered the tank. This greatly favored the work during the second stage as animals that were receptive to the early sessions showed a predisposition to other training.

Preferred foods were those with higher fat contents such as anchovies, mussels, squid, mackerel and shrimp. This information allowed us to improve the weighting of reinforcements, giving a better reward to new processes and more complex behaviors.

The process of desensitization to human presence was the most critical point with the rays passing from fear to confidence. This process lasted four months. Individuals exhibiting high stress levels began to swim excitedly and hit against the edges of the pool. After 6 sessions, some began to eat on the far side of the aquarist. Feeding progressed to locations closer to the aquarist, then off his foot, and finally out of his hand.

Once this level of confidence was achieved, the rest of the learning processes were resolved in a more dynamic way. The trainer then introduced a "clicker." The clicker is used as a bridge, telling individuals hearing the sound that they would then receive food. The use of a brightly colored acrylic target (seen in Figure 1) was then incorporated. Each contact with the target was followed by the sound of the clicker and a piece of food as a reward. The criteria were then established to make the rays follow that target, starting with short distance and then extending them to the desired end point. After 3 months of working with the target, the rays followed the aquarist in any direction asked of them, facilitating the introduction of the positioning ramp for further work with ultrasound (see Figure 4). However, initially, once the rays arrived at the base of the ramp as they followed the target, they showed some fear and moved away. To overcome this, high positive reinforcement was offered before they reached that critical point of fear. In this way they managed to reach the top of the ramp. Initially they were successful only when following the target, but they eventually responded when the aquarist called them, hitting the target at the top of the ramp.

Figure 2: A ray stationed on the platform at the top of the ramp.

The desensitization of the dorsal region of the rays was performed by the aquarist placing one hand on the nose and fingertips on the upper left back quadrant (Figure 3), without pressure and for a short period time. Over the course of the sessions, pressure and contact time were increased. The following elements were then introduced to simulate the ultrasound transducer. It started with the fingers, then a clicker, and progressed to a TV remote control (in a bag, simulating an ultrasound transducer). It took 6 months for indifference to the objects to be supported. It was noted that the manual contact (as in Figure 3) was easier for them to associate with objects, because the animals seemed to enjoy manual contact, which might even serve as secondary reinforcement. This secondary reinforcement facilitated the conditioning of rays to spend more time on the platform pending the ultrasound. Rays responded correctly to the touch of objects, but slower than they would to manual contact. They also showed some aversion to that contact.

Figure 3: Desensitization hand positions and the use of manual touch to simulate transducer positioning.

As the final step in the training process, the aquarist was placed behind the ramp and platform, and called the animal the hit the target against the ramp. Once the ray went up the ramp, the trainer held his nose with the left hand while the right moved the transducer over the back (Figure 4). Once the scan was obtained the sound of the clicker was given, the reward was offered and the ray was allowed to leave.

Figure 4: The final result of training. A ray submits to ultrasound examination while stationed on the fiberglass platform.

Obtaining all these results was aided through a parallel enrichment program, including the use of foraging puzzles (like a ball with food inside and a pipe with holes where the animal must move to obtain a food) (Figure 5), sensory stimulation (plants in the background and frozen fish blood), and environmental stimulation (gravel substrate and a pipe that altered the direction and intensity of a water stream). By doing this proactively and consistently, and making it a philosophy of work, the quality of life of the animals is improved and they become more permeable to stimulation and instruction using these environmental variables.

Figure 5: Plastic tube feeder puzzle provided for enrichment.

## Conclusion

The learning process was applied to these animals through training with the specific objective of reducing stress related to capture and the use of tonic immobility in research and medical situations. This favors both their everyday life in captivity and outcomes related to handling, because the animals do not show changes in their behavior as a result of these procedures. The development of stimuli and conditioning were performed with a specific objective to promote animal welfare and reduce the stress related to the research and / or veterinary treatment of this species.

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# CALCIUM CARBONATE AS A RESERVOIR FOR COPPER IN SEAWATER

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## Introduction

Nearly all seasoned aquarists know that heavy metals such as copper will be readily adsorbed by calcareous rock and substrata in the aquaria. Such interactions are described in detail in Spotte (1992); warnings against the re-use (with invertebrates or sensitive species) of live rock or gravel previously exposed to copper are ubiquitous in the common fish-keeping literature. Despite the universal acceptance of this truth we hold to be near dogmatically absolute there remain vast uncertainties. Last year a question was posed on the Aquaticinfo Listserv to our industry to which, it seemed, no one had an answer. Specifically: how much copper can one expect be adsorbed by a given amount of calcium carbonate? Conversely this question leads one to also ask: exactly how much copper can we expect to leach back out into the seawater of an aquarium?

Is a small amount of gravel that may have been immersed in water with a therapeutic dose of ionic or chelated copper potentially lethal in any volume of water, or is there a threshold at which the risk can empirically be minimized? Should a kilogram of aragonite be discarded because of prior copper exposure, or will the risk of using it in a large enough volume of seawater be negligible? A simple small-scale *ad hoc* experiment was in order to provide an informed approximation of the copper that may be bound in a given amount of rockwork or gravel.

## Methods

Six Pyrex® glass 1000ml acid bottles were cleaned with detergent (Oxy-Clean™), rinsed with alcohol (isopropyl), acid-washed (1M HCl), and rinsed clean with deionized water. Each bottle received roughly 100g of calcium carbonate that had been graded into various particle sizes using a series of sieves. Two forms of calcium carbonate were used, including three grades of aragonite (scleractintian origin) and two grades of calcite (molluscan origin). Scaled photographs were taken so that average particle size could be determined by measuring the widest point of each particle (n=25 per replicate). Calcium carbonate samples were all rinsed thoroughly and washed in 0.1M HCl for 60 min to ensure that the outermost calcareous surface of each particle was uniform in composition and clean of any biofouling or foreign particles that may interfere with adsorption of heavy metals. Each bottle received 500ml of an artificial seawater solution to which copper sulfate had been added to achieve a 5.85 mg/l concentration of ionic copper (as $Cu^{2+}$). Copper was quantified in seawater by use of a Hach DR2800™ spectrophotometer using the Hach CuVer2™ method no. 8026 (EPA no.3500Cu). All glassware used in water quality analysis was acid-washed (10.0M HCl) prior to use and rinsed three times with deionized water followed by a triple rinse with sample water before testing. Ten measurements were taken for each data point, and the results averaged. Samples were covered with a latex seal (to prevent evaporation) and homogenized (hand-swirled) daily to ensure that the copper-tainted water contacted the gravel particles evenly.

Figure 1. Different grades of calcite (top two) and aragonite (bottom four) used in the adsorption and leaching experiments. One of the aragonite samples (bottom left) was excluded from data analysis due to an unforeseen loss of liquid during the adsorption phase (i.e. the author is a klutz and broke a sample bottle).

*Copper Adsorbed*

The concentration of copper remaining in the seawater solution after 21 days was assessed by spectrophotometry (Hach™ DR2800 with method no.8026 as described above). Absolute copper (as $Cu^{2+}$) in the initial, and 21 day solutions was calculated by multiplying the concentration (in mg/l) by the total volume in liters. With these values the amount of absolute copper adsorbed (in mg) by each sample (per g) can be easily quantified as the difference between these two figures.

*Copper Leached*

After the amount of copper adsorbed was quantified, the initial 500ml of copper-tainted seawater was decanted and discarded. Each replicate received 500ml of deionized water with the pH adjusted downward to 6.5 (HCl added dropwise to DI solution on stir-plate), for maximum extraction of heavy metals. Solutions were re-tested approximately every 7 days and refreshed so that the total absolute copper leached over ten weeks could be quantified.

## Results

There was a significant difference in the copper levels of the solutions containing calcite versus those containing aragonite substrata (Student's t-Test t=4.5039; P=0.0102) indicating that much more copper was adsorbed (or otherwise sequestered) per unit of aragonite. The Aragonite samples had a mean $\Delta Cu^{2+}$ of 2.15±0.15 mg, calcite samples had a mean $\Delta Cu^{2+}$ of 0.20±0.17 mg. These data can be extrapolated to assume that overall aragonite will adsorb18.8-31.7 mg $Cu^{2+}$ $kg^{-1}$ substrate and calcite will adsorb 0.2-5.7 mg $Cu^{2+}$ $kg^{-1}$ substrate.

The amount of copper (as $Cu^{2+}$) leached back out into solution varied from 0.28-0.34mg (aragonite) and 0.20-0.37mg (calcite), equating to 13.8-16.3% and 75.5-78.4% copper recovery over 10 weeks (from aragonite and calcite, respectively). There were no significant differences in the amount of copper leached from all samples (calcite & aragonite pooled) as determined by single factor ANOVA (F=0.3776; Fcrit=2.5787), nor were there any significant differences when only aragonite replicates were included (ANOVA F=0.0858; Fcrit=3.3541) or among calcite replicates (Student's t-Test t=0.3508; P=0.3669). Though the amount of copper leached back as a percentage adsorbed was markedly higher from calcite. These data are summarized in Table 1 below.

Table 1: Summary of copper adsorption and leaching data for aragonite (n=3) and calcite (n=2) samples

| Sample | Material | Origin | Grain Size (mm) | Dry Mass (g) | Initial $Cu^{2+}$ (mg/l) | $Cu^{2+}$ Adsorbed (mg) | $Cu^{2+}$ Leached (mg) | $Cu^{2+}$ Recovered (% - 10 wk) |
|---|---|---|---|---|---|---|---|---|
| AG-01 | Aragonite | Scleractinian | 21.2 ± 1.0 | 104.3 | 5.85 | 2.43 | 0.335 | 13.8 |
| AG-02 | Aragonite | Scleractinian | 10.7 ± 2.3 | 105.9 | 5.81 | 2.00 | 0.325 | 16.3 |
| AG-04 | Aragonite | Scleractinian | 3.1 ± 0.4 | 106.3 | 5.87 | 2.01 | 0.280 | 13.9 |
| CG-01 | Calcite | Molluscan | 5.9 ± 1.2 | 106.8 | 5.76 | 0.49 | 0.370 | 75.5 |
| CG-02 | Calcite | Molluscan | 1.1 ± 0.5 | 106.0 | 5.83 | 0.26 | 0.200 | 78.4 |

## Discussion

It is well known that the mechanism of copper retention in calcium carbonate is by adsorption (Spotte, 1992) and formation of $CuCO_3$ (Papadopoulos and Rowell, 1989), both of which are surface interactions. Given the variable surface area and surface-to-volume ratio of the different sized substrata utilized we fully expected to see difference in the amount of copper extracted from the solutions and the amount eventually leached back out, though these data do not support that hypothesis. Given the limitations of this trial (limited sample sizes/replicates and the testing methodology) the possibility that finer substrata with greater surface area may retain more heavy metals cannot be conclusively ruled out and further study is warranted.

It is rather interesting that a significant difference existed in the amount of copper removed from solution by calcite or aragonite, yet there was no overall significant difference in the amount of copper leached back out into solution between the two species of mineral. This

Table 2: Maximum potential copper leaching from dataset collected. Note that this is a best estimate given the limited nature of this small-scale *ad hoc* experiment, use with caution.

## Theoretical Maximum Copper Leaching Potential (mg/l $Cu^{2+}$ per kg $CaCO_3$ as aragonite per unit volume seawater)

| | Gallons → | 5 | 10 | 20 | 50 | 100 | 200 | 300 | 400 | 500 | 1000 | 1500 | 2000 | 2500 | 5000 | 7500 | 10000 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Liters → | 18.9 | 37.9 | 75.7 | 189.3 | 378.5 | 757.1 | 1135.6 | 1514.2 | 1892.7 | 3785.4 | 5678.1 | 7570.8 | 9463.5 | 18927.1 | 28390.6 | 37854.1 |
| kg $CaCO_3$ | mg $Cu^{2+}$ | | | | | | | | | | | | | | | | |
| 1 | 31.7 | 1.6749 | 0.8374 | 0.4187 | 0.1675 | 0.0837 | 0.0419 | 0.0279 | 0.0209 | 0.0167 | 0.0084 | 0.0056 | 0.0042 | 0.0033 | 0.0017 | 0.0011 | 0.0008 |
| 2 | 63.4 | 3.3497 | 1.6749 | 0.8374 | 0.3350 | 0.1675 | 0.0837 | 0.0558 | 0.0419 | 0.0335 | 0.0167 | 0.0112 | 0.0084 | 0.0067 | 0.0033 | 0.0022 | 0.0017 |
| 3 | 95.1 | 5.0246 | 2.5123 | 1.2561 | 0.5025 | 0.2512 | 0.1256 | 0.0837 | 0.0628 | 0.0502 | 0.0251 | 0.0167 | 0.0126 | 0.0100 | 0.0050 | 0.0033 | 0.0025 |
| 4 | 126.8 | 6.6994 | 3.3497 | 1.6749 | 0.6699 | 0.3350 | 0.1675 | 0.1117 | 0.0837 | 0.0670 | 0.0335 | 0.0223 | 0.0167 | 0.0134 | 0.0067 | 0.0045 | 0.0033 |
| 5 | 158.5 | 8.3743 | 4.1871 | 2.0936 | 0.8374 | 0.4187 | 0.2094 | 0.1396 | 0.1047 | 0.0837 | 0.0419 | 0.0279 | 0.0209 | 0.0167 | 0.0084 | 0.0056 | 0.0042 |
| 6 | 190.2 | 10.0491 | 5.0246 | 2.5123 | 1.0049 | 0.5025 | 0.2512 | 0.1675 | 0.1256 | 0.1005 | 0.0502 | 0.0335 | 0.0251 | 0.0201 | 0.0100 | 0.0067 | 0.0050 |
| 7 | 221.9 | 11.7240 | 5.8620 | 2.9310 | 1.1724 | 0.5862 | 0.2931 | 0.1954 | 0.1465 | 0.1172 | 0.0586 | 0.0391 | 0.0293 | 0.0234 | 0.0117 | 0.0078 | 0.0059 |
| 8 | 253.6 | 13.3988 | 6.6994 | 3.3497 | 1.3399 | 0.6699 | 0.3350 | 0.2233 | 0.1675 | 0.1340 | 0.0670 | 0.0447 | 0.0335 | 0.0268 | 0.0134 | 0.0089 | 0.0067 |
| 9 | 285.3 | 15.0737 | 7.5368 | 3.7684 | 1.5074 | 0.7537 | 0.3768 | 0.2512 | 0.1884 | 0.1507 | 0.0754 | 0.0502 | 0.0377 | 0.0301 | 0.0151 | 0.0100 | 0.0075 |
| 10 | 317 | 16.7485 | 8.3743 | 4.1871 | 1.6749 | 0.8374 | 0.4187 | 0.2791 | 0.2094 | 0.1675 | 0.0837 | 0.0558 | 0.0419 | 0.0335 | 0.0167 | 0.0112 | 0.0084 |
| 15 | 475.5 | 25.1228 | 12.5614 | 6.2807 | 2.5123 | 1.2561 | 0.6281 | 0.4187 | 0.3140 | 0.2512 | 0.1256 | 0.0837 | 0.0628 | 0.0502 | 0.0251 | 0.0167 | 0.0126 |
| 20 | 634 | 33.4970 | 16.7485 | 8.3743 | 3.3497 | 1.6749 | 0.8374 | 0.5583 | 0.4187 | 0.3350 | 0.1675 | 0.1117 | 0.0837 | 0.0670 | 0.0335 | 0.0223 | 0.0167 |
| 25 | 792.5 | 41.8713 | 20.9356 | 10.4678 | 4.1871 | 2.0936 | 1.0468 | 0.6979 | 0.5234 | 0.4187 | 0.2094 | 0.1396 | 0.1047 | 0.0837 | 0.0419 | 0.0279 | 0.0209 |
| 30 | 951 | 50.2455 | 25.1228 | 12.5614 | 5.0246 | 2.5123 | 1.2561 | 0.8374 | 0.6281 | 0.5025 | 0.2512 | 0.1675 | 0.1256 | 0.1005 | 0.0502 | 0.0335 | 0.0251 |
| 35 | 1109.5 | 58.6198 | 29.3099 | 14.6550 | 5.8620 | 2.9310 | 1.4655 | 0.9770 | 0.7327 | 0.5862 | 0.2931 | 0.1954 | 0.1465 | 0.1172 | 0.0586 | 0.0391 | 0.0293 |
| 40 | 1268 | 66.9941 | 33.4970 | 16.7485 | 6.6994 | 3.3497 | 1.6749 | 1.1166 | 0.8374 | 0.6699 | 0.3350 | 0.2233 | 0.1675 | 0.1340 | 0.0670 | 0.0447 | 0.0335 |
| 45 | 1426.5 | 75.3683 | 37.6842 | 18.8421 | 7.5368 | 3.7684 | 1.8842 | 1.2561 | 0.9421 | 0.7537 | 0.3768 | 0.2512 | 0.1884 | 0.1507 | 0.0754 | 0.0502 | 0.0377 |
| 50 | 1585 | 83.7426 | 41.8713 | 20.9356 | 8.3743 | 4.1871 | 2.0936 | 1.3957 | 1.0468 | 0.8374 | 0.4187 | 0.2791 | 0.2094 | 0.1675 | 0.0837 | 0.0558 | 0.0419 |
| 100 | 3170 | 167.4852 | 83.7426 | 41.8713 | 16.7485 | 8.3743 | 4.1871 | 2.7914 | 2.0936 | 1.6749 | 0.8374 | 0.5583 | 0.4187 | 0.3350 | 0.1675 | 0.1117 | 0.0837 |
| 200 | 6340 | 334.9703 | 167.4852 | 83.7426 | 33.4970 | 16.7485 | 8.3743 | 5.5828 | 4.1871 | 3.3497 | 1.6749 | 1.1166 | 0.8374 | 0.6699 | 0.3350 | 0.2233 | 0.1675 |
| 300 | 9510 | 502.4555 | 251.2277 | 125.6139 | 50.2455 | 25.1228 | 12.5614 | 8.3743 | 6.2807 | 5.0246 | 2.5123 | 1.6749 | 1.2561 | 1.0049 | 0.5025 | 0.3350 | 0.2512 |
| 400 | 12680 | 669.9406 | 334.9703 | 167.4852 | 66.9941 | 33.4970 | 16.7485 | 11.1657 | 8.3743 | 6.6994 | 3.3497 | 2.2331 | 1.6749 | 1.3399 | 0.6699 | 0.4466 | 0.3350 |
| 500 | 15850 | 837.4258 | 418.7129 | 209.3565 | 83.7426 | 41.8713 | 20.9356 | 13.9571 | 10.4678 | 8.3743 | 4.1871 | 2.7914 | 2.0936 | 1.6749 | 0.8374 | 0.5583 | 0.4187 |
| 1000 | 31700 | 1674.8516 | 837.4258 | 418.7129 | 167.4852 | 83.7426 | 41.8713 | 27.9142 | 20.9356 | 16.7485 | 8.3743 | 5.5828 | 4.1871 | 3.3497 | 1.6749 | 1.1166 | 0.8374 |
| 2000 | 63400 | 3349.7032 | 1674.8516 | 837.4258 | 334.9703 | 167.4852 | 83.7426 | 55.8284 | 41.8713 | 33.4970 | 16.7485 | 11.1657 | 8.3743 | 6.6994 | 3.3497 | 2.2331 | 1.6749 |

| | |
|---|---|
| RED = | Above therapeutic range |
| Orange= | Therapeutic concentration (EDTA-chelated copper) |
| BLUE = | Therapeutic concentration (ionic copper products) |
| Green = | Below practical spectrophotographic analysis range |
| Grey = | Theoretical data, impractical kg/vol ratio |

All values in mg/l Cu2+

may indicate a higher propensity for copper to form insoluble precipitates such as $CuCO_3$ on the surface of aragonite versus calcite; or perhaps another unknown, insoluble form of copper sequestration. The duration of this limited trial may also artificially effect the perception of copper leached back out as the concentrations were only quantified for 10 weeks. As many of us know from personal experience copper can continue to leach back out in minute quantities for many months or even years (see discussion below regarding a reef tank); and if we were to assume that the trend of leaching (0.01-0.03mg/l $week^{-1}$) seen in the last few weeks of the trial for aragonite were to continue for a year the percent recovery for each aragonite dataset could potentially rise dramatically (to nearly 65% or more).

In order to make these approximations more relevant to the aquarist the maximum amount of copper retained was calculated over a wide range of substrate weights (in kg) which was then divided by a series of volumes to extrapolate a wide range of maximum copper concentrations (in mg/l) that one could expect to see by placing tainted gravel in aquaria (Table 2). It is important to note that these data are taken from the maximum amount adsorbed in the first phase of this investigation, not the amount of copper leached back into seawater in the second phase, and as such this represents a worst-case scenario. In practice the aquarist will probably see far less copper leached back out per kg substrate, though it is better to plan for a worst-case scenario rather than be taken by surprise.

While some approximations of the maximum amount of copper a given amount of substrate can be expected to harbor are presented here, these data do not definitively suggest whether or not said substrate should be considered forever tainted. As mentioned above dire warnings against the re-use of rocks, gravel, and plastic components are omnipresent in the popular fishkeeping literature, but I would posit that they are not absolute. Is it possible to extract copper from calcium to the point where it may be safe to use with invertebrates? While hard data to support this are lacking I am aware of several anecdotal cases where live rock and substrate have been re-used after sufficient time to leach out metals. Case in point is the author's own reef tank (Fig. 1).

Figure 1: What heavy metals? A variety of invertebrates thriving in a reef tank made from base rock that was once exposed to therapeutic copper, after nearly 2 years of EDTA chelation and activated carbon filtration.

This exhibit was one of the last completed during renovations of the aquarium in 2009-2010 and staff needed to place un-quarantined animals on exhibit (against their better judgement); which of course resulted in horrendous disease issues which were eventually only brought under control with the use of copper. After 2 years of EDTA chelation, fastidious removal of detritus and filamentous algal growth (both of which accumulate metals), and aggressive activated carbon filtration the very same rock and substrate once bathed in copper now support over 28 species of SPS and LPS corals (as well as soft corals, anemones, countless sponges, bryozoans, crustaceans, echinoderms, gastropods, et cetera). Therefore I would argue that calcium can be "cleaned" of heavy metals to allow its re-use if absolutely necessary, if one is willing to spend the time and effort on such an endeavor.

**Acknowledgements**

Thanks to Brian Nelson of the National Aquarium in Baltimore for posing a relatively innocuous question with the potential to burrow into one's brain and never rest until it is answered, much like that worm-thing they put in Chekov's ear in Wrath of Khan.

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Spotte, S. 1992. Captive seawater fishes: science and technology. John Wiley & Sons.

Bruce Koike

# CORN AND CLOWNFISH: MARINE SCIENCE IN IOWA

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**Kirk Embree, Instructor kirk.embree@dmschools.org**

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What happens when you mix 100 parts corn with 100 parts clownfish? Then you add in a few parts cowrie, several parts of corals, a pinch of cowfish, and two parts Cortez sting ray? Well, you get a horrible attempt at alliteration and something that probably doesn't taste too good. But, add in a hundred students or so and you get something amazing in the heart of Iowa.

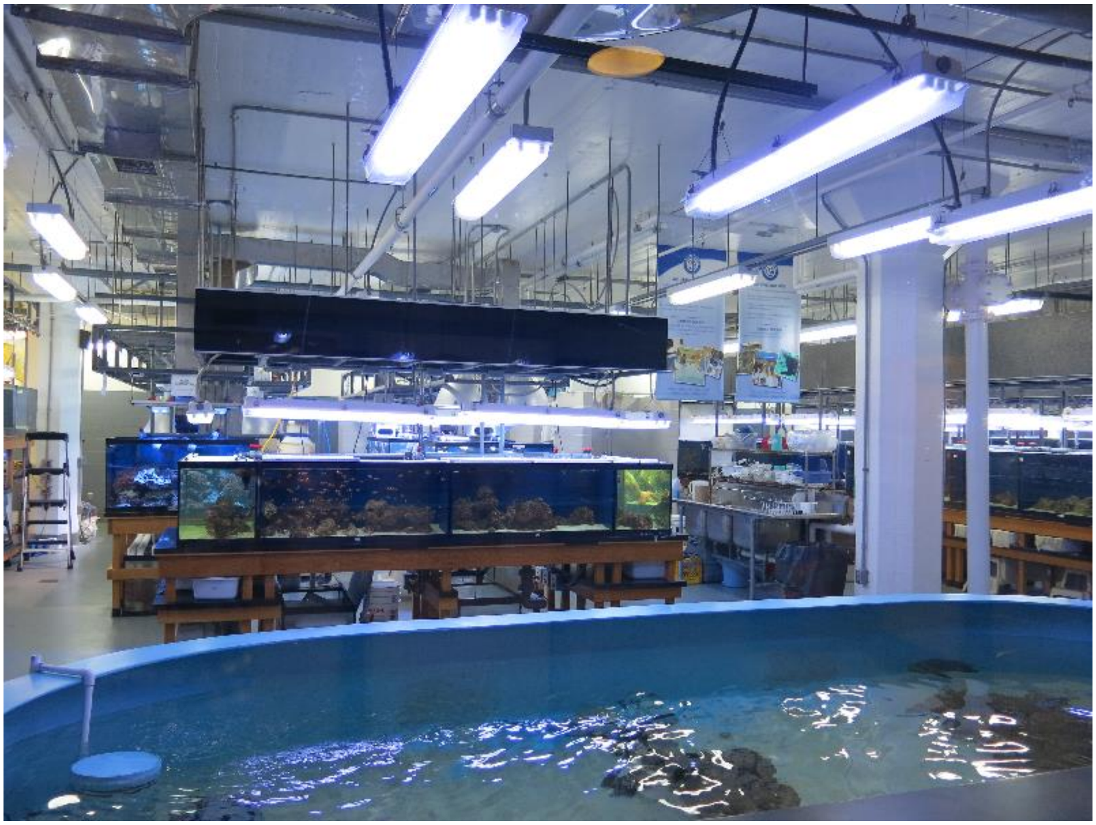


Figure 1: On the outside looking in on the Central Campus Marine Science program from the school hallway. The Ray Tank lies up front with our Aquarium Science side to the left of the white column, focusing on aquaculture, and the Marine Biology side to the right, focusing on behavior and diversity.

In a word, Central Campus is unique. On the front, it is a Career and Technical school… Here, students are given opportunities to follow their passions and not just learn, but practice future careers in a myriad of different professional fields from welding and aviation to nursing and teaching. In a real sense, the students run the school. Sure, there are teachers that train the students and oversee the classes. But in reality, the 1350 students at Central Campus do everything from repairing cars, to running a café, producing their own fashion show, building a house, and ensuring the technology and software in the school is working correctly. If the zombie apocalypse ever happened, we'd be all set to ride it out at Central Campus.

The Marine Science program is composed of two different courses: Aquarium Science and Marine Biology. ***All of the students run the Central Campus Marine Laboratory and Aquarium***. The program here at Central Campus goes beyond the course descriptions. Really, how can you possibly describe everything that our students do in a few sentences? Well, you can't. Here, we'd like to show you (with pictures) and tell you more about our program and how they make this program unlike any other marine science program in the world.

Figure 2: Students feeding one of the various organisms in our lab. On the left, students are feeding out flake and mysis to small fishes while the student on the right is feeding one of our epaulette sharks.

Technically, our day starts at 7:55AM when the first block of classes starts. Usually, though, students are here before that time and can be found out in the lab and aquarium checking on the approximately 130 marine aquarium systems we have running at any given time. This morning, they may be checking on quarantined fishes, a new fish, or just simply checking everything making sure the systems are functioning properly. Of course, the students should always check the nautiluses we have first!

Figure 3: In concert with feeding, the students also work on their daily husbandry tasks that include cleaning algae and intakes (left) and also ensuring their husbandry tools by their tanks are all clean and put back properly (right).

When classes begin, we usually have a quick update on the lab. Have any fish or invertebrates been moved? Are we treating any animals or tanks? Did anything new come in? Did anything die? Does anyone have any questions about what they are doing today? You see, students in our program make our program possible. While we train new students each year on how to care for the hundreds of different organisms that we maintain in the lab, after their 4-6 week training, they run the lab. We are their teachers for sure, but we are also their managers/curators/directors for the lab. That is the great thing about Central Campus. The students enable our program to grow. The students allow us to bring in awesome animals like nautiluses and breed a variety of different organisms such as clownfish and Banggai cardinal fish. It may sound cliché, but the students deserve all the credit for our lab.

Figure 4: The diet kitchen should be the place where you feel comfortable eating food off the floor, not that you would… Each day there are about 75 students in and out of the kitchen preparing food for each and every organism. These 75 students are feeding over 100 different tanks so adhering to protocols in the diet kitchen are critical to preventing cross-contamination. With a smile on their faces, the students must ensure that food items remain in the proper place, that the food dishes are washed properly, and finally that everything used that day is eventually disinfected before being used again.

After our morning updates, the students (we have about 150 total between us) begin to either feed their assigned tanks and/or work on husbandry. Similar to some aquariums, our students work on a rotation in the lab so that each month they are working with different animals, aquariums, and systems to give them a diverse environment to learn in and develop various skills. In one month you might be feeding nautiluses and kelp forest animals. The next month you might have to feed the brood stock room full of clownfish or one of the many live coral systems. In one month you may have to work on maintaining our Deadly and Dangerous exhibit or the Sting Ray Exhibit. The next month, you're focused on maintaining the Anemone Exhibit or the *Rhinopias* Exhibit. Here at Central Campus, our goal is to expose the students to as many different opportunities as possible.

Figure 5: The expectations in our program are high. We hold the students to high standards and in return, the students actually hold their fellow students to even higher standards. So, after making sure that every little starfish, sea horse, sting ray, and nautilus has been fed, and after making sure that every speck of algae is off of the glass, the students add that last shine and sparkle to their tanks by squeegeeing the outside glass. This seemingly small and 'easy' task is a perfect exercise to remind students of attention to detail.

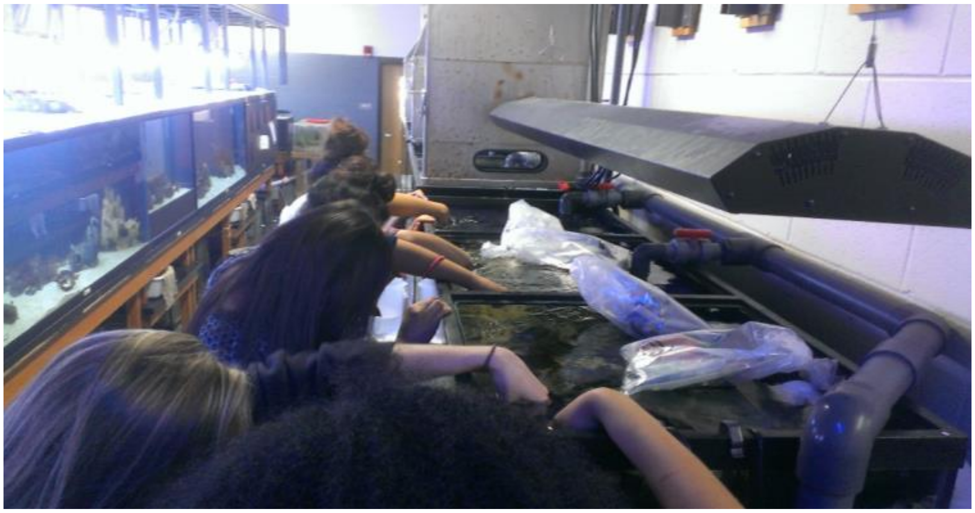
Although the Aquarium Science and Marine Biology classes are separate, we function as a whole to make our lab the 'Best of the Best'. Think of the good ole Halloween costume of two people being a horse. Together, the costume is awesome! Apart, all you're left with is two pieces that invariably will be made into glue or even hot dogs. Without both classes full of motivated students, our lab simply could not function.


Figure 6: Who doesn't love a good floor squeegee? Of course, where there are aquariums, there are spills. Thankfully, there are also a dozen squeegees in the lab. This seemingly mundane task is another great example of the commitment our students have to not just the facility but to their fellow students and their instructors. Rather than needing to be asked to help squeegee our floor, in most cases, students take it upon themselves to help with a spill without needing to be asked. There are few things better than coming into water on the floor and before you blink, seeing a dozen students with squeegees helping out to clean up the mess.

So, on a 'normal day' (is any day normal in an aquarium or laboratory?), the students ensure every organism (shark, nautilus, clownfish, and sponge) has eaten and they have also ensured that their tanks and systems are functioning properly and that all that wonderfully disgusting algae has been removed from the glass and walls of the tanks. But we all know that is not a truly normal day for any of us. So, what else do our students do throughout the year?

Figure 7: Acclimatizing new animals is always fun for our students to see the new animals and help get them from bag to tank. This is one instance that we encourage our students to be on their phones taking pictures and video of our new organisms to share them with their friends and family. Of course, sharing nautilus pictures to anyone and everyone is always highly encouraged!

Figure 8: Shipment days are always exciting. What could be more exciting than placing hundreds of snails into their tanks when they arrive? At Central Campus, it takes a village, or school or squad, to get all of the snails in the right place. It is this type of team work that we see every day in everything the students accomplish.



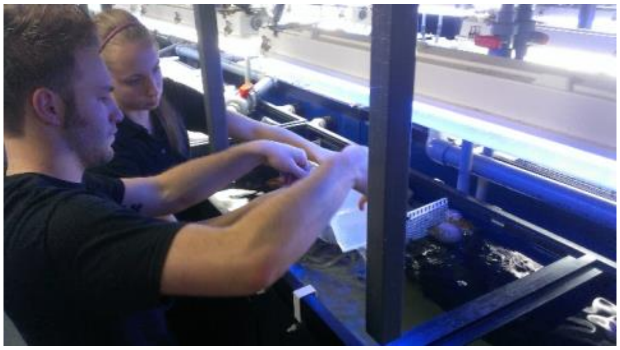


Figure 9: When an organism get sick, our students are able to learn about the entire process of diagnosing, treating, and monitoring these animals to ensure they regain their health. Students learn about conducting skin scrapings, gill clippings, and fin clippings to diagnose potential disease and infestations. After diagnoses, the students then fully participate in the treatment methods during the different protocols. From treating nautiluses to sharks, the students gain a diverse understanding of veterinary medicine and the importance of observing their tanks to catch health issues as soon as possible.

Figure 10: As technology changes and we continue to develop and grow, students are responsible for implementing the changes from new protein skimmers to LED lighting fixtures. Not only do our students learn about installing these lights (left) and controlling them via Wi-Fi, they also repair and maintain these lights themselves (below). Work like this is a testament to our overall program. Students here do not simply replace a light or pump, they disassemble the equipment themselves, determine the problem themselves, and fix what needs to be fixed, themselves. Our students know the outer and inner workings of all of the equipment in our lab.


Figure 11: With the right training and background, our students can literally do anything they want to their tanks. One key training skill they need is to learn how to aquascape their aquariums to make the rocks look however they'd like. Of course, stacking rocks can kill and scratch. So, our students practice their skills outside the tanks first, before stacking large rocks inside glass tanks with living animals.

Figure 12: Students also have the opportunity to use different types of microscopes to not only find out what tardigrades actually look like but also check out the intricacies of algae and copepods. Here, getting dirty is key so the students have their very own ‘dirty books” that are expected to get dirty during these dissections. As Dr. Barord tells them, “If you’re book doesn’t smell, then you weren’t doing it right”.

Figure 13: The students in our program have yet more responsibilities throughout the year as they also serve as guides and tour leaders around our facility for younger students (above), their peers during Sophomore Tours, and even adults (left). To prepare for these tours, that often times are weekly, the students must maintain the facility in pristine order to always ensure that the facility is looking the best. We nominate Student Ambassadors to serve as our leaders to guide people through our facility and share our program with a variety of different people.

Figure 14: Although Iowa is a bit far from any ocean, we take the chance to use our surrounding resources to practice exploring the water. Kayaking is another skill our students gain in our program by exploring the local lakes and rivers near Des Moines. Students that participate in the Field Studies course then continue their kayaking development in the ocean during the course.

Figure 15: Our students not only gain valuable experience in our program, they also gain college credit while still in high school. A student could potentially accumulate almost 20 credits while in our program. During Spring Break, students have the opportunity to participate in a Field Studies course that takes the students out of Iowa. Previous trips have been to the Bahamas, Florida, and most recently, California and down to Texas in 2016. During the trips, the students visit different zoos and aquariums in the area and are afforded amazing behind-the-scenes tours to not only find out how large public aquariums operate but also learn more about careers in marine science. We also visit the local ecosystems and investigate the different organisms that call these places their home. From tide pooling to kayaking to snorkeling, students participate in many activities to learn more about the ocean.

That really is just a scratch on the surface (not our aquariums) of what our students do in our program. Among other things, they also serve as Ambassadors and tour not only other students through our facility, but also invited speakers and guests, school board members, and literally anyone else who is interested in viewing our facility. In any given year, upwards of 10,000 people come through our lab and our students do a magnificent job at sharing their experiences with each and every person.

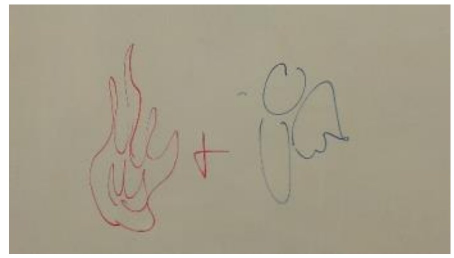


Figure 16: As part of the program, we use a variety of methods to introduce the students to the different life in the ocean. On any given day, students may be learning about how fragile the marine ecosystem is through "Ocean" Jenga (left), playing Pictionary (top) to review the different organisms in our lab, or they may be playing a Scientific Name-Match Game (below) to learn the scientific names of all of the organisms in the aquarium. Through all of these different activities, students utilize and develop different skills, such as critical thinking, when learning about the marine world.

So, what do you get when you cross the ocean with corn? Well, duh, an ocean full of corn. But you also get a new generation of students developing an appreciation for that salty body of water so far away and sharing their appreciation with their family and friends. What you really get is a better informed society about our planet as a whole. Perhaps there are programs like Central Campus, the more the better, but I think it's safe to say that all of our students that go through the program are impacted for the rest of their lives. At least from the standpoint of Dr. Barord, "I know that all of my students will at least know what a nautilus is after being in my class." But really, the students actually do so much more for ourselves, the program, and future students than they will ever know.

Of course, the students here would not have as many opportunities as they do without all of the support of our dozens and dozens of business partners, including many folks at zoos and aquariums throughout the country and even the world. The Marine Science Program at Central Campus is what it is because of all of your support. Thank you.


Figure 17: While some students may be doing a project on nitrate sequestration or puffer fish conditioning, other students may be doing a project on breeding grass shrimp or developing an Instagram page for our program. The dedication and creativity of our students in developing these projects is what ensures that our facility maintains its "Best of the Best" moniker and stays a few steps ahead.

## ***General Information for***

## ***RAW 2017***

## ***Boston, MA, USA***

*(note that Registration for RAW 2016 in New Orleans has CLOSED)*

**2017 Host institution:** New England Aquarium, Boston, Massachusetts, USA.

**When:** May 6-12, 2017

**Conference Hotel:** Seaport Boston Hotel
1 Seaport Lane, Boston, MA 02210,
(617) 385-4000
**Room Rates:** *~$343 for a two-person room.*

## BOOK REVIEW

### *The Salt Smart Guide to Preventing, Diagnosing, and Treating Diseases of Marine Fishes*

**Jay Hemdal**

**Print Version: 194 pages. $34.99 USD**
**eBook: $14.99 USD**
**http://www.saltwatersmarts.com/marine-fish-disease-guide/**

*Review by: Paul Poeschl, Aquarist, Shark Reef Aquarium at Mandalay Bay, Las Vegas, NV, USA*

### HEMDAL'S FISH DISEASE GUIDE A MUST FOR CAREER AQUARISTS

*The Salt Smart Guide to Preventing, Diagnosing, and Treating Diseases of Marine Fishes*, written by Jay Hemdal, is an excellent, inexpensive resource for the career aquarist. This book provides a commonsense perspective combined with a zoo curator's viewpoint on pathogens in aquarium fish by discussing their prevention, diagnosis, and treatment all while laying out medical methods that any aquarist could do. It covers frequently occurring diseases with microscope slides for reference, as well as the environmental factors that influence fish health. As a bonus, it goes into invertebrate disease, zoonotic disease, euthanasia, and collection techniques. All of these factors being extremely important to success in the aquarium field, I highly recommend this book to all my peers for their professional development and personal reference. The book can be purchased through the publisher, SaltwaterSmarts.com, or through Amazon.com.

## ENRICHING THE LIVES OF TOUCH TANK SOUTHERN COWNOSE RAYS (*Rhinoptera bonasus*) AT PHOENIX ZOO

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**Abstract**

The goal of an aquatic species behavioral enrichment program should be the same as that of any terrestrial species, which is to elicit natural, species-appropriate behaviors while reducing stereotypy.

A touch tank is a type of zoo or aquarium exhibit with the purpose of providing guests with a closer, more intimate experience with the animal species exhibited; often, however, the touch tank does not reflect the natural environment nor does it encourage species-appropriate behaviors. Furthermore, animal behavior in touch tank exhibits can be influenced and directly impacted by guest interactions (e.g., hand feeding near the surface of the water). Behavioral observations within a population of 31 Southern cownose rays (*Rhinoptera bonasus*) led to the development of a new behavioral enrichment and training program at the Phoenix Zoo's Stingray Bay. The program attempts to maintain a balance between stimulating natural behaviors and providing a more rewarding experience that enhances the connection between the guests and the animals. Interactions between animals and their new behavioral enrichment were recorded, evaluated and an ethogram was developed based on consistent behaviors observed within the population.

**Introduction**

Behavioral enrichment is a vital part of husbandry in any zoological or aquatic facility, allowing animals to exercise species-appropriate behaviors in a managed setting. Animal welfare and cognitive ability improve when behavioral enrichment is used; encouraging animals to make choices that allow new experiences aligning with their natural history. (Young, R, 2003).

Although there are ample behavioral enrichment resources for many species in managed settings, concepts for stingrays (specifically pelagic species like cownose stingray) are not yet widespread. While simulation of a natural environment is common at most zoos and aquariums, stereotypic behaviors can still occur since no exhibit can be an exact replica of the ocean (Smith, et. al, 2014). Touch tank exhibits are popular attractions at zoos and aquariums and provide guests the opportunity to directly interact with a seemingly misunderstood animal species. However, the typical design of a touch tank exhibit has the potential to evoke less natural behaviors in its inhabitants when compared to deeper, more complex and natural looking exhibits (pers. obs.). There are several unique factors about these types of exhibits influencing stingray behavior. These include the following:

- Daily human interaction with exhibit animals.
- Exhibit animals' dependency on hand feeding or feeding near the water surface.
- Exhibits are often of uniform and shallow depth.
- Exhibits often provide little to no decorations or furniture in the exhibit.

Salvanes et al. (2013) describe a correlation between behavioral enrichment and learning ability in juvenile Atlantic salmon (*Salmo salar*) in a managed setting. Fish exposed to behavioral enrichment made less mistakes in working their way through a maze. Experimental fish were also able to improve upon their accuracy with each trial at a rate substantially higher than the control group, a group not exposed to any enrichment. Consideration of these results prompted the Phoenix Zoo's aquarists to create a more complex behavioral enrichment program for the population of Southern cownose rays (*Rhinoptera bonasus*) at the Zoo.

Conducting behavioral observations of the population 11.18 cownose stingrays was vital to evaluating the successes and failures within this new behavioral enrichment program. Little is known about cownose ray behavior, and referencing manta ray ethograms (Georgia Aquarium, 2011) did not define behaviors seen within the Zoo's population of Southern cownose rays. Detailed information and articles about behavioral enrichment for touch tank animals are lacking. The Phoenix Zoo regularly used Kongs, forage balls, feeding tubes, and hula hoops for behavioral enrichment prior to the new program. In order to develop a new program, aquarists decided to observe and film the stingrays interacting with their current behavioral enrichment items. Animals were also filmed interacting with aquarists, guests, and each other.

Behaviors observed during filming were identified and defined to create an ethogram for future evaluation of the cownose rays. Observed behaviors were defined as follows:

<u>Solitary Behaviors</u>
*Foraging*: Animal is searching with mouth looking for food.
*Bury:* Animal lays flat on the sand and moves sand onto its back using mouth or body; animal then lays still on the floor of the tank with sand on its back. No food is present.
*Retreat:* Animal has been startled by something, a guest, object, and another animal or sometimes nothing that is obvious to the observer, and swims away very quickly from the item. Animal will sometimes splash during retreat. In rare or serious circumstances animal may try to breach (jump out of water).
*Pattern:* Animals are swimming in their normal, calm, clockwise motion around the tank.

<u>Social Behaviors</u>
*Guest:* Animal approaches guest against wall of exhibit and allows guest to touch when no food is present.
*Keeper:* Animal approaches keeper, follows keeper, or investigates keeper's activity. Keeper can be in or out of the water. No food is present.
*Feeding:* Animal chooses to interact with human (guest, keeper, guide) when food is present.
*Tight Schooling:* Multiple or all animals are swimming quickly and *very* close together. This is commonly seen when keeper is in the water trying to catch animals with a net.

<u>Social Breeding</u>
*Breeding Chase:* One or more males quickly following a female closely while trying to bite on her pectoral fin.
*Breeding Flip:* Male successfully bites onto the pectoral fins of the female and successfully flips her onto her dorsal side.

*Copulation:* Successful contact between the claspers of a male and the cloacae of a female. This can be difficult to see, but occurs almost immediately after breeding flip.

<u>Social Aggression</u>
*Block:* Animal uses body to prevent other animal(s) from gaining access to item/food. Animal will either push/swim into another or swim higher/over top of another animal. Animal receiving block behavior is not able to access food/item.
*Submission:* An animal specifically receives blocking behavior from another and swims away, giving up the chance to have access to the desired object, food, or interaction.

<u>Manipulation</u>
*Inspection:* Animal approaches object to within 15 cm, then swims away. During inspection animal will sometimes bump their rostrum or brush pectoral fins against object before swimming away**.**
*Oral Inspection:* Animal swims over top of and positions head and mouth near object. The lobes that surround the mouth should be protruding and moving around the object. If food is involved then that is *foraging*, not oral inspection.
*Favored Use:* Animal changes usual (circular) swim pattern to use specific behavioral enrichment item or an animal uses item and then immediately turns around to reuse item. This would not apply if keeper puts item within the normal circular swim pattern in which the stingrays swim.

<u>Possible Abnormal Behaviors</u>
*Spiraling:* Animal is seen swimming in very tight (small), repetitive circles with no obvious direction or gain to the animal. This does not include swimming in their normal circles around the tank or turning to change direction.
*Spyhopping:* Animal is vertical in the water and moving in an up and down motion with the head bobbing in and out of the water. Animal is usually doing this against the wall of the tank.

The above ethogram has proven beneficial in allowing aquarists to monitor ray behaviors. The ethogram can be used to define the manner in which an animal chooses to interact with new enrichment. Aquarists can now choose an enrichment item to entice specific behaviors to stimulate animals in different ways. Staff can also better define unusual behaviors and intervene to extinguish undesirable or potentially harmful behaviors.

**Treating Possible Abnormal Behaviors**

The Zoo had a very successful breeding season between December 2014 and April 2015. Sixteen cownose stingray pups were born on exhibit during this time and moved into a 10,000 gallon isolation tank behind the scenes. Although all 16 were deemed healthy by the veterinarian, there were three pups that exhibited spyhopping, a behavior that Zoo staff now believe to be unnatural and harmful. The first two pups that began to exhibit this behavior were born close enough to each other that they were housed together with 13 other pups in the isolation tank. During this time, both animals would find different parts of the tank to move up and down along the wall repeatedly. Aquarists tested water quality and inspected life support equipment but could not find anything abnormal in these parameters that one might expect. The rest of the pup population at this time showed no signs of this behavior.

These first 15 pups were placed at other facilities by March 2015, after which an additional pup was born in April. This final pup born in April was also moved to the isolation tank for monitoring and rearing. Aquarists noticed that this pup also exhibited the spyhopping behavior and spent so much time exhibiting this behavior that his ventral side received multiple abrasions (Fig. 1). Even with the increasing number and severity of abrasions, the pup continued the behavior. Aquarists and veterinarians could find no cause for this behavior, and staff decided to intervene.

Figure 1: Photograph illustrating the abraded ventral surface of a Southern cownose ray pup after repeatedly spyhopping.

Staff decided to try utilizing pool noodles (cylindrical, polyethylene pool toys) around the circumference of the isolation tank where the water meets the wall (Fig. 2) to prevent the animal from spyhopping. The hypothesis behind this decision was that the behavior would cease if the pup had no access to the areas of the tank where the behavior was exhibited. When tying the noodles to the sides of the tank, the pup was observed swimming to different areas of the pool, possibly in an attempt to try finding a spot to spyhop. After swimming to all sides of the tank and gently touching his head to the noodle, the animal ceased spyhopping and began to forage and swim normally around the tank.

During the months between September 2015 and November of 2015, the Zoo had another 15 pups born on exhibit. All pups were placed in a separate nursery on exhibit so the isolation tank could be prepared for an animal acquisition. The pups had access to a sand substrate and dive rings for enrichment at all times and had interactions with the animal care staff daily. These animals spent the majority of their first day burying in the substrate and resting. Aquarists spent a few min every day socializing with and teaching pups to swim through the dive rings. Pups quickly learned to interact with the dive rings as taught without assistance. These pups also showed more interest in interacting with the aquarists and were hand feeding consistently as early as four days old. Aquarists observed that the pups being raised in this environment did not exhibit any of the spyhopping behavior from the previous season and no pups sustained the ventral injuries seen from the previous year. The observations from comparing the behavior over the two seasons seem to support the hypothesis that the presence of substrate, enrichment or exhibit furniture and consistent interaction with aquarists at least play a role in preventing the spyhopping behaviors. The comparison of these two seasons has led aquarists to believe that spyhopping is a stress-based or abnormal behavior that is either highly influenced or dependent upon the animals' environment.

Figure 2: Isolation pool of Phoenix Zoo's Stingray Bay after lining the pool with pool noodles in an attempt to extinguish the abnormal and undesirable spyhopping behavior observed in Southern cownose ray pups.

**Behavioral Enrichment**

Animal behavior during interactions with enrichment items led to the observation that four distinct behaviors were consistently seen within the population during enrichment interactions. These four behaviors were observed often enough that they were categorized as the behaviors that defined whether or not an enrichment item was a success within the population, and became the four main behaviors that aquarists worked to encourage in a managed setting. Aquarists have observed that if the first three of the following behaviors are seen consistently, that the fourth behavior, favored use, is a likely outcome and the enrichment item will be used on a regular basis:

1. Foraging
2. Inspection
3. Oral Inspection
4. Favored Use

**Foraging Behavioral Enrichment**

Most touch tank animals obtain the majority of their diet from public feedings where guests are encouraged to purchase food for a close encounter. Animals become accustomed to foraging at the surface of the water and approaching guests for food, which is an unlikely behavior in the animals' natural habitat. Aquarists supplement public feedings with foraging enrichment in an attempt to encourage the animals to exhibit more natural foraging behaviors. Burying the food in the substrate is another good example of promoting benthic feeding and sifting behaviors. Aquarists can also opt to give the animals live food to encourage more natural foraging (Smith et. al, 2014).

The stingrays at the Zoo already had access to artificial foraging behavioral enrichment such as forage balls, feeder tubes and Kong® toys. The main issue with all of these objects was that one animal could easily monopolize the item, preventing the rest of the population from having a chance to use the enrichment or have access to the reward. Zoo staff also felt that animals were able to manipulate these objects so quickly that the items were deemed too simplistic to provide meaningful or long-lasting enrichment to the animals.

The goal of the new foraging behavioral enrichment was to increase foraging time and encourage multiple animals to participate. Aquarists created a tetherball set using PVC pipe to form the pole and connected forage balls to this via string (Fig 3). The forage balls were moving at more unpredictable angles as they dangled by the string, making the animals work harder and longer to obtain the food (unpubl. data).

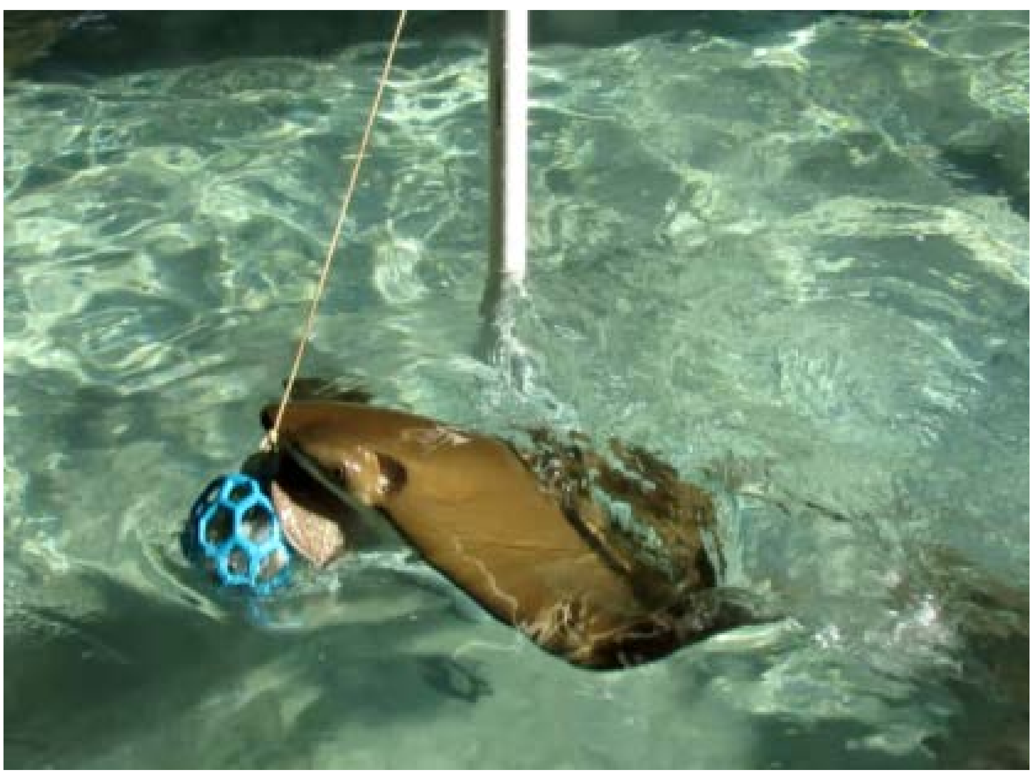

Figure 3: Cownose stingray interacting with tetherball enrichment.

Another foraging behavioral enrichment device created for the stingrays was a 'painting machine,' modeled after an object seen in a video at the St. Louis Zoo. The painting machine consists of a forage ball that is connected to PVC pipes (Fig 4). When animals try to manipulate the forage ball to access the food the PVC pipes also move, which in turn moves the paintbrush across the canvas (Fig 5). The paintbrush is shielded by a milk crate to protect the animals and water from possible paint spills. This particular behavioral enrichment item has been very popular with guests, was featured on the local news, and is has potential to create future revenue due to the high volume of requests to purchase the paintings.


Figure 4: Photograph of the stingray painting foraging device made of PVC.


Figure 5: Photograph illustrating how stingrays manipulate painting machine.

**Tactile Behavioral Enrichment**

The ability to provide animals with ample foraging behavioral enrichment can be restricted by potential water quality issues. Overfeeding fish has the potential to cause health issues and/or ammonia and nitrite spikes. If ammonia and nitrite levels are too high, or there are life support issues, then an aquarist may need to decrease the amount of food going into the tank to prevent further ammonia or nitrite spikes. The possibility of these issues can be limiting to food enrichment, which makes tactile behavioral enrichment a good substitute as animals receive stimulation without the possible adverse effects on water parameters. Hula hoop enrichment was already practiced at the Zoo. Aquarists hold the hoops in the water and the stingrays would repeatedly alter their typical swim patterns to swim through the hoops. Aquarists also observed via film, that the stingrays in the holding tank would repeatedly alter their swim patterns to swim into airline tubing suspended in the tank connected to airstones to aerate the water body. These same animals would even pick up the airstones connected to this tubing and drag them around the tank. After observing these two behaviors, a new behavioral enrichment device was created to combine the two enticing activities. The "carwash" enrichment (Fig. 6) consists of an archway built completely out of PVC piping that has airline tubing suspending downward from the top of the arch. This item is considered safe to leave in the tank for the entire work day without direct supervision from the aquarists. When deployed, the stingrays interact with this object to the point that they actually drag it around the entire exhibit.

Figure 6: Photograph of the "carwash" tactile enrichment device.

After observing the repeated, positive interactions between the stingrays and the carwash, aquarists designed a larger obstacle course that lengthens the time of tactile behavioral enrichment. The obstacle course was made with one archway exactly the same as the carwash but was also connected to vertical pool noodles. The animals would swim through the carwash portion and continue through the pool noodle “forest”, pushing the noodles around as they exited. This particular tactile enrichment was considered successful due to the animals’ repetitive swimming through the course.

**Training as Behavioral Enrichment**

When reviewing video footage of provided hula hoop enrichment, a female stingray (Annie) was observed picking up a hula hoop by positioning the edge of the item in the center of her rostrum and swimming upward (Fig 7). Once the hula hoop was secured by Annie, she tried to swim towards an area where there were fewer animals. Based on her interest in this particular hula hoop, aquarists decided to see if she could be trained to retrieve this item on cue (Fig 8).

Figure 7: Photograph of Annie picking up hula hoop during filmed enrichment session.

Retrieval training with Annie proved to be very successful. She learned target training quickly, and within thirty minutes of the first training session she was successfully delivering the hula hoop to the aquarist. Annie has also developed different methods of manipulation with the hula hoop, helping her to successfully approach and deliver the object from different angles when the cue is given.

While consistency was being established with Annie’s retrieval training, a male stingray (Phil) began to follow her during sessions. Ultimately, Phil began to interfere with the training sessions by positioning himself between the aquarist and Annie, blocking her access to the hula hoop. Sometimes he would even steal the hula hoop from Annie and swim away with it. Though he was never rewarded for this behavior, and the hula hoop was pulled during his approach, this did not deter his efforts to interfere. Aquarists decided to begin a training session with Phil immediately after Annie retrieved the hula hoop. Phil was ignored completely during the training sessions with Annie and immediately rewarded with target training at the conclusion of her sessions. Phil’s behavior eventually changed to where he would hover near the aquarist and only approach when his target was placed in the water.

Phil’s target training was also successful and he eventually advanced to ‘agility set’ training, where he was trained to weave through vertical PVC poles set in the tank by aquarists (Fig 9). The training sessions with Phil were shorter to accommodate his seemingly shorter attention span. The agility set training has been successful so far, and Annie eventually began to follow him through the set trying to repeat the behavior.

Figure 8: Photograph of Annie manipulating hula hoop during retrieval training.

Annie’s retrieval training became more advanced after Phil’s interference was under control. Aquarists decided to try a different item for retrieval- a smaller, weighted dive ring. The dive ring is held by the aquarist and the same cue is given under the water for retrieval. When Annie was consistent at holding the ring with her rostrum and following the aquarist, an underwater camera was added to it. Annie was then given the cue to come get the camera from the aquarist and swim the circumference of the tank until the same cue was given to return (Fig 10). These camera training sessions have been highly successful. Annie did not hesitate once with the additional weight or shape at the base of the dive ring. She is able to drop the camera at any point during the session because the dive ring rests loosely on top of her rostrum, but she consistently carries the camera until the return cue is given.

Figure 9: Photograph of Phil during a training session with the agility set.

The rapid rate of learning demonstrated by both rays in the training sessions outlined above illustrates the intelligence and potential capabilities of the species. Future training plans are being considered by the Zoo to expand on management and behavioral enrichment of the species. Similar sessions and plans should also be considered by other institutions holding this, and similar, species. We have found that their reputation of "simple" animals does not approach their true ability to respond to training and learn.

**Conclusion**

Since training Annie and Phil, aquarists have observed that these two animals are the first animals to approach new behavioral enrichment devices and are typically the first to figure out the correct way to use each item. They also developed the most social behaviors with guests and staff. The training sessions have truly enhanced the lives of the rays, but have also improved the experience of our guests. In fact, guests who visit on a regular basis remember these individuals and ask about them during visits.

Since enacting the behavioral enrichment program, the frequency of stress based behaviors observed during the busy season at the Zoo has noticeably decreased, suggesting a positive effect on behavior. Prior to implementing the expanded behavioral enrichment program, animals had a tendency to swim very closely together in smaller circles and avoid the sides of the tank during busy times. The addition of behavioral enrichment during these times has appeared to decrease tight schooling and spiraling behaviors. The animals were seen interacting with the behavioral enrichment items for a period of time and then resuming normal swim patterns around the sides of the tank where the guests are able to reach them. Aquarists are planning a more in-depth evaluation on this behavior during the busy times using the ethogram. If the behavioral enrichment is able to decrease the stressful swim patterns and encourage animals to swim closer to the edges of the tank, then the experience improves for the guests as well. Guests have also mentioned how much they enjoy seeing the animals interact with the different enrichment items, and that watching these interactions taught them more about the intelligence of the species. If paid exhibits are thriving, then the entire zoo community benefits from the funds that are received. Behavioral enrichment can be used to improve the welfare of touch tank animals, while also being an important promotional tool for an exhibit that generates revenue.

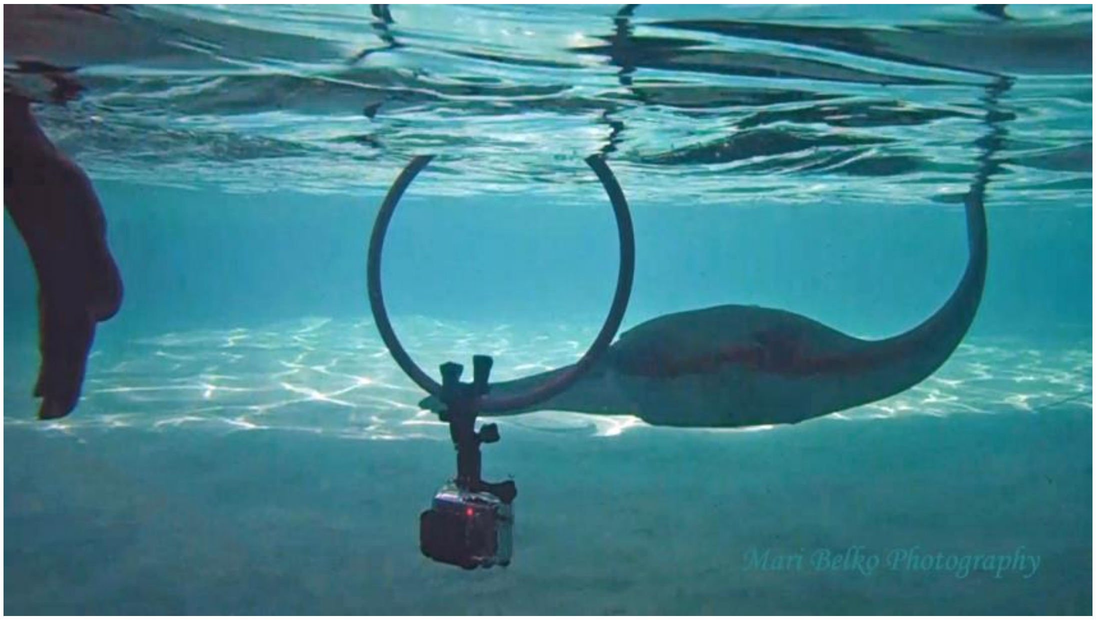


Figure 10: Training session with Annie, retrieval of an underwater camera. Photo credit: Mari Belko

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# RAW 2015 ABSTRACTS

**Regional Aquatics Workshop, March 3-5**
**Monterey Bay Aquarium, Monterey, CA, USA**

**Welcome and Introduction**
Paul Clarkson

**A History of the Monterey Bay Aquarium**
Jon Hoech

**Animal Professional**
(Recorded all presentations. Available at animalprofessional.com)

## Tuesday, March 3

***Session 1: New Projects and Exhibits***

### Mini Aquariums Coast to Coast: Catch-and-Release Public Aquarium Model

Melanie Knight and Ruby Banwait
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Melanie Knight, Founder of the Petty Harbour Mini Aquarium describes in her TEDx talk the power of bringing the ocean to eye level. Ruby Banwait, the PHMA Founding Curator, makes this happen every day. Hear from this dynamic duo about the two sides of starting a public aquarium; 1) the planning, fundraising and community engagement and, 2) the life support system planning, plumbing, pumps, animal collection and care, exhibit design and animal handling protocols. This highly visual and practical talk will show-and-tell how a Mini Aquarium in Petty Harbour, Newfoundland came alive.

### Exploring Explorer's Reef: Or, How to Design, Create, and Implement an Interactive Experience for up to 60,000 Guest Visits a Day!

Mike Price
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At SeaWorld San Diego connecting guests to the wonders of the marine environment is fundamental to the purpose of the park. We believe a direct connection inspires people to take an active part in protecting ocean health and conserving marine habitats. In support of this goal, in March of 2014, SeaWorld San Diego opened Explorer's Reef, a massive, 3-acre transformation of the marine park's arrival experience into a "world beneath the waves". Explorer's Reef features touch pools that allow guest interaction with bamboo sharks, cleaner fish, rays, and various reef fishes within minutes of their arrival. The four pools are divided by species, with two bamboo shark pools, a cleaner fish pool, and a ray pool. A state-of-the-art life support system provides an environment that maximizes the health and wellbeing of the animals and serves the uniqueness of interactive pools. The biggest challenge was determining how to deliver an interactive attraction that could handle more than 60,000 visits on peak days. Success came using a combination of interactive guidelines, cross-departmental team development and an openness to flexible adjustments as needed. Visitor response to Explorer's Reef was an overwhelmingly positive. Guest Satisfaction scores set some of the highest recorded in the park's history. Ancillary benefits of the attraction were increased visitor time at adjacent merchandise opportunities and increased length of stay in the park. Explorer's Reef is unique opportunity to inspire millions to connect and care for the natural world while achieving both animal care and business goals.

## Cold, Deep and Dark: Designing, Constructing and Stocking a Native California Reef Exhibit

Margarita Upton and Nick Yim
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In 2012, the Steinhart Aquarium determined that it wanted to add more medium sized exhibits—between 2000 and 10,000 gallons—to its collection. In 2013, the opportunity arose to renovate an existing space which resulted in the replacement of several small temperate exhibits with a single 2500 gallon temperate display. The theme selected was a living California coast deep rocky reef, inspired by the reefs typically found at 120-200 feet in the Cordell Bank National Marine Sanctuary; an underwater plateau located approximately 50 miles northwest of San Francisco. The waters off the California coast are known for their rich productivity, which yields an incredible density of invertebrate and fish life that we intended to replicate. The goal in designing the display and associated systems was to be able to sustain a living, temperate, non-photosynthetic reef tank in a closed system.

One of the biggest challenges in planning this exhibit was to design a life support system that would allow us to mimic the high productivity, thereby allowing us to be able to feed a dense population of non-photosynthetic invertebrates and fish, while also maintaining adequate water quality. The other main challenge we faced was how we would safely collect animals and re-create the proposed habitat, without compromising the safety of the animals or divers. This presentation will outline the process from exhibit design through collection and LSS construction to the final product. Opened in June 2014, as it continues to grow and develop over the coming years, we hope this exhibit will help our guests realize and appreciate that there are beautiful living coral reefs just offshore of California.

**Sponsor Presentation – Tenji, Inc.**

**Using Ultrasound Technology to Monitor the Captive-Reproductive Cycle of Female Broadnose Sevengill sharks, *Notorynchus cepedianus*, at Aquarium of the Bay**

Michael Grassman
Aquarium of the Bay
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Ultrasound examinations have been used on several species of elasmaobranchs as a noninvasive means of monitoring the reproductive cycle. Information on follicle development in captive broadnose sevengill sharks, Notorynchus cepedianus, has previously been collected from individuals in Australia, but the current case-study is the first reported account of using the technology on this species in North America. Initial observations of changes to body shape and size of a mature female sevengill were first made in the autumn of 2011, and the first ultrasound conducted in April of 2012 confirmed the presence of developing follicles. A subsequent ultrasound in March of 2013 showed an increase of follicle size from 3.3 cm to approximately 5.12 cm, and a third ultrasound in November of 2013 revealed many of the follicles were 7.0 cm diameter or greater. During this study period, all sevengill sharks in the collection were trained to respond to auditory and visual cues to voluntarily enter an isolated acclimation pool for examination. Throughout the early winter months of 2014, the shark's girth began to decrease noticeably, and her final ultrasound in March of 2014 confirmed her follicles were not fertilized and were in the process of being reabsorbed. The shark was eventually released back into San Francisco Bay in June of 2014. This case-study represents the collection of valuable data on the captive reproductive cycle of female sevengill sharks and may be applied to future attempts of captive reproduction with this species.

**Not Another Octopus Enrichment Talk: A Quantitative Approach to Enrichment**

Jordan Salyers
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Behavioral training and enrichment are common practices used to enhance the wellbeing of many species at zoos and aquariums. Multiple training and enrichment models exist, however many of these models do not answer an important question: is the enrichment program working? At the Virginia Aquarium, we have developed a method to collect quantitative data from an enrichment session with the common octopus, Octopus vulgaris. Specifically, a goal for the enrichment program is established and a numerical scale is assigned to specific behaviors. The data are manipulated and analyzed in Excel, and then used to mathematically eliminate unsuccessful enrichment items. This allows the aquarist to enhance the enrichment program for an individual over time based on desired behaviors. Though our program has been developed for the common octopus, the techniques can easily be adapted and applied to other species. Notably, the method of data collection, the process by which an enrichment item is assigned for a session, and how the data informs the future of the enrichment program allow an aquarist to answer the question: is the enrichment program successful and of value to the animal's wellbeing?

## Don't Shoot the Cobia!: Training *Rachycentron canadum*

**Michelle Coley**
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Training has become an integral part of collection maintenance and care, often done with larger species such as sharks, rays, and turtles. Large game fish are often displayed in multi species exhibits and maintained with a larger collection as a whole. The cobia (Rachycentron canadum) is a large game fish displayed within Chesapeake Bay and Atlantic Ocean exhibits. The challenges of keeping cobia in a mixed species exhibit can be aggressive feeding that often results in them out competing other animals and causing them to dominate the feeding. Cobia can also cause distractions and interruption to training of other large species, such as sea turtles, if they are not stationed during a feeding. It can be challenging to train this species within a mixed species exhibit as there are other animals around, causing potential distraction and loss of "reward". With the use of a striped pole, we have been able to minimize stress and successfully move this animal from one exhibit to another as it grows and create a feeding station that allows a concentrated focus from the animal. There has also been success in getting the cobia to follow a swim pattern before returning to the target to get food during a feeding. Auditory cues have also begun to be investigated within this training process to determine the potential auditory capabilities of this species as there is minimal documentation on the subject. Based on our experience with training Rachycentron canadum to come to a target, we have found the large group feedings have minimal distractions and this has allowed us more opportunity to effectively and efficiently manage this species and other large fish. We hope that our experience with training this type of animal allows other institutions to implement the same practice and allow greater opportunity for growth and success.

## A Touching Experience

Ashleigh Clews, Meredith Meyers and Leigh Ann Clayton
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Touch tanks and elasmobranch training are both hot topics in the public aquarium industry. This presentation discusses training methods employed with stingrays and skates acquired for a National Aquarium touch exhibit opening in 2015.

At concept development, there was a clear expectation that guests would readily be able to touch animals in an exhibit that supported exceptional animal health. Staff identified deliberate respondent and operating training pre-opening as critical to meeting these goals. Purposeful training was identified as the only way to increase the likelihood animals would voluntarily participate in touch interactions. Multiple staff members from quarantine, animal programs, veterinary, and husbandry groups were involved in training approximately 25 animals over a period of 18 months.

About half of the total animals were quarantined and held in a large tray system. Here, techniques included staff putting hands in water during feeding and offering food by hand. Most animals readily approach hands, touched hands, and permitted acceptable levels of tactile interaction without detailed positive reinforcement training plans. For animals that did not meet behavior goals, more detailed plans were utilized.

For holding systems too deep for staff to reach animals, target training with artificial hand targets was used. Additional work with one ray in particular has focused on the goal of training this ray to swim onto a platform, be lifted towards the surface and stay on the platform while being touched.

Challenges included staffing levels/training experience, quarantine/medical needs and enclosure access. This presentation focuses on how these challenges have been addressed, the training methods employed and the animals' progress on each of these training goals.

**Sponsor Presentation – TRACKS Software**

## Tuesday, March 3
### *Session 3: Elasmobranch Husbandry*

**Sponsor Presentation – Dynasty Marine**

### ***Mobula hypostoma*** **– You're Not Cool Unless You Have a School**

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This presentation in intended to walk the listener through the whole process of obtaining Mobula hypostoma in more detail than has previously been presented on. Last year Dynasty Marine successfully collected a school of 6 Mobula hypostoma that are currently on display at Aquarium Encounters. The talk would follow the natural sequence of events required to get these animals on display. It would start with information on permitting from the state. The collection process would be briefly touched upon. Then acclimation, quarantine, and teaching to feed in captivity would be discussed. We would go over prophylactics used and dosages administered. Then transportation of the animals both by land and in the air would be explained. Finally, long term holding and discussion on the great interactive display animal that they can be.

**Husbandry R&D Efforts with Common Thresher Sharks, *Alopias vulpinus*, and Opah, *Lampris guttatus:* No One Said This Would Be Easy**

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*No abstract provided*

**How to be a Smooth Operator with *Gymnura micrura***

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Smooth Butterfly Rays (*Gymnura micrura*) are a very interesting ray species that are rarely exhibited in Aquariums because they are considered difficult to keep in captivity. They are a small ray species found in the Eastern and Western Atlantic, which earn their name from their butterfly shaped wings and their "fluttering" swimming motion. They are predatory rays that explode out of the sand to attack prey items, making for exciting feeding presentations. These rays also have an unusually short tail, and lack a barb, which makes them a great addition to touch tanks.

Since Smooth Butterfly Rays are such a unique species, and readily available to us locally, the North Carolina Aquarium at Pine Knoll Shores decided to start collecting and working with them in 2012. We made several attempts to solve the problems associated with feeding them in captivity. Through trial and error, we have found very successful ways to get them eating. Due to their shy nature and predatory style of feeding, we had a few hurdles to jump when displaying them in a touch tank exhibit. After a few more modifications to their husbandry, such as feeding them separately in a simple acrylic box, we started seeing great success in keeping these animals.

These rays are fantastic exhibit animals, especially in a touch tank scenario. Armed with a little bit of information, any aquarium could successfully add this species to their collection.

**Thinking Outside the Box Truck - Long Distance Transports for Elasmobranchs: Department of Transportation Compliance and LSS logistics**

Barbara Bailey, Monika Schmuck, Shannon Mahoney, Caitlin Crowley, Lydia Glenn, Nina Fisher, Julie Cavin
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Long distance transports for elasmobranchs take a great deal of planning, but are further complicated when complying with Department of Transportation regulations. In late July, early August 2014, the New England Aquarium transported six *Rhinopterus bonasus* (cownose ray) to South Carolina Aquarium in exchange for two adult female *Sphyrna tiburo* (bonnethead shark).

In order to comply with DOT 'hours of service' regulations for truck drivers, the transport teams needed to be launched in stages. To limit the amount of stops for water quality testing and adjustments and visual checks, a sophisticated life support system and animal monitoring capabilities were designed, all operated from the cab of the truck. This system included; inverter power, mechanical and biological filtration, pH buffering system, water sample tap, dosing capability, dissolved oxygen monitoring and control, webcam feed to a laptop and use of a go pro for underwater video. This presentation will outline the details of this transport.

## Sawfish Mega-Move

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More than a decade after Cairns Marine first started providing juvenile sawfish, public aquaria now face the challenge of managing animals that have matured to young adult status, attaining lengths of up to 14 feet. Whilst the majority of animals remain at the original aquarium that they were shipped to, some animals have outgrown their exhibits, others have become problematic with some of their tank mates and some need to be moved in order to form pairs and capitalize on potential breeding opportunities as part of studbook programs.

Moving a 12 foot sawfish is problematic at the best of times but what if the aquarium that the animal has to be transferred to is international and the only way the movement can be achieved is via standard commercial passenger aircraft without an attendant? There are no precedents and this would be the first time anyone has attempted to move a sawfish this size in an aircraft.

This presentation follows the transfer of a 12 foot sawfish from Reef HQ in Townsville where it has been living since it was collected in the Norman River back in 2003. The destination is the massive open ocean display at Resorts World in Singapore. Significant logistical issues have to be overcome including capture of the animal from a multi-species exhibit, relocation to Cairns via road transport, temporary housing at Cairns Marine, air transport on a standard commercial aircraft, and unloading into an aquarium with significant access constraints. Airfreight is a major issue given the length of the Sawfish exceeds the length of an aircraft pallet and typical transport containers are not big enough.

Given there are other animals and facilities in a similar position around the world, this is a timely opportunity to share the good and the bad about this epic world first and assist aquaria to better manage their collections.

## Sponsor Presentation – Cairns Marine

## Tuesday, March 3
### *Session 4: Husbandry*

**Sponsor Presentation – McRoberts Sales Co. Inc.**

**Managing Diversity in a Complex Predatory Amazon Exhibit**

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The Amazon flooded forest is one of the most dynamic tropical freshwater habitats in the world with an amazing diversity of fish. This diversity can make for a truly awe-inspiring exhibit in a public aquarium. However, the challenge is to create a display that successfully balances showing off the truly impressive array of large predators and smaller species, giving the public an accurate representation of the Amazon. This presentation will detail the process by which the Steinhart Aquarium's Flooded Forest exhibit team successfully approached this exhibit challenge.

Previous attempts to add smaller, schooling fish to the exhibit resulted in them becoming food for the larger predators, especially the nocturnal red-tail catfish (*Phractocephalus hemioliopterus*). Therefore it was determined that any successful introduction of new fish must be predicated by the removal of those predators. Subsequently, the red-tails were removed from the tank, the new species were introduced and allowed to acclimate, and then the catfish were slowly re-introduced to the exhibit.

After 12 months and all six catfish back in the tank, all the newly introduced species successfully co-exist with the red-tail catfish. Although losses did occur, the impact was not enough to diminish the value of the added fish.

***Pleine eau* Seaweed: Habitat-based Aquascaping at Monterey Bay Aquarium**

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The Monterey Bay Aquarium is a habitat based aquarium. Most of the permanent exhibits at the Monterey Bay Aquarium are based on specific ocean habitats. These habitats are designed based on habitat accuracy and an in-house aesthetic commonly called aquascaping. This talk will discuss aquascaping at the Monterey Bay Aquarium and how it includes collection, design, and husbandry of algae, invertebrates, and seagrass.

## Cnidarella - Not a Fairy Tale

Marie Collins
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One genus a majority of all aquariums have in common is one pesky Cnidarian—*Aiptasia*! These stinging hydroids are often a common thread upon discussion groups and are often a difficult invertebrate to conquer. Once *Aiptasia* starts to bud, and it is abundantly known to reproduce very quickly, it can be a major challenge to eliminate. Especially if you don't have a *Berghia* nudibranch colony growing at your facility. There have been many discussions, commonly amongst reef keepers, on best practices to destroy them. This presentation will discuss the many trials and treatments done at SEA LIFE Aquarium @ LEGOLAND California Resort, but also amongst the SEA LIFE Aquarium group as a whole. A survey will reveal the most successful methods of successes and failures among the world's largest aquarium brand, with 50 aquariums worldwide.

There are three main ways to reduce this species—through biological, mechanical, and chemical methods. Biological successes can be attributed to *Aiptasia*-eating filefish, *Acreichthys tomentosus, Berghia nudibranchs*, *Chelmon rostratus, Lysmata wurdemanni*, and even *Asterina miniata* (in ambient temperatures). Mechanical or physical removal of species can be done by the use of a heat gun or an electric laser, called an "Aiptaser." This essentially removes the polyps by use of an electric wand by using low voltage electrical current to create hydrogen gas from the saltwater. Thus, chemical removal has been done by using other chemicals such as "Aiptasia X", vinegar solutions, bleach solutions, and most recently the use of chloroquine. Chloroquine has been the most successful method of chemical reduction of hydroids at our facility from doses at 10-25ppm. This method has been used extensively on sensitive species (juvenile *Hippocampus* sp., *Heteroconger* sp. and other scale less fishes). Photographs and videos will be shown to document the potential uses of the treatments.

## Promoting *Chrysaora quinquecirrha* Growth without Using Jellies. Is it Possible?

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*Chrysaora quinquecirrha*, the Atlantic sea nettle, is a jellyfish species common in the east coast of the United States, such as the Chesapeake Bay. In captivity, it is considered essential to provide other jellies as food to achieve success in *C. quinquecirrha* growth from ephyrae to adults and to maintain the adults. In order to test if it was possible to raise *C. quinquecirrha* without using jellyfish as food, an experiment using Moon jellyfish (*Aurelia aurita)* and Atlantic herring (*Clupea harengus*) mash as food for the ephyrae was conducted. The respective growth rates, as well as mortalities, were studied. Results showed that although the herring mash diet did not promote the transition from ephyrae to juvenile stage, the jellyfish mash diet had higher mortality. A different experiment using adults instead of ephyrae was conducted to test the effect of these two diets. Observations indicated that the adults fed with the herring mash developed a "stronger" and "firmer" mesoglea.

**Let's Get Kraken: Managing a Diverse Collecion of Octopuses**

Chris Payne
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The new cephalopod gallery, Tentacles: Astounding Lives of Octopuses, Squid and Cuttlefishes has allowed us to work with over ten species of octopuses from around the world. We have worked through several challenges while learning different husbandry methods and techniques for each species and how each species best fits into our exhibit needs.

The exhibit features five octopus exhibits but we accommodate several other species behind the scenes in a number of different holding areas. Since the initial inception of research and development for the gallery, we have worked with 11 confirmed species and 3 unknowns. These animals are held in tanks ranging from 10 gallons to our largest exhibit, 483 gallons.

The greatest challenges are the short life spans, sourcing the animals, and finding a balance between exhibitry and proper animal care.

**Sponsor Presentation – Mazuri**

## Wednesday, March 4

***Session 5: Propagation***

**Sponsor Presentation – Quality Marine**

**Project Coral - Captive Broadcast Coral Reproductive Research at the Horniman Museum & Gardens**

Michelle Davis
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In 2013 the Horniman Museum & Gardens became the first institution globally to predictably and purposefully induce broadcast coral spawning events in captivity. Reacting to this achievement the museum official founded Project Coral, an umbrella, multiyear coral reproductive research project with two broad aims.

- To understand how climate change affects the ability of broadcast corals to reproduce.
- To develop techniques to enable the sexual reproduction of corals in captivity to facilitate the sustainable aquaculture of coral.

Utilising two microprocessor controlled coral research systems at the museum a number of experiments, in collaboration with our partners are being conducted. Each experiment is / will focus on a specific area of coral reproduction and will contribute significantly to our understanding of the larger aims of Project Coral.

In the initial stages protocols will be developed to reliably induce spawning events. These will then provide the foundations for more direct environmentally relevant research investigating the impacts of climate change on broadcast and brooding coral reproduction and our understanding of coral reef resilience.

## Mating, Birth, Larval Development and Settlement of Bargibant's Pygmy Seahorse, *Hippocampus bargibanti* (Whitley, 1970), in Aquaria.

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Pygmy seahorses are among the most charismatic inhabitants of coral reefs. Their diminutive size, impressive coloration and rarity make them attractive to recreational divers, underwater photographers and videographers. However, very little is known about their life history and ecology due to their relatively recent discovery and the difficulty of studying them in the wild.

In May of 2014, Steinhart staff successfully collected a mated adult pair of *Hippocampus bargibanti* (Whitley, 1970) from the Anilao region of the Philippines. These were subsequently transported to Steinhart Aquarium, California Academy of Sciences, San Francisco in order to conduct research on their husbandry and life-history. The pair of seahorses mated in their aquarium and gave birth to three broods of larvae. A portion of these were raised through settlement to a maximum of 120 days from birth. This presentation will describe the collection, transport, and husbandry of the adult seahorses, as well as larval husbandry, process of host coral selection, settlement, and associated color change of juveniles.

The results of this study mark the first time that a pygmy seahorse species has been bred and reared in captivity, offering opportunities to study brood size, gestation period, growth rate, host selection and settlement. Despite our successes, we were unable to raise the juveniles beyond 22.2 mm SL, which was 73% of the maximum size of the adults collected (30.5 mm SL), indicating a need for additional study of their feeding and nutritional requirements post-settlement.

## The New Seadragon Propagation Program at the Birch Aquarium at Scripps

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The Birch Aquarium at Scripps has a long history of working with seahorses and other syngnathids since 1994. These charismatic animals have become important ambassadors to emphasize conservation of fragile marine habitats. The popularity of weedy and leafy sea dragons in aquariums prompted us to initiate a propagation program for sea dragons in 2013.

Our sea dragon propagation program involves many aspects to ultimately attain success. These are the questions we asked ourselves: What would be an appropriate sea dragon propagation space? What type of life support would we need? What should the proper nutrition and feeding regime be? How will we be able to observe behaviors? Would our visitors have access to see these sea dragons? What about possible inbreeding?

These questions and more will be discussed. How we moved forward and the current developments with our sea dragon propagation program will be presented.

### Alternative Methods in Strobilating *Aurelia aurita*

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Strobilation is the metamorphosis from schyphistoma to strobila in schyphozoan jellyfish. In nature this is induced by abiotic factors such as fluctuations in temperature, pH, and salinity. In a controlled setting, changing temperature is the most common method for inducing strobilation. This, however, may not always work. Lugol's solution which is a combination of elemental iodine and potassium iodide has been used historically in public aquariums to strobilate schyphozoan jellies. Recent changes in laws regarding iodine have made obtaining Lugol's solution more difficult. I tested the effectiveness of more common forms of iodine against a drug known as 5-Methoxy-2-methylindole. 5-Methoxy-2-methylindole gave more consistent results by strobilating polyps of *Aurelia aurita* due to its structural similarity to the protein CL390 which is thought to be a strobilation inducer in that species.

### The Frugal Rotifer Reactor: Raising Rotifers on a Budget

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*Brachionus plicatilis* (or L Type Rotifers) are commonly used as live foods for corals, sea jellies and hatchling fish. Keeping a large culture going for any length of time is often a challenging experience. Over the years the Cabrillo Marine Aquarium has gone through many trials and tribulations while raising rotifers and hope to share some techniques that will save you time and money. We can reliably expect to harvest approximately 30 million rotifers for a paltry $1.25 per day using yeast as a main food source and supplemented with algae paste. Looking for a smaller alternative to a large rotifer reactor? We will also share with you our 70 liter DIY rotifer reactor build.

### Sponsor Presentation – TJP Engineering

**Sponsor Presentation – Ecoxotic**

## Bigfin Reef Squid (*Sepioteuthis lessoniana*) Display and Culture Through Multiple Generations

Bret Grasse
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In the aquarium industry, displaying cephalopod species can be both challenging and rewarding. Some cephalopod species are more suited for captive conditions than others because of their biological and environmental requirements. Squid display can be particularly challenging due to their short life span, high metabolism, cannibalistic tendencies, and paralarval sensitivity in culture. Although most squid species have been problematic to culture and keep in captivity, bigfin reef squid (*Sepioteuthis lessoniana*) are an exception. Here we examine enclosure design, egg care, feeding strategies, and shipping considerations for successful bigfin reef squid display and culture through multiple generations.

## "Are You My Daddy?" A Closer Look at Parthenogenesis and Artificial Insemination in Zebra Sharks (*Stegostoma fasciatum*)

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For the last three years, the Aquarium of the Pacific, in Long Beach, CA has been raising zebra shark pups (*Stegostoma fasciatum*). Although there is one sexually mature male at the facility, genetic testing has revealed that all pups are the result of parthenogenesis. Since parthenogenesis decreases genetic diversity in captive populations, the vet staff at the Aquarium of the Pacific, led by Dr. Lance Adams, developed a technique to artificially inseminate zebra sharks. This procedure successfully produced two healthy zebra shark pups. During this presentation, I will discuss the different environments/exhibits in which parthenogenesis has occurred. In addition, I will present data collected from eggs, unborn neonates, and pups, as well as suspected genetic deformities found in nine parthenogenetic pups. Lastly, I will describe physical differences between parthenogenetic pups and artificially inseminated pups.

## The First Successful Captive Breeding of Common Shovelnose Rays (*Glaucostegus typus*)

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The common shovelnose ray, *Glaucostegus typus*, is a species of guitarfish native to Australia and the Indo-Pacific. The species is listed as vulnerable by the IUCN due to overfishing, bycatch and habitat destruction. The Downtown Aquarium in Houston, TX houses 2.1 adult common shovelnose rays. In October 2013, the female gave birth to 11 premature stillborn pups. This represented the first documented captive breeding of the species. One year later, in October 2014, she gave birth again, this time to 17 live pups. This presentation will discuss care of the adults, the birthing event, care of the pups and future research.

## The Use of Assisted Reproductive Technologies in Breeding Programs for Elasmobranchs in Aquaria

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Despite the use of assisted reproductive technologies (ARTs) in terrestrial and aquatic species, very little work has been done with elasmobranchs. ARTs such as sperm collection and quality assessment, sperm cryopreservation, artificial insemination, and monitoring female reproductive condition and gestation could potentially be used to complement existing breeding programs for elasmobranchs in aquaria. As a greater emphasis is placed on self-sustaining aquarium populations, ARTs will become an increasingly important component of breeding programs for elasmobranchs in aquaria. Ongoing research at Sea Life Melbourne Aquarium, Australia, since 2004 aims to create a basis for future use of ARTs in elasmobranchs in aquaria worldwide. This is to ensure sustainable captive populations of elasmobranchs, as well as having the potential for conservation of species in the future. The achievements to date as well as future areas of research will be discussed.

## Sponsor Presentation – Reed Mariculture

**Sponsor Presentation – RK2**

**Sketch It Up: An In-house Approach to Life Support System Design and Development**

Micah Buster, Meghan Atkinson, Clare Hansen, Ryan Hannum
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As part of the Life Support Operation and Design course in the Aquarium Science Program at Oregon Coast Community College, students were teamed up to deliver a term project with the instructions to design a hypothetical life support system for a provided scenario. The project included design criteria and calculations, specifications on equipment, drawings, a budget, operational SOPs and schedule through installation and start-up. This presentation will be delivered by one of these student teams with a primary focus on how the utilization of online available 3D modeling tools such as Google SketchUp can aid husbandry and exhibits personnel in the design and development of in-house projects for life support systems and exhibits. The use of such tools can help facilities reduce design costs, communicate to stakeholders, ease installations, improve operational efficiencies and benefit staff development. The students will also share their experience in developing their term project in relation to applications and lessons learned on how to possibly approach life support project design for the benefit of in-house projects at aquarium facilities.

**Design, Construction, and Operation of a Modular Sulfur Denitrification System**

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Nitrate accumulates in recirculating aquarium systems as a result of filtration processes such as bacteriological denitrification and ozone sterilization. Nitrate control is an important aspect of aquarium water quality control and life support management due to the harmful effects of nitrate on sensitive aquatic organisms. Operators of closed aquatic systems have typically managed nitrate levels via water exchange. However, biological denitrification has become an attractive method of nitrate remediation in aquaria due to the costs of transporting or mixing seawater in large-scale aquarium applications. The Life Support Department at the California Science Center has been investigating the efficacy of a sulfur denitrification system designed to remove nitrate in a 200,000 gallon living kelp forest exhibit. Key design factors include hydraulic retention time, nitrate loading rate, and nitrate removal efficiency. Designers implemented strategies to mitigate potentially harmful water quality effects resulting from incomplete or partial denitrification.

## Act Like You Own the Place Before You Even Do

Love Ruddell and Joe Arlotto
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This was a presentation I gave to AALSO about new construction projects and the importance of having a Life Support Technician involved from the beginning. It weighs the importance of the position and knowledge vs not having someone on site during projects. It reflects and follows a major undertaking we had here at the Zoo for our Elephants and what could have been done differently. It also explains what an LSS technician should be able to provide a facility and the importance of LSS and animal care staff working together and understanding each other. The presentation explains many misunderstood aspects of a new construction project (change requests, RFI's, inspections, types of engineers etc.) and as a technician and animal care person how to survive a process like this in the most positive manner. I feel this information allows upper management to understand and reflect on the stress the front line employees feels during this process and allows your front line staff to understand more of what they don't see in a project.

## Techniques for Manufacturing Artificial Seawater

Bart Shepherd, Laurie Kormos, Matt Wandell, Arnel Bautista
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For nearly ninety years, Steinhart Aquarium's marine exhibits relied upon natural seawater obtained through a Ranney collector system at San Francisco's Ocean Beach. Despite a recent renovation of the beach pumping station, incoming water quality was highly variable. Because of the inconsistent quality of incoming seawater, specifically low salinity and high phosphate, a decision was made in 2010 to switch to synthetic salt water. In order to optimize this change, a team of biologists, life support operators, and animal health personnel conducted a thorough review of available commercial and institutional salt mixes used in public aquaria throughout the United States. The development of this formulation consisted of careful identification of suppliers, thorough water chemistry analysis and biological testing on live organisms including delicate reef-building corals. Formulations vary and can be tailored to fit local source-freshwater chemistry, the location of the facility in relation to commercial suppliers of major ingredients, and other specific institutional factors.

## Life Support Systems and Exhibit Design for Pelagic Gelatinous Species

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Pelagic gelatinous animals like cnidarians, ctenophores, and pteropods present a number of challenges for captive display: they are often difficult to culture or capture in the wild, their bodies are physically delicate, they have unique Life Support System (LSS) needs, and they require specialized enclosures and food. In order to successfully design exhibits for these animals, it is essential to understand the biomechanics of the different phyla of gelatinous organisms. Through the use of fluorescein dye injected into the pathway of swimming jellies, we can elucidate the ideal flow regime and enclosure design for displays through the visualization of these flow patterns. Kreisels (pseudo, true, or stretch) are better suited for ctenophores, pteropods, and "weak swimming" cnidarians, while "active" swimming cnidarians can be maintained in a variety of tanks shapes and flow regimes. The fluorescein dye technique is also helpful for visualization of the flow fields around system injection boxes and allows one to find areas where flow may damage jellies. LSS components are critical to a well-designed system. Foam fractionators have a large impact on the concentration of DOC (dissolved organic carbon), which negatively affects the uptake of dissolved nutrients for some gelatinous species. Other key LSS components, such as de-gas towers, light (both for exhibit lighting and as a component of LSS), heat exchangers, pumps, kreisels and other tank designs, and the use of LSS bypasses are essential to good design.

**Sponsor Presentation – YSI**

## Wednesday, March 4

### *Session 8: Husbandry*

**Sponsor Presentation – Two Little Fishies**

**Floating Freshwater Treatment System for Giant Black Seabass (*Stereolepis gigas*)**


Erin Lyman
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For years the Monterey Bay Aquarium has successfully used routine, prophylactic freshwater treatments to treat monogenean infestations on the three Giant Black Seabass located in the Monterey Bay Habitats exhibit.

These specimens are getting large with the biggest one weighing in at just under 300 lbs. In an effort to decrease the handling stress on these endangered animals as well as protect husbandry staff from injury, we created a floating bath technique so that the freshwater bath could be performed in the saltwater exhibit without having to remove the animal from exhibit and into the service area. The treatment process is much quicker, requires half the staff with no heavy lifting and the animals are returned to exhibit faster making it easier on animals. This floating treatment structure is easy to build and can be used for a variety of treatments and on any large animals.

### A Submersible Hyperbaric Chamber for Decompression of "Twilight Zone" Fishes

Matt Wandell
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The California Academy of Sciences is currently engaged in exploration of mesophotic coral reefs between 50-150 meters depth (aka the "Twilight Zone") and is committed to a future exhibit of fishes and corals from this habitat. One of the challenges with collecting and exhibiting fishes from this depth is preventing barotrauma from the relatively rapid ascent to the surface. Methods such as swim bladder venting with a needle or caging the fishes were considered less ideal than a slow and controlled ascent in a hyperbaric chamber. In this presentation we will describe how we adapted a boat/land-based hyperbaric chamber design from Joe Welsh of the Monterey Bay Aquarium into a compact and submersible hyperbaric chamber that can be easily staged underwater during rebreather dives. Once on land, the system relies on a diaphragm pump that exchanges water under pressure and an adjustable pressure relief valve to regulate the pressure inside the chamber. Early results have been encouraging and examples of its use will be presented. Future plans for modifications and deployment will also be discussed.

### The Effectiveness of Compression Treatment for Deep-Sea Demersal Fishes Using a Gravity-Produced Pressure Chamber

Hiroko Takaoka
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Rapid reduction of water pressure is the most serious limiting factor for collecting and keeping deep-sea fishes in aquariums. To treat the symptoms of decompression sickness, so-called barotrauma of the fishes collected from the deep ocean below 200m, we have designed a gravity-produced chamber in Okinawa Churaumi Aquarium. This chamber system consists of a 190L and a 3000L tank on the ground floor of the aquarium which hold 4 smaller satellite tanks, respectively on the 1st floor (1.5m), 2nd floor (5m), 3rd floor (10m) and 4th floor (18.5m) . It is a circulating system, each tank with inlet filters to help preserve water quality, and with shut-off valves on each floor to create variable pressures in the holding tank. Live deep-sea fish for exhibition are collected by rod & reel and baited traps, both methods commonly used by Okinawa's local deep-sea commercial fisheries. Collected fish are quickly put into cold water tanks with oxygen, sometimes releasing air from their air bladder first, and then transferred to our gravity-produced pressure chamber.

This system has helped us successfully keep over 100 species from depths of 200 to 650m from around Okinawa, such as *Triodon macropterus*, *Cephalopholis igarashiensis* and *Parmaturus pilosus*.

This chamber has shown great effectiveness, therefore we have been able to focus on other challenges, such as displaying the fish and technical developments for reproduction.

**A Comparison of Various Disperal Methods of Live Aquatic Food Items and Liquid Food Supplements Using a Laser Nephelometer to Monitor Changes in Food in an Aquarium over Time**

Dave Smith
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Goal: To evaluate the effectiveness of three methods of liquid food (nauplii, live algae, liquid supplements) dispersal in an aquarium display based on the following criteria:

1. Distributes food items in a manner that does not impact visitor experience nor exhibit husbandry.
2. Food is fed out in significant densities so that exhibit filter feeders can acquire enough food to meet nutritional requirements.
3. Food is fed out over multiple intervals to closer resemble natural food availability throughout the day.
4. Live food items are maintained in optimal conditions ensuring good water quality and low mortality.
5. Food items are fed out in a cost effective manner that reduces excess food items going down the drain without being consumed.

Food density and duration of food in the water column will be determined by turbidity levels using a laser Nephelometer.

I am comparing three different methods to distribute naups/algae to the exhibit over the course of three days:
Method 1: Addition of naups/algae directly into to tank 2x/day.
Method 2: Add the naups to the "standard" auto feeder used at MBA with a water timer controlling how fast the food items drain out of the display.
Method 3: Use a water timer on a FSW line to supply batches of water to the auto feeder. The "modified" auto feeder has a Hartford loop, allowing the feeder to flush out food while maintaining a constant volume of water in the auto feeder.

**Collection, husbandry and transport of *Naucrates ductor***

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This is a report on the capture, transport, and husbandry of pilotfish, *Naucrates ductor*. The objective of this work was to investigate and essay the most adequate process for capturing, transporting and maintaining pilotfish, while gaining valuable understanding on their behavior. Collection was done in the Azores archipelago, approximately 25 nautical miles off Horta, the capital of Faial island. They were captured using a standard fishing rod and hook, after attracting blue sharks with bait. Such is done because pilotfish normally associate with larger animals, particularly blue sharks in this region. 76 individuals were then transported by boat, with regular water changes to the Porto Pim Aquarium, where they were maintained for 2 months. After this, they were transported by sea over 3 days inside a 40 feet shipping container, while swimming in round polyethylene tanks equipped with both mechanical filtration and foam fractionation. This transport was then followed by an 8 day long transport by road along Portugal, Spain and multiple public aquaria in France. These individuals endured the entire transportation with no losses and showed remarkable resistance to extreme temperature fluctuations, amongst other critical changes. The multiple challenges faced upon all stages of the process (i.e. collection, holding, transport and introduction) are discussed in detail, especially the moment of introduction, where massive losses (due to predation) were regrettably frequent in all institutions. Transports in sealed plastic bags were later conducted and results on both simulated and real shipments are presented and discussed in detail as well.

## Thursday, March 5
### *Session 9: Animal Health*

**Sponsor Presentation – Fritz Aquatics**

**Quantification Method for *Decacotyle floridana* after Praziquantel Treatment in Spotted Eagle Rays**

Charlene Burns
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Aquariums are experiencing morbidity and mortality challenges with a monogenean fluke, *Decacotyle floridana*, found in the spotted eagle ray, *Aetobatus narinari*. Praziquantel baths have been a longstanding treatment against external monogeneans but are not often effective in eliminating these infestations completely. In order to better evaluate the effectiveness of treatment, a standardized method to quantify monogeneans removed from rays after praziquantel baths was developed. Treatment water is filtered to collect the monogenes, the sample is then suspended in a sugar base solution, and microscopically evaluated to quantify the number of monogeneans. Using this technique, we can better determine how successful trial treatments are against this resilient parasite.

**Identifying an Effective Treatment for Lobster Shell Disease**

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Shell disease is an infection of emerging concern in lobsters and other crustaceans, both in aquarium exhibits and in wild populations. Shell disease results in disfiguring erosive lesions and, in severe cases, death. Currently, there is no effective treatment for shell disease. The existing best practice is limited to waiting for the lobster to shed the diseased shell and hoping that the new shell avoids infection. The New England Aquarium's Lobster Research and Rearing Facility maintains hundreds of juvenile lobsters and conducts research to optimize lobster husbandry and health. With the aim of identifying an optimal treatment for shell disease in captive American lobsters, we compared the effectiveness of five different treatment methods, including betadine, formalin, malachite green, vitamin E oil, and freshwater, as well as an untreated control (n = 8 lobsters per treatment). Lobsters were intentionally punctured on one claw to simulate a wound leading to shell disease. Starting one week post-puncture and continuing over the course of one molt cycle (approximately 90-120 days), each lobster was treated 2-3 times per week with a bath (formalin, malachite green) or topical application (other treatments). The lobsters were analyzed every two weeks for the progression of shell disease on each animal (number of body areas affected) and the spread of lesions on the puncture wound (change in affected surface area). We expect the results to indicate an optimal course of treatment and to suggest further measures to improve the management of shell disease in lobsters and other crustaceans in public aquariums.

## The Effects of Deslorelin Implantation on the Reproductive Steroid Hormones of Female Freshwater Singrays

Jolene Hanna
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This study was designed to define the reproductive steroid hormone response within female freshwater stingrays once they had been challenged with deslorelin; a GnRH agonist and effective contraceptive used predominantly in mammalian species. Changes occurring within two principal hormones associated with reproduction in elasmobranchs (17-β estradiol and progesterone) were monitored for a 12 month period. Six female Potamotrygon castexi hybrids currently housed within the Newport Aquarium animal collection were placed within the parameters of the project. Four of the six animals received deslorelin implants while the remaining two animals were used as controls for baseline hormone dynamics. The primary goal of the study being the characterization of the length of estrous suppression associated with the implantation of deslorelin in an elasmobranch species. Secondary goals included changing the ability or inability to house heterospecific freshwater stingray species within the same exhibit without concerns of hybridization.

## Aulani System Sanitation Protocol 2.0

Eric Curtis, Marj Awai, Jeff Sedon, and Kirk Murakami
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Eric.W.Curtis@Disney.com

In 2014 at RAW, Marj Awai presented our changed quarantine procedures and several hypotheses as to why we so far have had no significant disease outbreaks on exhibit despite significant "Biosecurity" challenges. Fast forward to 2015 and we will discuss our experience with the *Cryptocaryon* life cycle in our quarantine systems here at Aulani, a Disney Resort and Spa, and the resulting changes in system sanitation protocols whenever pathogens are suspected. The details of our new multistep sanitation protocol will be discussed but include draining, acid cleaning, freshwater, 60 ppm chlorine, drying, filling and adding bacteria to the system. We will cover some other possible reasons for the lack of disease outbreaks including water calcium level. Our salt water well has significantly elevated calcium levels (average 757 ppm vs. 418 ppm for Ocean). We will present some interesting ways that elevated calcium levels in water can affect fish along with possible ways it might also affect protozoa.

**Medical Aquarist, What Is It and Why You Want One**

Rebecca Bray and Sarah Halbrend Monterey Bay Aquarium
Mystic Aquarium and Monterey Bay Aquarium
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As advances in Aquatic Medicine continue to be made, particularly in the area of fish and invertebrates, there are more and more successful ways that Veterinarians and Aquarists can address animal health concerns as they arise. How does a facility effectively manage the number of health concerns that a collection of thousands of individual animals can generate?
With a Medical Aquarist.

This talk will cover the evolution of the Medical Aquarists' role at Mystic Aquarium and how it has aided in the overall care of our collection; how it has drastically improved the communication between the Fish & Invertebrates Department and the Veterinary team; how to potentially implement the position at other facilities; and the basic requirements of the position.

**Sponsor Presentation – Brightwell**

## Wednesday, March 5
### *Session 10: Conservation and Research*

**Sponsor Presentation – RocknReefs**

**Advancements in the Management of Sea Star Wasting Syndrome in Aquaria**

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In the State of Washington, Sea Star Wasting Syndrome (SSWS) was first observed during the summer of 2013. It has now spread to sea star populations all along the West Coast of North America. SSWS events have occurred in the past, but the range and severity of the current outbreak is unprecedented. The Point Defiance Zoo and Aquarium (PDZA) is geographically located on Puget Sound, and has been significantly impacted by SSWS with the loss of over 80% of exhibit sea stars. Success in halting, and in some cases reversing the syndrome has been observed in PDZA's sea star collection with the use of Sulfamethoxazole Trimethoprim immersion baths. This presentation will briefly review what is presently known about SSWS, give an overview of the SSWS research program at PDZA, and offer recommendations for moving forward in successfully managing SSWS in closed and open sea water aquariums.

## Communication in Flamboyant Cuttlefish (*Metasepia pfefferi*): Investigating Body Patterning

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Cuttlefish are known for their ability to quickly alter their appearance to camouflage or to communicate information to predators, prey and conspecifics. Like any form of communication, a visual communication system is limited by the number of signals that are recognizable between individuals. To better understand the behavior of these animals, their signaling systems and thus their body patterns have been extensively studied in some species of cuttlefish. Unfortunately, they have never been thoroughly investigated in the Flamboyant cuttlefish (*Metasepia pfefferi*) and thus all behavioral and communicatory inferences are based on studies of other species. This study aimed to identify all of the components of *M. pfefferi* body patterns that are visible to the human eye and to determine the most probable number of patterns used by this species. It was determined that aquarium-raised *M. pfefferi* generated 89 chromatic, 10 textural, 16 postural and 8 locomotor components which were combined to create 11 distinct patterns. Unlike other species of cuttlefish, none of the 11 most probable patterns appeared to be useful for camouflage in their current environment, suggesting that this species used its appearance-altering abilities to communicate more frequently than to camouflage. Similarly, 8 of the 11 identified patterns contained at least one "moving" component in which the colors on the skin appeared to travel on the animal's mantle. In other species, these "moving" components were generally only seen during feeding or aggression, but *M. pfefferi* utilizes them frequently in a variety of contexts. These findings indicate the necessity to study the body patterns and communication methods in various species of cuttlefish to better understand their species specific behavior.

## Pecos Pupfish *Cyprinodon pecosensis* Conservation at the Fort Worth Zoo

Robyn Doege
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The Fort Worth Zoo works towards conservation efforts for our native Texas species in particular. The Pecos pupfish *Cyprinodon pecosensis* is one of the aquatic species we work with. This species *was* once one of the most abundant fish in the Pecos River system. Today, there is only a 3.2 mile stretch of Salt Creek that holds non-hybridized individuals making them vulnerable.

In 2010, I was put in charge of finding Pecos pupfish specimens to be displayed in one of our Texas Wild Exhibits. The zoo had previously housed this species years ago with some success, but the collection was not sustained. During the drought of 2012, Texas Parks and Wildlife was concerned that the pools and streams in the Pecos Salt Creek region would dry up by summer's ends. Due to this drought, the Fort Worth Zoo offered to help house specimens as a refuge population. Since that time, TP&W and the Fort Worth Zoo have worked together keeping a small breeding population on site.

Repatriation into the wild has begun with the construction of new pools on private land during January of 2014. The Rillito Springs Pecos Pupfish Project was constructed. It added two new pools where pupfish were reintroduced. At this time, Texas Parks and Wildlife is working alongside New Mexico Department of Game and Fish, New Mexico State Parks, U.S. Bureau of Land Management, and U.S. Fish and Wildlife Services to come up with a conservation agreement to protect this species historic range.

## The Role of Aquariums in the Recovery of Australian Grey Nurse Shark Populations

Mark Smith (New England Aquarium)*, Rob Jones*, Rob Townsend, Carolyn Hogg, Craig Thorburn, Trevor Long, Kate Willson, Marnie Horton, Paul Hamilton, Sebastian Schmidt, David Blyde, Stephen Menzies and Jon Daly
msmith@neaq.org

The grey nurse shark, *Carcharias taurus*, referred to as the sand tiger in the USA and ragged-toothed shark in South Africa, is an iconic species in Australia. Once abundant, the Australian East Coast population is now listed as critically endangered under the Environment Protection and Biodiversity Conservation Act, with population estimates ranging from 1,000 to 2,000 individuals. Primary threats to the species were, historically, recreational spear fishers and commercial fishers. Threats now constitute recreational fishers, commercial fishers and habitat degradation. It is now common to see specimens with fishing gear trailing from their mouth and anecdotal estimates place this incidence as high as 50% of the population. In 2002 the Federal Government released a DRAFT Recovery Plan listing six primary anthropogenic threats to grey nurse sharks, including “collection for aquaria”. The report recommended a moratorium on the take of grey nurse for public aquaria, which has been in effect since 2002. In 2013, a group of concerned stakeholders convened a meeting to discuss measures to mitigate and reverse the population decline of East Coast grey nurse. During this meeting regulators acknowledged that existing measures had not reversed the population decline, and further, that partnering with public aquaria was crucial to successfully addressing this troubling trend. With teams of skilled personnel, and a visitor-ship exceeding 10% of the Australian population, the aquarium community can provide a critical support role for the implementation of the Recovery Plan in three primary areas: (1) research and research support, (2) intervention / rescue, and (3) advocacy and education.

## Seahorse Roundup: Establishing a Genetic Baseline for the Lined Seahorse, *Hippocampus erectus*

Nancy Pham Ho and Steven Yong
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Seahorses are consistently listed as one of the most popular exhibit animals at public aquariums. However, they're not just another pretty face. Seahorses are often considered an indicator species for healthy coral reefs and sea grass beds. Their popularity makes them an excellent topic for conservation, research, and outreach, which are high on the list of priorities for our institutions and Species Survival Plans®. This presentation will review a wild population genetics research project led by Vero Beach Marine Lab researchers and the lined seahorse SSP. We will report on preliminary results, challenges, how this data will be used, and possible future opportunities. We will also discuss how this project involved collaboration not only between public aquariums and a marine lab; but also incorporated participation from government agencies, aquaculture facilities, and citizen scientists.

**Sponsor Presentation – Reynolds Polymer**

## Wednesday, March 5

### *Session 11: Conservation and Research*

**Sponsor Presentation – Seachem**

### Spawn Induction of *Acropora cervicornis*

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During the coral spawn of 2014 in the Florida Keys, The Florida Aquarium, the University of Florida and The Coral Restoration Group came down two weeks prior to the natural spawn event to examine various strategies to chemically induce spawning in Acropora cervicornis. Dr. Mark Flint, Dr. Josh Patterson and I set up a battery of self-contained systems that were used to house fragments of A. cervicornis collected directly from the Coral Restoration Foundation's Tavernier nursery and selected for their mature gamete bundles. Mr. Ken Niedermeyer of the Coral Restoration Foundation confirmed genotype and maturity.

Five chemical cues proven to have positive effects in induction of spawn in other invertebrate species were tested on over 100 coral fragments.

Methodologies included serial replicated assessment of various concentrations. Results were consistently supportive of chemicals known to be successful in mollusk reproduction.
Future experimentation will further refine these techniques, chemicals, dosages and application to coral restoration efforts using sexual reproduction.

### Around the World and Back to Save Nautiluses

Gregory Jeff Barord
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Nautiloids have survived all five mass extinction events in Earth's history on their own. However, without human intervention and help, nautiloids may not survive the current extinction event taking place. While unregulated fisheries appear to be the most significant factor of nautilus population decline, the effects of climate change may also affect nautiluses and are even less understood. Thus, nautilus conservation research has become vital to providing effective regulation and management of the remaining nautilus populations. But what is nautilus conservation research? Well, with an organism largely unknown to the world and with little information on its biology and behavior, nautilus conservation research is many, many things. It means grant writing and airline traveling and trap setting and video watching and genetic testing and maze running and teaching, to name a few. Over the last few years, our research team has traveled near and far to collect data and create awareness for nautilus conservation efforts. As we get closer and closer to our goal of saving nautiluses, our research expands further and further. Recently, our research has expanded to Central Campus High School in Des Moines, Iowa. Yeah, that's right, I said Iowa. Having recently taken over the Marine Biology program at Central Campus, one goal for this program is to connect high school students to the 500 million year old nautiloids and provide opportunities for these students to conduct their own research to help save nautiluses. Here, I will provide updates on our research efforts in the field (most recently from Vanuatu and Philippines), current laboratory work at Central Campus, and our future plans to continue engaging the next generation in nautilus conservation and the importance of our oceans in general.

## It's All About HEAD Pressure and Pump Efficiency: Building Energy Efficient LSS

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When budgeting and planning for renovations where does energy consumption fit? What are the most important energy inputs and where can savings be maximized? Here we demonstrate the importance of placing energy consumption as the top priority during a complete renovation at the Audubon Society of Rhode Island, Environmental Education Center's aquarium. We designed life support systems for exhibits of 600 to 2000 gallons that consumed 50% less power, require less consumables (water and filter bags), produced less system heat, and reduced noise by 90%. This was accomplished by using low head efficient pumps, installing insulated sumps, insulating tanks, and engineering life support systems away from the outdated high-energy consumption sand-filter.

## LED Lights and Corals: Truth Is All in the Photos.....and Data!

Grant Anderson
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As more aquarists are making the leap from traditional metal halide lit coral ecosystems to LEDs more data is needed. Over the past 18 months we have been continuing to gather data and evaluate LED lights for consistency in PAR output, color spectrum, spread, operational costs, and reliability. For this study we have continued to measure PAR values to show the amount of PAR concentrations and losses over the course of 18 months. We have also been monitoring the spectrum consistency to see which LED lights stayed in the correct color needed for coral health and growth. In doing the following two measurements we have also determined the spread value loss for such LED lights. These three very important measurements are what keep our corals healthy and growing nicely. When any of these parameters change, coral health concerns emerge. LEDS are not changed yearly like the metal halide counter parts; this leads to making sure the above parameters stay within a strict coral growth and health guideline. When evaluating which LED light is correct for a certain application you need to make sure it is also reliable. With 18 months under moist, warm, and salty conditions which lights stood up to the harsh environments coral exhibits lights are kept in? We also evaluate the significant cost savings in chillers, maintenance, AC costs, and general electrical consumption our facility achieved. By adding yet another new manufacturer to our testing grounds we feel we have finally broke into the 1000 watt equivalent LED. Not being a cannon style we were very skeptical, however the truth is all in the data. The photos taking over the 18 month study will conclude corals grow exceptional well while also maintaining health so as long as the LEDs maintain a consistent output in above parameters

**Sponsor Presentation – Kessil**

## Wednesday, March 5
### *Session 12: The End!*

**Sponsor Presentation – Living Color**

**Smack of Plastic Jellies?**

Julianne E. Steers
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Our oceans and waterways are polluted by plastics and other synthetic non-biodegradable materials. Marine debris is commonly mistaken by marine species as prey, such as jellyfish. While many medusae have been viewed in aquaria for a few decades, few have seen the plastic bag variety circulating around in captivity; however, in the wild the environmental consequences of this species are many. The Ocean Institute always strives to produce effective methods of environmental education; a visual of the issue at hand speaks volumes. Through trial and tribulations, a successful display of plastic bags that resemble jellyfish is now possible for any budget and utilizing existing systems. Material selection, methods for creating your very own smack of jellies, and their husbandry is key to establishing this impactful exhibit. Apparently, plastic bags do look like jellyfish—to a sea turtle…and humans too.

**Creating a Plan for Knowledge Management in the Aquarium Industry:
Why Reinvent the Gravel Vac?**

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The aquarium industry, like many others, is nearing a time of great generational turnover. The outgoing generation put public aquariums on the map and has made our industry what it is today. In A Note on Knowledge Management, Artemis March writes that a large amount of professional knowledge is the property of individuals, not the firm. All too often, when an employee leaves an organization, their expertise leaves with them (1997). Although our industry is one of shared ideas, methods, and knowledge, it is the critical thinking skills - the essential components of a capable, skilled person who can work in an autonomous environment, learn from failure, and deliver results - that have not only advanced us to where we are today, but are also the most difficult to pass on. In our present situation of impending generational turnover, the significance and necessity of taking action to impart as much knowledge as possible is crucial to the development of our industry. We have the opportunity to proactively strategize and implement methods of preserving our industry knowledge for the next generation of aquarists. This paper will address the concept of knowledge management and analyze significant examples in the hopes of being a catalyst for further progress.

# A BRIEF GUIDE TO AUTHORS

***Updated 2016***

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

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

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

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

## All Articles Must Adhere To The Following Basic Format:

- Use Times New Roman 12 point font throughout (except figure and table legends as noted below).
- A4 users please reformat to 8 ½ x 11 inch documents.
- Keep the resolution of photographs LOW. High resolution photos make the PDF file huge and are compressed anyway.
- **Format the title section with the line spacing set on 1.5 lines (not another method) and using centered, boldface font. Only the title should be CAPITALIZED (except italicized *Scientific namesii*).**
- Double-space after your "institution name" to begin the body of your text. It should look like this:

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

**Jill Fishhead, Senior Aquarist jfishhead@fishstinking.com**

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

*Continued….*

**Text Format**

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

**Figure Legends**

Please use the following format:

Figure 1: Legends should appear under the photo or graph in this format in 10 point font, aligned with the sides of the image or figure (center or justify). Photographs should be pasted into the document in the proper location by the author. All photos MUST be formatted as low resolution files, 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 space above or below lines. I have to remove all of these. Start with a single spaced Word template.
- Use the "enter" key for all spaces ("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.
- 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, apocrypha, and serious technical articles are equally appreciated.