DRUM and CROAKER

A Highly Irregular Journal for the Public Aquarist

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TABLE OF CONTENTS
Volume 38, 2007

cover photo: from Daniels et al .

DRUM AND CROAKER 35 YEARS AGO

Richard M. Segedi

(from the 1971 issue)

Phase-out of the National Fisheries Center and Aquarium

In the annual budget message to the Congress there is the recommendation that planning for the National Fisheries Center and Aquarium be discontinued. This means that all activities directed toward accomplishment of this project shall cease. Minimum funds will be provided to close out all Fisheries Center activities.

The National Aquarium in the Department of Commerce Building will continue to function.

Aquarium Symposium - An End to the Confusion

William P. Braker, John G. Shedd Aquarium

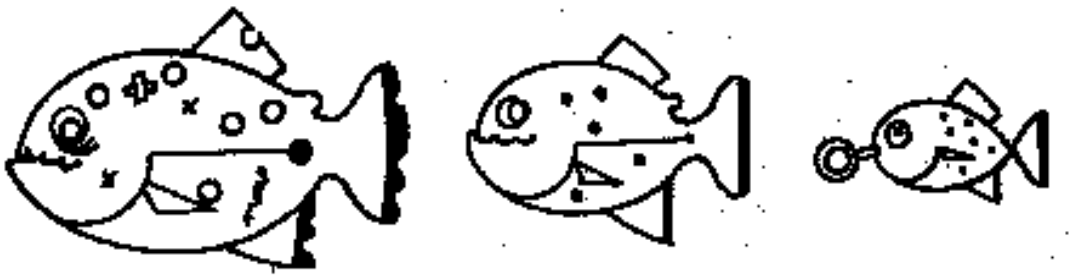
Two national meetings attracted a good representation of aquarists last year: the American Society of Ichthyologists and Herpetologists meetings in New Orleans and the American Association of Zoological Parks and Aquariums meetings in Buffalo. It was apparent, in both places, that there was much concern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

After much correspondence and due deliberations it is the consensus of this committee that we should all meet in Salt Lake City on September (?) 1971, with the AAZPA *(2007 Editors note: AAZPA is now known at the Association of Zoos and Aquariums (AZA)*.

Second Generation Piranha

Louis Garibaldi National Fisheries Center and Aquarium

On November 23, 1970, fourteen baby piranha (species) removed from our piranha display tank. The spawning of piranha (for which we take no credit) is still an achievement, but this was a noteworthy spawning because the parents were obtained from Shedd Aquarium several years ago as fry which resulted from a spawning of their wild-caught fish. [drawing below by Craig Phillips 1971]



BIO-MOS® IMPROVES THE GROWTH AND SURVIVAL OF CULTURED EUROPEAN LOBSTER

C. Daniels, D. Boothroyd, S. Davies, R. Pryor, D. Taylor and C. Wells

The National Lobster Hatchery, Padstow, Cornwall, UK.

In a recent series of experiments conducted here at the National Lobster Hatchery, the carbohydrate Mannan oligosaccharide (Bio-Mos® donated by Alltech Ltd) was used as a dietary supplement to explore its potential for improving growth and survival of larval lobsters (*Homarus gammarus*). This product has been shown to enhance the growth and survival of other cultivated marine species, including shrimp and sea bream. Among domestic livestock it enhances growth and survival by increasing the efficiency of the digestive tract, so improving food breakdown and nutrient uptake.

Photo 1. Researcher Carly Daniels holding a berried female

Bio-Mos® is a prebiotic: a non digestible food ingredient which beneficially affects the health of the host by selectively stimulating the growth or activity of one more bacteria in the digestive tract. Prebiotics such as Bio-Mos® also possess immunostimulation properties. Firstly Bio-Mos® has been reported to augment and arouse the natural immune system of organisms to which it has been fed, and it will also bind specifically with pathogenic growth-inhibiting microbes, preventing their attachment to cells within the gut, whilst leaving beneficial bacteria unharmed. Given its successful use with other marine species it was suspected that Bio-Mos® could potentially reduce bacterial diseases in and increase survival rates of lobster larvae in culture. Aiding the development of a healthy immune system should increase survival which is very important to the success of livestock output in both agriculture and aquaculture. It is especially important in lobster culture since crustaceans lack a specific immune response and rely on non-specific defences during their larval stages, where highest mortality occurs

As part of its mandate to conduct research into the enhancement of lobster stocks, the National Lobster Hatchery routinely receives berried (egg bearing) adult female lobsters from local fishermen during the summer. (See also Hatchery International Vols 6-1; 7-1) Newly hatched larvae are collected daily from the hatchery broodstock tanks and transferred into 60l kreisel cones. (800 - 1000 larvae per cone) and fed once daily with enriched *Artemia* larvae at a concentration of 5 per ml. Once the larvae have moulted to become characteristically lobster

shaped (stage IV) they are transferred from the kreisels into individual rearing pens to prevent cannibalism. These juvenile lobsters are fed daily on Selco™-enriched *Artemia* supplemented with frozen adult brine shrimp.

During the Bio-Mos® study, twelve kreisels of larvae were observed. Three controls were fed upon *Artemia* nauplii enriched only with A1 DHA Selco™ (INVE Aquaculture), while larvae in the other nine cones were fed upon *Artemia* nauplii enriched with a mixture of A1 DHA Selco™ and Bio-Mos® at three differing concentrations; 2ppt, 20ppt and 200ppt (3 replicates of each). A standard enrichment solution of 6g of Selco™ and 3ml of water was enhanced with the three measures of Bio-Mos® (Table 1) for enrichment of up to a maximum 100,000 *Artemia* in a 10L enrichment vessel.

Photo 2. 12 Kreisel cone recirculation system used to rear larvae from hatching through to stage IV.

After stage IV and transfer to the rearing tanks, the normal rearing protocol was followed, including feeding on Selco™ enriched *Artemia* supplemented with frozen adult brine shrimp.

Several parameters were measured including: survival to stage IV, mortality to stage V and VIII, developmental growth to stage V and VIII and bacterial conditions in the *Artemia* culture medium. Cumulative survival and mortality data were analysed by one way ANOVA and

Kruskal-wallis non parametric tests respectively. Morphological aspect length data was analysed using one way ANOVA.

Table 1: Experimental enriched *Artemia* diets

Feeding regime	Measure (g) of Bio-Mos® added to standard enrichment solution
Control	0
2ppt	0.0072
20ppt	0.072
200ppt	0.72

Immunostimulants show positive and negative effects

The results showed that the addition of Bio-Mos® at 2 and 20ppt to A1 DHA Selco™ enriched *Artemia* improved the survival of lobster larvae to their first juvenile stage (IV) of development, but that survival was significantly lower among larvae fed 200ppt Bio-Mos (fig 1) verified by significant differences in survival between the four feeding regimes, with P-values <0.001.

It was also discovered that the addition of Bio-Mos® at 20ppt to larval feed improves survival and growth during juvenile stages of development (stages IV to VII). With significant differences found between post stage IV survival for juveniles pre-fed the 20ppt Bio-Mos diet and all other diets (P<0.013 against control, P<0.005 against 2ppt, P<0.041 against 200ppt).

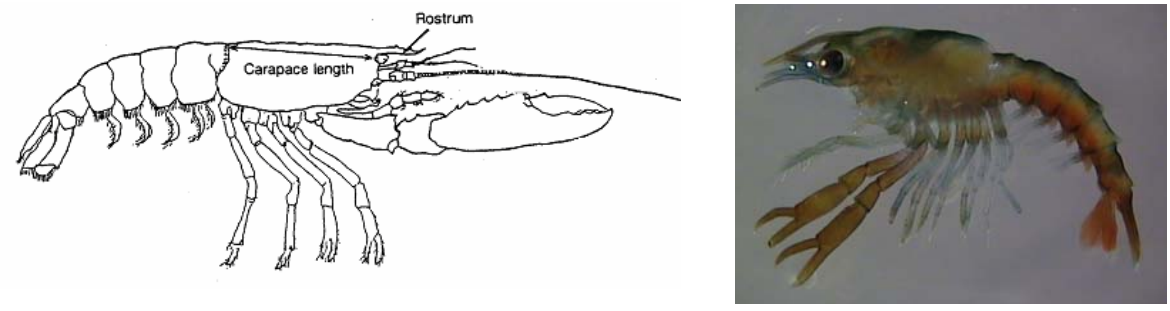


Photo 3. left: Diagram showing major morphological aspects of the European Lobster (H.gammarus), measured for this study (Modified from MAFF, 1991). Right: Photo of lobster larvae ready for morphological analysis.

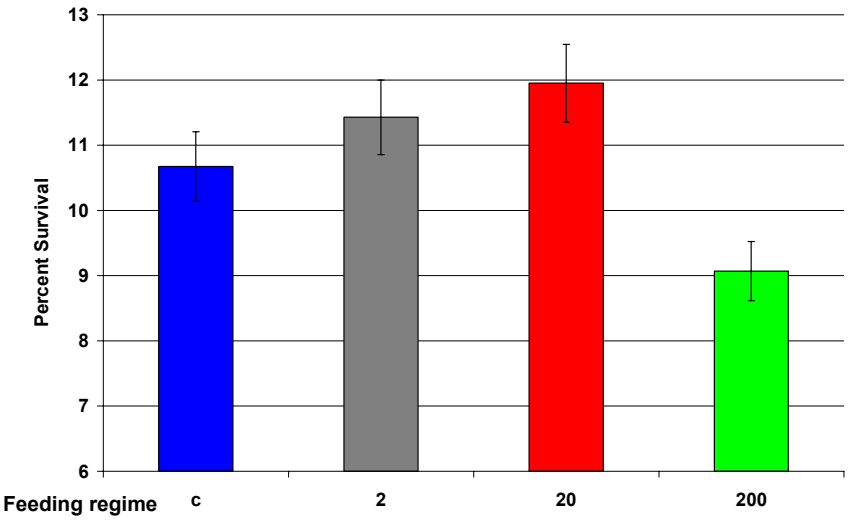


Figure 1. Survival (mean of three replicates) of lobster larvae to stage IV under four separate feeding regimes. 5% Error bars shown.

Feeding Bio-Mos® at higher concentrations (200ppt), not only increased mortality to the early juvenile stages, but subsequent development to stage VIII was reduced. The average carapace length of stage VIII juveniles pre fed a diet containing Bio-Mos® at 200ppt was between 0.4 - 0.6mm less than those pre-fed the other larval diets.

Bio-Mos® also appeared to reduce bacterial abundance (no of *Vibrio spp.* colonies per ml of water) in the *Artemia* culture medium (Fig 2) and so in larval food supply.

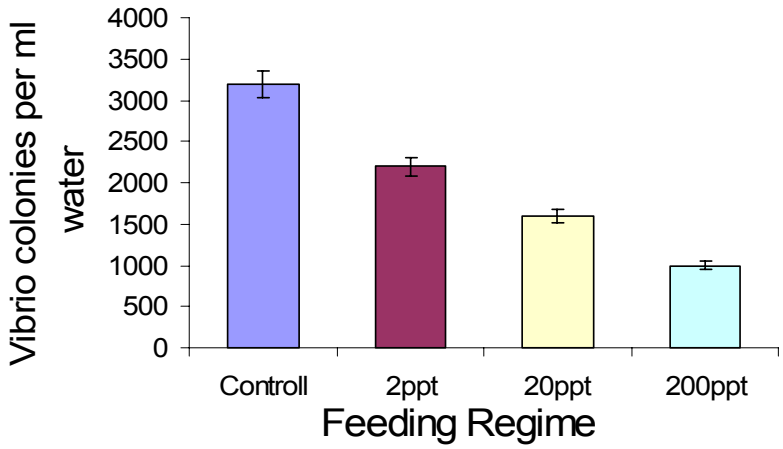


Figure 2. Estimated number of vibrio spp. colonies per ml of culture water. 5% Error bars shown.

Bio-Mos® added at 20ppt to lobster larval feed, from hatching through to stage IV, significantly decreased mortality to that stage and, giving a higher survival rate to stage IV, V and VIII of growth. However Bio-Mos® fed at a concentration of 200ppt during larval stages lowered both growth and survival of lobsters to stage IV, V and VIII.

The causes of mortality were not analyzed. However, reduced mortality does suggest enhanced disease-resistance with Bio-Mos®, which has been shown in other species such as *Litopenaeus vannamei.* Adverse effects on survival at 200ppt would appear to counter this theory, but could be due to negative effects on the nutritional value of *Artemia* rather than on the lobsters themselves. This is supported by a lower mortality between stages V-VIII among lobsters prefed a diet that included Bio-Mos® at 200ppt, and also significantly lower growth rates. Such suppressed development could be attributed to a lack of essential nutrients caused indirectly by high Bio-Mos® supplementation.

The use of multi-functional diets may be new in aquaculture but it shows the potential to develop diets that meet basic nutritional requirements and also extend beyond this to improve disease resistance. Diet formulations that have both the correct balance of key nutrients and also possess immunostimulatory properties, (which may well be species specific) could have widespread application.

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BENZOCAINE IN THE EUTHANASIA OF THE GIANT PACIFIC OCTOPUS, *Enteroctopus dofleini* (WÜLKER, 1910)

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Abstract

The use of the local anesthetic benzocaine as a rapid alternative in the euthanasia of the giant Pacific octopus, *Enteroctopus dofleini*, has been suggested in the literature, though its use has yet to be documented. This investigation subjected two senescent, female specimens of *E. dofleini* to immersion in concentrations of benzocaine ranging from 500 to 3500 mg/l. Various stages of anesthesia were documented in both specimens, leading to eventual medullary collapse and death. Benzocaine was found to be an effective anesthetic at concentrations above 1000 mg/l. Concentrations above 2500 mg/l caused terminal anesthesia within 45 minutes, with concentrations above 3500 mg/l inducing medullary collapse within 15 minutes. This method proved to be a relatively rapid, humane method of euthanasia in comparison to other common practices.

Introduction

Euthanasia is a topic of great debate in aquaria and other zoological collections. Owing to the semelparous nature and short life span of the Cephalopoda, the subject of euthanasia presents itself as a regular necessity in the management of captive populations. This is especially true due to the increased popularity of the giant Pacific octopus, *Enteroctopus dofleini* (Wülker, 1910), with 24 institutions exhibiting the species in 1999 (Carlson and Delbeek); and numerous other facilities (including that of the authors) having acquired specimens since that survey. There are 5 methods of euthanasia described for the fixation of cephalopods as museum specimens (Roper and Sweeney, 1983). The suitability of these methods and two others as they pertain to specimens in zoological collections has also been discussed in the literature (Anderson, 1996). Anderson (op. cit.) concludes that the two preferred methods of humane euthanasia for octopods are ethanol and freezing; he also mentions that the less expensive fish anesthetic benzocaine may be effective, though it has not been proven.

Methods

On 26 July 2006 a female specimen of *E. dofleini* was removed from its exhibit and brought to the quarantine facility of the Aquarium at Moody Gardens. The animal was approximately 2 years old, roughly 20 kg, and had been senescent for 4 months. The specimen was held in 40 l of tank water from its exhibit. Benzocaine (reagent grade, Sigma-Aldrich) was added (in ethanol solution) in successive dosages of 20, 20, and 120g, to produce concentrations of 500, 1000, and 3500mg/l. Aquarium staff and Dr. Phillip G. Lee of the National Resource Center for Cephalopods monitored ventilation, sucker adhesion, muscle tone, arm movement, and response to tactile stimulus continuously throughout the experiment. At the point of

presumed death the vessel was flushed with clean seawater to reduce the benzocaine concentration by 50% in order to see if the specimen would revive. A cursory necropsy followed the presumed death of the specimen. The ligature connecting the cap to the mantle was severed so that the branchial and systemic hearts could be inspected for the cessation of cardiac contractions. This procedure was repeated on 07 December 2006, on a female *E. dofleini* weighing 2.5 kg that was approximately 1.5 years of age and had been in senescence for 5 months. The concentration of benzocaine used in the second trial was 2500 mg/l, from a stock solution dissolved in ethanol. The notes and photos taken from both trials were correlated to the various stages and planes of clinical anesthesia documented in fishes (Brown, 1993; Stoskopf, M.K., 1985).

Results

The total time for the first trial was 70 minutes for three different dosages added. The initial concentration added was 500 mg/L which was chosen as a start point as it is twice the concentration recommended to euthanize bony fishes (Noga, 1996). The first dose induced a light sedation equivalent to stage I/ plane 1 as characterized by slight loss of reactivity to visual and tactile stimuli with normal respiratory rate and muscle tone at the 10 minute mark. When the concentration was increased to 1000 mg/L, the specimen progressed into a deep sedation (stage I, plane 2) as evidenced by loss of reactivity to visual and tactile stimuli and a decrease in respiratory rate, though it was still responsive to positional changes. After 45 minutes the animal had entered a state of light narcosis (stage II, plane 1) including a characteristic brief excitement phase that consisted of resumed mantle and arm movement just prior to lack of all responsiveness. At 50 minutes, the container was flushed with 50% clean seawater in order to gauge whether the anesthesia had become terminal. After the water was added the specimen revived instantly. A third dose of 120 g benzocaine induced a surgical plane of anesthesia as characterized by total loss of reactivity and cessation of ventilation within five minutes. After 15 minutes the animal was presumed to be in a state of medullary collapse (stage IV); and the container was flushed with clean seawater once again. The specimen did not revive and a cursory necropsy confirmed death. This information is presented in a concise form in Table 1.

The total time for the second trial was 45 minutes at 2500 mg/l, and due to the efficacy of this concentration in inducing clinical anesthesia the dosage was not increased. After 8 minutes of immersion a slight loss of both equilibrium and sucker adhesion was noted along with a slight decrease in ventilation. These changes indicate the animal was most likely in a state of deep sedation (stage I, plane 2). At 10 minutes the specimen entered an excitatory phase characteristic of light narcosis (stage II, plane 1). A slight, involuntary emission of ink was noted at 15 minutes, most likely indicating that the animal had transitioned into a state of deep narcosis (stage II, plane 2). After 20 minutes a total loss of arm movement, coupled with continued loss of equilibrium, and near total loss of sucker adhesion indicated the induction of a light stage of clinical anesthesia (stage III, plane 1). After 30 minutes the specimen exhibited a total loss of muscle tone, continued decrease in sucker adhesion, and a marked decrease in ventilation rate, indicative of a state of surgical anesthesia (stage III, plane 2). Finally, after 45 minutes a total loss of ventilation was noted, indicating a state of medullary collapse and imminent failure of the branchial and systemic hearts (stage IV). This information is summarized in Table 2.

Table 1. Timeline of benzocaine anesthesia trial 1, 26 July 2006. Benzocaine indicated as concentration in seawater, ethanol as percent solution, notes regarding anesthesia correspond to Brown (1993).

Time (Min)	0	10	30	40	45	50	51	55	70
Benzocaine (mg/l)	500	500	1000	1000	1000	500	500	3500	3500
Ethanol (%)	0.5	0.5	0.8	0.8	0.8	0.4	0.4	2.0	2.0
Notes:	*1st Dose*	*Light Sedation*	*2nd Dose*	*Deep Sedation*	*Light Narcosis*	*Flush w/ SW-50%*	*Revival "Normal"*	*3rd Dose*	*Collapse*
Anesthetic Stage/Plane:	S-0	S-I/P-1	S-I/P-2	S-I/P-2	S-II/P-1		S-0	S-III/P-2	S-IV

Table 2. Timeline of benzocaine anesthesia trial 2, 07 December 2006. Benzocaine and ethanol indicated as in Table 1, notes regarding anesthesia correspond to Brown (1993).

Time (Min)	0	8	10	15	20	30	45	50
Benzocaine (mg/l)	2500	2500	2500	2500	2500	2500	2500	1250
Ethanol (%)	1.4	1.4	1.4	1.4	1.4	1.4	1.4	0.7
Notes:	*1st Dose*	*Deep Sedation*	*Light Narcosis*	*Deep Narcosis*	*Light Anesthesia*	*Surgical Anesthesia*	*Medullary Collapse*	*Death*
Anesthetic Stage/Plane:	S-0	S-I/P-2	S-II/P-1	S-II/P-2	S-III/P-1	S-III/P-2	S-IV	S-IV

Discussion

Benzocaine as an agent of euthanasia proved an effective and humane alternative in the terminal anesthesia of *E. dofleini*. The drug produced states of anesthesia relatively rapidly without any overt signs of irritation or agitation. Benzocaine acts as a local anesthetic by suppression of neurotransmission via disruption of sodium ion channels (Haydon, et. al. 1986), though is capable of acting as a general anesthetic in cephalopods due to the exposure to the ambient water their microvillus epidermis affords them. In this investigation benzocaine was shown to induce various planes of anesthesia in *E. dofleini* up to clinical narcosis at concentrations of 1000 mg/l or less. Rapid onset of surgical anesthesia (to the point where the animal was totally non-responsive with greatly reduced respiration) was achieved at concentrations above 2500 mg/l (within 45 min) and 3500 mg/l (within 15 min). A summary of the anesthetic stages for *E. dofleini*, as used in this study, is presented in Appendix I. Because the giant Pacific octopus is not commonly used as a laboratory animal, the sample size in this investigation was limited to two individuals; these results, although compelling, should be viewed as tentative regarding the widespread use of benzocaine as a general anesthetic. And while this sample size was limited, it is, however, comparable to those of other studies on anesthesia in cephalopods (Messenger et. al. 1985).

One of the main drawbacks to the use of benzocaine is its lack of water solubility, and the required use of a non-polar solvent (ethanol or acetone) to produce an aqueous solution. In this case mixing the stock solutions proved difficult and time consuming, and there was some precipitation when the stock solution (at 25°C) was added to the exhibit water at 16°C. In this

case ethanol was chosen as a solvent, though it cannot be determined what, if any, synergistic effect the ethanol had on anesthetizing the animal. In this experiment the concentration of ethanol never exceeded 2%; which is well below the most liberal suggested dosage of 10% for euthanasia (Scimeca, 2006), and still below the most conservative concentration of 5% (Anderson, 1996). Due to the amount of time spent preparing stock solutions (over 45 min.), and the difficulty in dissolving these solutions in cold water, the hydrochloride form of benzocaine (which is water soluble) may prove a more practical alternative. Benzocaine hydrochloride, however, is not as readily available, and is more expensive.

While it is difficult to determine whether any given method is more or less humane, benzocaine worked relatively rapidly without any observed signs of stress to the animal. While freezing and ethanol remain viable methods of euthanasia for octopods, benzocaine offers a rapid alternative with reduced expense (in comparison to ethanol alone), and little risk to personnel involved. It also offers the possibility of a more complete necropsy, as freezing would obviously destroy and distort many tissues and key anatomical features. Further investigation into the specific effects of benzocaine on cephalopods and into synergistic effects produced when used in combination with ethanol are needed, though this study shows its effectiveness as both an anesthetic and an agent of euthanasia.

Acknowledgements

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Appendix I

Stages of anesthesia in the giant Pacific octopus, *Enteroctopus dofleini*, adapted from the work of Brown, L.A. 1993. Anesthesia and Restraint. In Fish Medicine, Stoskopf, M.K. ed. W.B. Saunders, 882 pp.

Stage	Plane	Category	Behavioral Response
0		Normal	• Swimming, movement, and equilibrium normal. • Response to visual/tactile stimuli. • Ventilation rate normal. • Good muscle tone, strong sucker adhesion.
I	1	Light Sedation	• Depression in response to stimuli. • Ventilation, sucker adhesion, equilibrium, muscle tone, and swimming behavior normal.
I	2	Deep Sedation	• Strong depression in response to stimuli. • Decrease in ventilation. • Response to positional changes. • Muscle tone/equilibrium reduced.
II	1	Light Narcosis	• Excitement phase, characterized by normal mantle and arm movement, and increased ventilation, followed by marked decrease in response and sucker adhesion.
II	2	Deep Narcosis	• Total loss of equilibrium. • Decrease in muscle tone. • Slight response to stimuli. • Decrease in ventilation, returning normal.
III	1	Light Anesthesia	• Partial loss of muscle tone. • Loss of arm/tentacular movement. • Near total loss of sucker adhesion. • Significant decrease in ventilation.
III	2	Surgical Anesthesia	• Total loss of muscle tone. • Extremely low ventilation rate. • Total loss of response.
IV		Medullary Collapse	• Cessation of ventilation. • Branchial hearts in state of arrest.

WHAT ARE THEY THINKING?
A LOOK INTO THE COGNITIVE ABILITIES OF STINGRAYS

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Introduction

Target training or conditioning of stingrays is a common practice in aquariums today. The fact that stingrays are capable of learned behaviors is well established, but how they learn is not as well understood. Our stingrays in the Shallows and Shores exhibit at the Albuquerque Aquarium were conditioned a year ago to feed at stations assigned to the two main species: southern stingrays (*Dasyatis americana*) and cownose rays (*Rhinoptera bonasus*). For this project, we identified three potential cues that could trigger the feeding behavior: visual target, location, and order of feeding. We hoped to determine which one variable or combination of variables triggers the desired response in the stingrays.

The Shallows and Shores exhibit is a 10,600 gallon, 33 ft x 24 ft x 3.5 ft tank that houses 24 stingrays of 3 different species (13 southern stingrays, 10 cownose rays, and 1 Atlantic stingray [*Dasyatis sabina*]), as well as 6 other species of Gulf of Mexico fishes.

Figure 1: Shallows and Shores exhibit.

Prior to September 2005, we broadcast 4 quarts of food twice a day over the exhibit, but were concerned about how much food each ray was getting and whether each was getting enough variety. As a solution, we identified each ray and conditioned them to eat by species at targets 6 feet apart, with one person feeding. This feeding method enabled us to monitor the diet of each ray. We began the experimental feeding protocol in September 2006 by isolating and varying the components of our target feeding and testing the rays' responses to each situation. From this study, we hope to gain insight into stingray cognition, leading to more efficient use of training programs.

Normal Feeding Protocol

Cownose rays:

- Fed first on right side of exhibit.
- Black plastic triangle (target 1) is held under clear plastic board with a magnet.
- Target 1 is held by the feeder under water and all cownose rays that come to target are fed. Any southern stingrays that come to target 1 are ignored.
- After five minutes, the target is removed from water.

Southern stingrays:

- Fed second on the left side of exhibit.
- White plastic cross (target 2) replaces black triangle underneath clear plastic board.
- Target 2 is held by the feeder under water and all southern stingrays that come to target are fed. Any cownose rays that come to target 2 are ignored.
- After eight minutes, the target is removed from water.

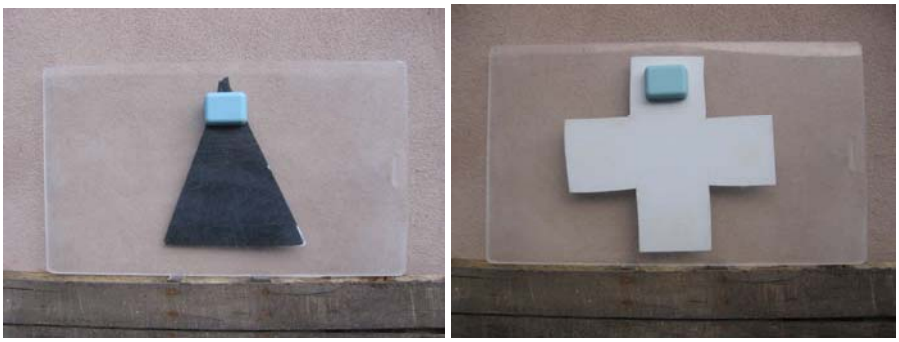

Figure 2: Targets 1 & 2.

Figure 3: Cownose ray and southern stingray stations.

Notes:

- The Atlantic ray is fed at whichever target she comes to because she is a sporadic eater. She is not included in this experiment.
- One particular southern stingray (F3) comes to and sits at the cownose target during every normal feeding, even though she is never fed there.
- Normal food consumption is 60 shrimp and either 15-20 capelin or 10-15 squid for cownose rays and 40-60 squid and 40-60 capelin for southern stingrays.

Experimental Feeding Protocol

From September 7 to October 19, 2006, we performed a series of six tests, each isolating one variable. Each test was performed on a Thursday and was followed by a week of normal feeding, with the exception of Test 5 which was followed by two weeks of normal feeding. We chose this schedule because the rays are easily conditioned and we wanted to observe the initial response to altered feeding parameters. All of the tests were video recorded to use as a reference; given the same time limit as normal feedings (5:00 minutes for cownose rays; 8:00 minutes for southern stingrays); conducted at the normal feeding time of 11:00-11:30 am with the exception of test six, which was conducted at 3:30 pm; and performed by the same feeder with the exception of Test 5, which used two feeders. The amount of food eaten by each species during each test was also recorded so that it could be compared with normal food consumption.

Test 1: September 7, 2006. 11:00 am

Question:

Will the position of the target on the board affect the rays' feeding behavior?

Procedure:

Keep order of feeding and location constant but change target component by placing targets 1 and 2 on top of, instead of underneath, clear plastic board.

Results:

Cownose Session: Placing target 1 on top of the board made little difference to the cownose rays. They bumped into the target more, but this did not cause discouragement from eating. Total amount eaten included 30 shrimp and 25 capelin, which is less than normal. F3 was aggressive towards the cownose rays during this session.

Southern Session: Placing target 2 on top of the board caused the southern stingrays to frequently get caught underneath the target. Otherwise, this was a normal feeding, with the southern stingrays eating 60 squid and 44 capelin.

Discussion:

Since minimal changes were observed in this test, we can assume that the rays are not sufficiently affected by target placement to discourage them from eating. We attribute the decrease in food consumption by the cownose rays to F3's increased aggression during this feeding rather than to the altered target placement.

Test 2: September 14, 2006. 11:30 am

Question:

Will the absence of a target affect the rays' feeding behavior?

Procedure:

Keep order of feeding and location constant but change target component by not using it.

Results:

Cownose Session: This feeding was better than normal. The cownose rays came to their location without any hesitation and ate as normal (60 shrimp and 20 capelin). F3 did not come to the cownose station at all.

Southern Session: This was also a very good feeding session for the southern stingrays. They fed at their station without hesitation, ate a normal amount (58 squid and 50 capelin), and had no interference from the cownose rays. The session ended early (at 7:00 minutes) because the southern stingrays ate faster than normal, causing us to run out of food.

Discussion:

The removal of the target did not cause confusion, leading us to assume that both species rely more on the combination of location and order of feeding than on the target itself. F3 did not come to the cownose station, as she always does during the normal feeding protocol. Was it because the target was absent? Is target 1 her cue to come over? This implies that the target may have some value, at least to F3.

Test 3: September 21, 2006. 11:30 am

Question:

Will the rays respond when the order of feeding is reversed?

Procedure:

Keep location and target constant but change order of feeding component by feeding the southern stingrays first and the cownose rays second.

Results:

Southern Session: There was a delayed response from the southern stingrays. They did not approach the target as aggressively but still ate the normal amount (60 squid and 38 capelin). The cownose rays rushed target 2 and were a hindrance to the entire feeding.

Cownose Session: The cownose rays ate less than normal (37 shrimp and 25 capelin) with no interference from the southern stingrays, including F3.

Discussion:

By rushing target 2, the cownose rays ignored the visual target and location, suggesting that they rely heavily on order of feeding. The southern stingrays' hesitation to approach target 2 at their normal location implies that they, too, rely on order of feeding. The smaller amount eaten by the cownose rays is assumed to be due to discouragement from not eating first. The fact that the southern stingrays did not interfere with the cownose ray feeding suggests that they stop coming to the target once they are full.

Test 4: September 28, 2006. 11:30 am

Question:

Will the rays recognize their target in the wrong location?

Procedure:

Keep target and order of feeding constant but change location component by feeding the cownose rays on the left side of the exhibit and the southern stingrays on the right side of the exhibit.

Results:

Cownose Session: The cownose rays responded right away to their target but were discouraged
towards the end of the session due to interference from the southern stingrays. F3 sat on target 1 for the entire feeding, and F2 (another large female southern stingray) came to the target right away but did not stay the entire time. By the end of the session, most of the southern stingrays were interfering with the cownose feeding. The cownose rays ate only 60 shrimp and 10 capelin, which is less than normal.

Southern Session: There was a delayed response from the southern stingrays when it was their turn to feed. Throughout the session, they came up sporadically to their target but still ate a normal amount (60 squid and 38 capelin). The cownose rays interfered throughout the entire session, even though they had already been fed.

Discussion:

The rays exhibited extreme confusion during this test, with both feedings being chaotic. The cownose rays came up right away to their target, unfazed by the different location. Were they responding to the target, or is this reaffirmation that order of feeding is most important to them? The fact that they came up for the southern session as well implies that the location is more important than the target in this session. Due to the southern stingrays' sporadic feeding at the cownose location and their interference during the cownose feeding, we can assume location is most important to the southern stingrays in this test. The hesitation by the southern stingrays at the beginning of the cownose feeding suggests order of feeding was a factor as well, making the target the least important component for the southern stingrays in this test.

Test 5: October 5, 2006 11:30 am

Question:

Will the rays respond normally when both species are fed at the same time? Will the addition of a new stimulus (second feeder) affect the feeding?

Procedure:

Keep target and location constant but change order of feeding component by using two people to feed both species at the same time. The person feeding the cownose rays will stop after 5 minutes, while the person feeding the southern stingrays will stop after 8 minutes.

Results:

Cownose Session: For this test, we had to use a makeshift board out of a clear container lid since both species were being fed at the same time. We decided to use the makeshift board for the cownose rays because we thought they would be less affected by the lid than the southern stingrays. The cownose rays kept bumping into the raised lid, but they still ate a normal amount (70 shrimp and 15 capelin). F3 came over to this station immediately and stayed for 30-45 seconds before trying the other side. She never came back afterwards. No other southern stingrays came to the cownose side.

Southern Session: The southern stingrays came to their target right away and ate a normal amount (60 squid and 55 capelin). The session ended early (at 7:30 minutes) because the southern stingrays ate faster than normal, causing us to run out of food. The cownose rays did not come up to southern station.

Discussion:

This test supports our theory that order of feeding is the most relied-on variable for the cownose rays and that location is the most relied-on variable for the southern stingrays.

Test 6: October 19, 2006 3:30 pm

Question:

Will the rays react to targets during a non-feeding time without the presence of food?

Procedure:

Keep target, location, and order of feeding constant but remove food stimulus and change the time of day.

Results:

Cownose Session: All of the cownose rays came to their target repeatedly for the entire session, although no southern stingrays appeared.

Southern Session: Five minutes into the session, one male southern stingray (M5) came up to the target and continued coming back for the rest of the session. No other southern stingrays came to the target. Two cownose rays came up to southern target initially but left soon after.

Discussion:

The cownose rays continuously responded to their target without receiving a food reward. One explanation for this is that the target is a conditioned stimulus which elicits a conditioned response. The majority of the southern stingrays did not approach their target. An explanation for their lack of response could be that they understand approaching the target leads to a food reward so they will not approach it if they are not hungry. Looking into sensory system and metabolic differences between the two species may provide an alternate explanation.

Conclusion

These tests were part of a preliminary study to gain insight into the cognitive abilities of our captive stingrays. Based on the discussions above, we have come to the conclusion that the most important variable may be different for the cownose rays and the southern stingrays. We found the cownose rays relied most heavily on order of feeding, but the results from Test 6 suggest that other variables may affect their response since there was no food present. The southern stingrays seemed to rely most on location, although Test 4 suggests they also use order of feeding and F3's response in Test 2 suggests that the target does have some merit. In order to provide more conclusive results, repeats of these tests and additional in-depth analyses would need to be conducted. A future goal would be to use three groups of stingrays, train each to a feeding protocol using a different variable, and then compare the results of each group. A review of stingray biology, including morphological and physiological differences between species, could help identify anatomical limitations and learning patterns. This might help explain why our two species reacted differently to the testing conditions. With this project, we have increased our knowledge of stingray behavior and look forward to expanding our ideas with future studies, hopefully leading to a better understanding of the cognitive ability of stingrays.

Acknowledgments

We would like to thank Brian Dorn for his help with developing the initial training protocol and Holly Casman for her continuous support and encouragement, especially throughout this project. We would also like to thank the husbandry staff at the Albuquerque Aquarium for their feedback and our editing staff for last minute revisions.

BOOK REVIEW

ADVANCED MARINE AQUARIUM TECHNIQUES
Jay Hemdal, 2006. 352 pp.
T. F. H. Publications, Inc., Neptune City, NJ
$35-60 US

Review by Pete Mohan, Animal Care Manager, Akron Zoological Park

I have known the author since we met prior to the founding of the Regional Aquatics Workshop in 1989, not too long after Jay became Curator at the Toledo Zoo's Aquarium. At that time many of the aquarium department curators in the Ohio and Great Lakes area were relatively new in their positions, a bit green, and hungry to share information that would help us all better manage our collections. We discovered that Jay was (and continues to be) involved with many small but interesting experiments designed to test various husbandry changes or improvements on his exhibits. This book references many of these projects. Jay has taken an interesting approach to gathering information for this volume, and has purposefully written a guide that focuses primarily on his personal approach to aquariology, only relying on other publications to a limited degree.

Every new aquarist should pick up a copy of *Advanced Marine Aquarium Techniques*. While Spotte's "Seawater Fishes" remains the key in-depth scientific reference, Jay's book is a handy quick-reference that allows the problem-solving aquarist to rapidly search through many possible options for life support, water quality, disease treatment, and feeding techniques. The book is especially valuable because each technique has already proven useful in a public aquarium setting. It also has one clear advantage over other professional books: you don't need to search though lots of techno-babble to get the practical information you need NOW. While the section on animals is necessarily short, it features a number of unusual species that are usually seen only in public aquariums, and includes useful husbandry tips.

If you are looking for some quick ideas on how to modify a system or process at your facility, keep Jay's *Advanced Marine Aquarium Techniques* within easy reach.

Schedule and details available soon at: http://www.rawconference.org/index2007.htm

The Regional Aquatics Workshop (RAW) is the premier annual gathering of public aquarium professionals. The 21st Regional Aquatic Workshop (year 18) will be hosted by the Pittsburgh Zoo & PPG Aquarium, in Pittsburgh, PA. The main conference will take place from Tuesday, June 19th to Friday 22nd. As has been customary, the annual working meeting of all AZA aquatic conservation groups will take place the day before RAW on Monday June 18th.

Tentative Schedule:

- Sunday, June 17th – Registration available, tentative baseball game Pittsburgh Pirates vs. Chicago White Sox (1:35pm)
- Monday, June 18th – AZA TAG and CAP meetings in AM; AQIG meeting in PM; informal evening.
- Tuesday, June 19th – Intro to RAW sessions; Evening at the Pittsburgh Zoo & PPG Aquarium
- Wednesday, June 20th – RAW paper sessions in AM and PM; evening dinner on the Gateway Clipper (cruises the Pittsburgh Rivers)
- Thursday, June 21st – RAW paper session in AM; Aquarist Olympics in PM at ICC
- Friday, June 22nd – Optional white water rafting trip. (not included w/conference registrations)

CONTACTS / INFORMATION

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Conference registrations are $35 before May 17, 2007 and $50 thereafter. The conference will take place at the Station Square Sheraton Hotel. The room rates are $99 per night plus tax. Parking is $16 per day. For booking information please go to:
http://www.starwoodmeeting.com/Book/zoo.

RAW 2006 ABSTRACTS
Regional Aquatics Workshop, May 15 - 19
Moody Gardens, Galveston, TX

Abstracts compiled, edited, and matched to actual presentation schedule
by 2006 co-organizer: Paula Kolvig
pkolvig@moodygardens.com

[Abstracts were submitted for all but six of the papers presented. However the titles and presenters are provided for missing abstracts.]

Monday, May 15
Pre-RAW AZA Conservation Group Meetings

For complete minutes of all AZA TAG meetings, and copies of any materials distributed, please contact Doug Warmolts, Beth Firchau (MFTAG), Paul Loiselle (FFTAG), Steve Bailey, Jay Hemdal (LVSSP), Pete Mohan (AITAG) or Mike Brittsan (CRCAP)

ZIMS - What It Will Do for Your Institution

Presented by Tim Carpenter, Seattle Aquarium
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The ISIS Zoological Information Management System (ZIMS) will have a marked impact on how aquariums operate on a day-to-day basis. This new software application goes beyond the simple acquisition of data concerning your collections and focuses on how that data can be utilized to make your operations more efficient.

What can ZIMS do for aquariums?

The management of groups within collection management software has long been a point of contention. While managing groups is important for genetic management, you have not had reliable software to help. Now, ZIMS will give you the ability to track a variety of information for groups, merges and splits. With ZIMS, you will even be able to track two groups within the same enclosure,

Workflow is another key component of ZIMS that has been missing from all of the other applications that have been developed to help you maintain information about your collections. ZIMS workflow includes automation functionality that will provide user-role message notification

based on pre-defined events and activities. For example, ZIMS will notify a veterinarian if a medical test result is out of the reference range and send a message to the registrar that a new animal data has been entered and should be reviewed.

ZIMS will allow you to include environmental data within the same system that maintains information concerning your collections. This will enable a more detailed level of analysis regarding all of the data around an enclosure.

All aquariums can benefit from ZIMS. Please join us to learn how.

Tuesday, May 16

Opening Session/General Interest/Cephalopod Topics

How to Keep Your GPO Happy

Roland C. Anderson, The Seattle Aquarium, Seattle
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The giant Pacific octopus (GPO – *Enteroctopus dofleini*) is the world's largest species of octopus. It can grow to over 400 pounds, although common large weights in aquariums are 60-80 pounds. Other species of octopus have similar outliers at the heavy end. In the wild, GPOs range in nearshore waters down to about a thousand feet deep, from Southern California all along the west coast to Alaska and down to Japan. It can be distinguished by the longitudinal wrinkles along the body, flat papillae, a white streak through each eye, and a single white spot in front of the eyes.

It lives three to four years at the end of which it reproduces and dies. Both males and females will die at the end of that time even if kept solitary. Females lay eggs and guard them six to eight months until they hatch. Males die shortly after mating. Females die about the time the eggs hatch. It is relatively easy to maintain GPOs given a large enough tank and standard water quality parameters.

Octopuses should be given enrichment based on their intelligence. They are more intelligent than any other invertebrate, but how smart are they? Their intelligence is difficult to measure since they are adapted for survival in the wild and since they are so different from us. We have some indicators of their intelligence based largely on what they can learn to do in captivity, such as opening "prey puzzles."

Cephalopod Work with Waikiki Aquarium and Georgia Aquarium

Bruce Carlson, Georgia Aquarium, Atlanta

Oval Squid (Sepioteuthis lessoniana) Husbandry at Oceanário de Lisboa

Vanda Lobo, Oceanário de Lisboa, Esplanada D. Carlos I, Doca dos Olivais, Lisboa, Portugal
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In 2003 a new exhibit in the Tropical Galleries at Oceanário de Lisboa was developed, aiming for fluorescent corals and tropical squid. Since 2002 we are trying to culture the oval squid for this exhibit. On this study we will show in detail what we could accomplish so far in the husbandry of this squid. Several but important items will be shown: the life support system necessary for each life stage, the water quality, egg maintenance, hatch survival, the type and amount of food from early hatch until adult size, space considerations and squid development, reproduction behavior and egg laying. Presently we are trying to complete the first cycle in order to achieve the first F1 generation. It seems to be a good and easy species to culture providing certain conditions, but the main problems are to get good viable eggs from them and feed the hatchlings, which may well be the most important and decisive factors for their culture.

Sepia Pharonis Husbandry Advances

Adam Daw, National Resource Center for Cephalopods, Galveston

"Reef Protection International"

Drew Weiner, Reef Protection International
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Reef Protection International (RPI) has conducted research on the marine aquarium trade resulting in the creation of the RPI Reef Fish Guide, a pocket guide for the responsible hobbyist. Learn about the RPI Reef Fish Guide campaign and how public aquariums may use this guide to enhance existing conservation and education programs. The goal is to increase public awareness about the ethical implications of keeping coral reef fish as pets and provide those entering the hobby, as well as existing hobbyists, with the knowledge to make informed buying choices that contribute to coral reef conservation.

Ethics and Invertebrates – Where Do We Draw the Line?

Roland C. Anderson, The Seattle Aquarium, Seattle
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Jennifer A. Mather, University of Lethbridge

We humans tend to look at the care of zoo and aquarium animals with an anthropocentric eye. For example, enrichment is now a given for charismatic megafauna. In zoos, pandas, bears, and gorillas have been given enrichment for many years. The same is true for marine mammals in aquariums. Lately, there has been much discussion on an aquarists' email list about the possible necessity of enrichment of fishes. But with the exception of intelligent octopuses with their well-developed eyes, there has been no discussion about the quality of life of other so-called lower

invertebrates. Recent studies have shown that invertebrates show stress and pain under adverse conditions. As recently as 1964 a popular cooking book advised us to cook a live Maine lobster by placing it in a pan of cold water and bringing to a boil (it now advocates killing a lobster humanely before cooking). There is now evidence that despite their small brains (comparable is size to an insect's) lobsters are smarter than once thought. They easily crawl in and out of lobster pots and they recognize individual cohorts. The mantis shrimp arguably uses tools. If intelligence is the factor we use to judge whether an animal is deserving of ethical treatment and enrichment, then the bar gets lower the more we learn about each one.

But what about animals without brains such as clams and oysters, animals we harvest and cook alive, animals we feed alive to marine mammals and animals we keep to show the public? When we keep them alive in aquariums, what responsibility do we have to make their lives comfortable? Where do we draw the line?

Institutional Collection Planning for Public Aquariums

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Having a comprehensive, well conceived collection plan aids public aquarium curators in maximizing the impact their animal collection has on their visitors. Resource allocation and communication are also improved when a high-quality collection plan is in place. The Association of Zoos and Aquariums now requires that all accredited facilities have an approved institutional collection plan. Examples of Departmental, Institutional and Regional collection plans are presented to serve as a possible framework for further development of similar strategies at other facilities

Propagating Nautilus in Artificial Sea water

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Nautiluses are a unique type of cephalopod that are frequently overlooked by the public due to their seemingly simplistic snail-like appearance. At the Henry Doorly Zoo for the past eight years, ongoing attempts have been made to breed, collect eggs and hatch nautilus in a closed system using artificial seawater. There have been many trails and tribulations along the way with promising signs of success. My goal is to continue developing an incubation technique that will yield a high hatch rate and a holding system that will support longevity in captivity.

Making of Adventure Aquarium

Nicole Grandinetti, Adventure Aquarium
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In September of 2004 the New Jersey State Aquarium closed its doors. Over the course of the next nine months Steiner Entertainment transformed the aquarium into Adventure Aquarium.

Steiner entertainment is a company that creates experiential leisure time businesses. The Aquarium went from a non-profit organization into a business run by Steiner Entertainment. Steiner also owns the Newport Aquarium and Majestic Theatres. Adventure Aquarium added a 70,000 square foot expansion, unveiling two major exhibit additions; Shark Realm featuring a forty foot tunnel, and West African River Exhibit – the first North American aquarium to exhibit hippos. There is also a Jules Verne gallery, and a swim with the sharks experience area. Along with the addition of the new building the aquarium renovated the original building by updating almost all of their exhibits with new themes, LSS, fabrication, graphics, and many new animals. Adventure Aquarium opened its doors in late May of 2005 with a whole new experience for their guests. Adventure Aquarium recently received its AZA accreditation and will launch six new guest experience programs this summer. Adventure Aquarium's goal is to become a world class aquarium where amazing experiences happen.

Extreme Duration Hyposalinity in the Control of *Neobenedenia melleni* (Monogenea: Capsalidae), with Notes Regarding the History of Treatment in Public Aquaria

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Neobenedenia melleni has long been regarded as one of the most enigmatic and notorious Monogenean parasites in public aquaria and aquaculture. The extreme lack of host specificity, direct life cycle, and rapid multiplication in captive environments contribute to the pathogenicity of the species to aquarium fishes. The species has also gained a reputation as being extremely difficult to control in large exhibits, as the eggs are inordinately resilient to common chemotherapeutic agents. The history of the species has been defined by, and interwoven with the development of the modern public aquarium; the species was first described from the New York Aquarium in 1927, and 88 of the 116 subsequent published host records have come from captive fishes. Although modern chemotherapeutic agents such as Praziquantel offer an advantage over the parasite, the exhibit size of many modern public aquaria preclude its use, due to the expense. The Aquarium at Moody Gardens first used extreme duration hyposalinity to combat *N. melleni* in 2004, on a group of approximately 200 infected Florida Pompano, *Trachinotus carolinus*, in the quarantine facility. The salinity of the holding tank was lowered to 17‰ for 25 days, immediately stopping host mortalities. After the treatment the host group has remained free of *N. melleni* until the present day. In the summer of 2005 the million gallon Caribbean exhibit at the Aquarium at Moody Gardens suffered an outbreak of *N. melleni*, resulting in notable scratching and flashing of nearly all the exhibit fishes, and significant mortalities. Owing to the expense of Praziquantel treatment, and the prior success of lowered salinity, extreme duration hyposalinity was again utilized. The exhibit was lowered to 17‰ for 30 days. On day 2 of treatment, mortalities ceased, and since treatment no specimens of *N. melleni* have been recovered from fishes of the exhibit. The fishes of the exhibit suffered no ill effects or noticeable stress throughout the treatment. A historical perspective of treatment methodology from various public aquaria is also presented. Aggressive hyposalinity treatment thus seems to be an effective option to controlling *N. melleni* in large exhibits where Praziquantel treatments are less practical.

An Introduction and Other Interesting Factoids for Exhibition

Chrystal Crain, National Resource Center for Cephalopods, Galveston

Reproduction in Captive Giant Pacific Octopus, *Enteroctopus dofleini* :
An Elusive Circle Closing?

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Challenges in rearing viable offspring of the giant Pacific octopus remain despite increased interest in public aquariums and research laboratories. In May 2004, mating occurred between a five year old male and a four year old female within an aquarium in public view. First obvious development in the resulting clutch of eggs was observed in November 2004, and hatching commenced in April 2005. The eggs developed in ambient seawater with two small groups separated from the maternal brood to facilitate access to eggs for examination. Significant visible milestones in development were documented. Hatched paralarvae 5-7mm in length were reared in two separate aquariums which were similar to kreisel-style aquaria used to rear jellyfish. Several types of local wild and commercially-available planktonic foods were offered but it was noted the paralarvae were foraging on prey items larger than zooplankton. Shaved portions of clams and euphausid shrimp were tried which increased the feeding response (Marliave, 1981).
Paralarvae were reared 3 months post-hatch, reaching 8 mm total length. Suggestions are offered for improving the survival of paralarvae by addressing abrasion in the rearing vessels and by enhancing nutrition during the pelagic larval stage.

Wednesday, May 17

Elasmobranchs, Water Quality/Life Support / General Interest

Captive Husbandry for the Genus Potamotrygon,
Emphasis on P. henlei, P. leopoldi, and P. menchacai.

Dave Hoffman, Atlantis Marine World
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There is a unique diversity that lives in the Amazon rainforest, by having breeding populations we can conserve these animals. Right now there are about 26 identified species of freshwater stingrays that live in the Amazon. Several institutions already have breeding populations of *Potamotrygon motoro, P. leopoldi,* and *P. castexi.* Recently, there has been success in breeding *Potamotrygon menchacai* and a new species the pearl ray, *Potamotrygon sp*.

Brazil and other South American countries have quotas or bans in place to keep many ray species in their native environment. Although these quotas have helped, many stingrays are exported illegally.

Explaining some simple techniques for feeding and certain things to watch for, we can help keep these animals healthy. Ray enthusiasts should know which stingrays are easier to care for and which ones take more effort so they will hopefully stay away from them. Every stingray has its own breeding and social behavioral characteristics. The three stingrays that will be focused on are *Potamotrygon leopoldi*, *P. henlei*, and *P. menchancai.* These rays have proved to be the most sought after for public aquariums and the hobby trade.

Using certain husbandry techniques that are not available to field researchers, aquariums will help propel our success along with the success of the researchers. Over the years, several husbandry procedures/techniques have been implemented in many institutions. I will be explaining the procedures, problems and behaviors from seven years of experience, so we can maintain a level of excellence in keeping these animals alive.

The Use of Ultrasound in the Monitoring of Reproduction in Freshwater Stingrays, *Potamotrygon species*.

Erica Clayton, John G. Shedd Aquarium

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There has been recent field research on reproduction in Freshwater Stingrays in Brazil. These rays exhibit a reproductive strategy called matrotrophic viviparity with the female developing trophonemata for fetal nourishment.

The Shedd Aquarium has been able to learn a lot about freshwater stingray reproduction, using ultrasound as a tool, that complements what is known from data collected on wild Potamotrygons. Stingrays are easily taught to 'station' for feeding, which can be used to get an animal to present for the procedure. At Shedd, in most cases water level is lowered so that staff can easily stand in the water. Most of our rays will stay still for the procedure, which in noninvasive and does not appear to cause the patient any stress. This allows us to tell when a female is ovulating and when pups start to develop. We have been able to track growth of ova and pups, and plan on continuing this research in the future. We have also been able to record pup nursing in utero. In addition, since most of our females are left on exhibit to pup, it is a useful tool that tells us how many pups need to be collected from exhibit when the female gives birth. We are able to get more accurate gestation time because we do not need to guess when a female became pregnant, and as we collect more data it allows us to better know when we need to start looking for pups. By comparing this with behavioral observations, we are able to get a more complete picture of freshwater stingray reproduction in a captive environment.

The Use of Ultrasonography in Managing Reproductive Activity of *Rhinoptera bonasus* in Captivity

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The reproductive cycle of captive as well as wild cownosed rays, *Rhinoptera bonasus*, is not well documented. A variety of values for duration of gestation, age of sexual maturity and inter-pregnancy intervals are mentioned in the literature. Rays as well as other elasmobranchs are ectothermic and as such are greatly affected by the temperature of their exhibit as well as other environmental parameters such as salinity, photoperiod, diet, etc. These variables may account for different values reported from a variety of institutions.

Periodic and regular ultrasonography of female cownosed rays can accurately define the reproductive cycles of exhibit animals. It is quick, easy to do, and non-invasive. Ultrasonography can be coupled with plasma chemistry testing of various hormones to further define the animal's cycle. *R. bonasus* is viviparous and carries its embryo in a uterus lined with villi referred to as trophonemata. The embryo lives off of its yolk sac in the early stages of the pregnancy but ingests an organically rich histotroph secreted by the villi during the remainder of the pregnancy. While cownosed rays do not have a placenta, there does seem to be a level of human chorionic gonadotropin (HCG) present in their blood when they are gravid. This hormone can be easily measured and employed to detect pregnancy at an early stage.

Ripley's Aquarium of the Smokies in Gatlinburg, Tennessee does a reproductive census of its *Rhinoptera.* Using the ultrasound, a staging protocol has been developed that allows the staff to keep track of each individual's reproductive cycle. When rays are close to parturition they are moved to a nursery area. After birthing, they are returned to the exhibit. This monitoring program allows the animals to remain on display as much as possible but helps ensure the maximum survivability of pups. It also has provided information that helps define the cycles of this species in captivity. A number of rays have been sampled and blood testing for HCG has been correlated to an animal's reproductive status as determined by ultrasound. Because the results of this on-going program have proven so useful from a practical as well as academic point of view, the program has been expanded to include a large collection of Southern rays at the Ripley's Aquarium in Myrtle Beach.

In-House Shark Collection and Proactive Stress Conditioning for Transport Sensitive Elasmobranchs, Oklahoma Aquarium

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On June 2005, the Oklahoma Aquarium in conjunction with PredatorWorld, conducted a three-week shark collection trip to the Texas gulf coast. The purpose for this trip was to cost-effectively obtain young and; therefore, more durable and transportable specimens for public exhibit and husbandry research. Various elasmobranch species were collected and eventually transported to their destinations of the previously mentioned institutes. Methods of collection and transport were tested along with the utilization of various metabolic drugs such as dexamethazone and sodium acetate. During the ongoing husbandry of these animals, the staff at the Oklahoma Aquarium implemented a proactive stress conditioning strategy for the inevitable transport of three transport sensitive shark species; *Carcharhinus leucas, Carcharhinus limbatus, Carcharhinus brevipinna.* The purpose of this conditioning is to reduce metabolic stress and; therefore, increase survival rate during and after the relocation of these species from a current holding facility to their final exhibit tank. This transport would be conducted when these animals have attained an adult size and; therefore, have a significantly increased sensitivity to handling and transport stress.

Kiddie Pools: Thinking Outside the Box.

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Scenario 1: Sandbar Shark Transport and Physicals.

Two moderately large sandbar sharks, housed in a 45,000 gallon Quarantine tank, have finished their quarantine and needed to be moved to their exhibit as well as receive their exit physicals. Access to this tank is somewhat limited and requires maneuvering up or down narrow steps. In our opinion, based on previous animal moves, trying to move a large non-sedated or under-sedated animal down these stairs could be potentially harmful for both aquarist and animal. Knowing this - what do we do?

Scenario 2: Bonnethead Shark Physicals.

Three adult bonnethead sharks are done with quarantine and need to have their exit physicals and be tagged for identification. From previous cases with bonnetheads we've seen that they can injure their eyes while being anesthetized or during recovery by bumping or

rubbing their eyes on the sides of the tank or transport. They also tend to show evidence of recovery (by struggling and swimming off) then relapse into apparent sedation with reduced responsiveness. Knowing this – what do we do?

Answer to both scenarios: Use an inflatable Kiddie-Pool of course.

Scenario 1: Sandbar Shark Transport and Physicals. After careful consideration of multiple options we felt that our best option would be to use an 8' diameter inflatable plastic kiddie-pool to anesthetize the sharks, one at a time, in the tank they were in. The sharks were given diazepam 1 hour before starting to drop the water and about 4 hours before catch-up. Once the tank levels reached the desired depth (3 feet) the inflatable pool was put into the tank. House air was used to partially inflate the ring around the pool. Two aquarist stepped on the edge of the pool's inflatable ring and used jump guards as barriers to create a pathway into the pool. Three other aquarist work with jump guards to separate and direct individual sharks into the pool. As the sharks swim freely into the pool the 2 aquarist step off the ring. It immediately pops to the surface and is topped off with air. The jump guards are placed on top to act as a cover. A pump was used to further fill the pool to an estimated volume (700 gallons). MS222 (~125ppm) is added to the water. Once the shark is suitably sedated a sling is used to hoist the shark over the tank wall and down the stairs to a shark transport container (filled with 100 gallons water and 75ppm MS222). The sharks were rolled in the transport to their new exhibit. Their exit physicals were done – morphometrics, body weight, blood draw, cloacal lavage, ultrasound, gill clip, transpondered, etc. The sharks were woken up in the circular med-pool attached to their exhibit. Repeat above with second shark. Both went off without a hitch and according to plan.

Scenario 2: Bonnethead Shark Physicals. Used the same 8' diameter inflatable kiddie-pool to anesthetize one of the sharks. First shark had some issues. Left the other 2 alone for about 2 weeks then repeated with them but also used a 12' diameter inflatable kiddie-pool as a wake up. Second time it went according to plan.

The Captive Handling and Restraint of the Freshwater Sawfish: When Not to Say "1, 2, 3, Go!"

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Sawfish are now relatively common in aquaria worldwide and because of their size, power and toothy rostrums handling them in a confined, captive environment poses serious safety risks to the aquarist and the animal. As opposed to animals collected in the wild with nets or long lines, captive specimens usually are fresh and strong because they have not been exerting themselves for any length of time. Over a period of three years the Aquarium of the Pacific has kept two Freshwater Sawfish, *Pristis microdon*, and as part of annual physicals, transfers and medical evaluations we have developed various techniques to capture and handle sawfish up to 2.5meters long and 70kg. Video segments will show the varying degrees of success of each technique.

Denitrification for Recirculating Systems

Phillip Lee, National Resource Center for Cephalopods, Galveston

Getting full Performance from Packed Columns,
Or, How to Get Your Monies' Worth

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Packed columns are used in many applications. For life support systems they are primarily used for degassing or biofiltration. The following factors affect the performance of packed columns: type of packing or media, amount of packing, shape of the vessel, ratio of air to water, water temperature, and distribution of air and water through the tower. Water distribution is the most commonly neglected and least understood aspect of the design of packed columns. Poor water distribution is the primary cause for poor performance and high maintenance costs for packed columns. A well-designed column utilizes even water distribution across the entire packing surface. For a biofiltration system, this means that all of the microorganisms will have an equal and consistent supply of nutrients and oxygen. For a stripping column, even distribution means there will be a uniform water film on all of the packing. Poor water distribution can lead to the following problems: loss of performance due to dry surfaces in the packing bed, loss of performance due to air bypass through the dry areas, erosion of the packing due to heavy hydraulic loadings, plugging of the packing due to scale deposits in the intermittently wetted areas. Distribution nozzle selection is based on the shape of the media bed, desired distribution pattern, flow rate and available head.

Use of Ionic Constituents to Approximate Total System Volume

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While the total volume of most major systems in public aquaria is known with certainty, occasionally the volume of a particular system may be in question. The total volume may be affected by filtration retrofitting, displacement of water by additional major tank structure, or deviation from original blueprints during construction of new exhibits. When a large system is established and cycled with livestock added, often there is no practical way to approximate the volume short of completely draining and refilling the tank. This study evaluates the practicality of spiking the system water with non-toxic ionic salts which can be measured via spectrophotometry, electrochemistry, or traditional wet chemistry with the animals still on exhibit. Assuming that the starting concentration of the selected ionic constituent is known, the addition of a known amount of the selected stable salt will yield a final concentration that can be used to calculate the approximate system volume. Preliminary results from trials using sodium phosphate are

presented, and the practicality of other compounds is discussed. This method shows promise in evaluating the volume of aquarium exhibits with minimal expense, minimal time expenditure, and without having to remove the exhibit specimens. One negative consequence of this procedure is the resulting high concentrations of the spiked ions in the system. Our solution to this problem is to perform a high volume water change following the spiking and testing to reduce the ionic compound through dilution, a benefit of having a ready supply of low cost replacement seawater available.

Ozone Equipment Selection

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Ozone is ozone? If this is the case, why are there so many ozone vendors out there trying to sell us equipment and all stating their equipment is better than the rest? Is the lowest price ozone system on a pound per pound (grams/hour) basis truly the best buy?

Some ozone manufacturers try selling us equipment at the lowest possible price. Others sell the highest quality at the highest price. The sales people for each side battle… when the smoke clears the consumer is usually left wondering what quality equipment they got based on the price they paid. Time and repairs tell the true cost of ownership.

A non-biased view of the various ozone equipment product offerings that are currently available is presented in this presentation. Guidance will be offered on which products to choose versus others when it comes to your particular water quality objectives. Equipment selection, longevity, serviceability, and repair costs all factor into the guidance selection process. The end result is better equipment for the application, a longer equipment life, and a less problematic life support system, all at a lower cost.

The result is a simple process to keeping selection of your current ozone system one that will satisfy you not only today although for years to come.

Ozone Application and Management in the Aquatic Life Support System (LSS)

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Mark Smith, Cosestudi

Ozone's chemical properties make it a valuable resource for maintaining water quality in aquatic life support systems. When applied carefully ozone provides excellent control of microorganisms, dissolved organic molecules and other contaminants that threaten aquatic animal health. However when improperly applied and controlled, ozone can be toxic to aquatic life and can even pose a threat to human health. In solution, ozone and some of its by-products are collectively referred to as total residual oxidant (TRO). TRO should be maintained at or near zero

in the aquatic animal pool to prevent chronic (and acute) exposure to oxidant. Redox (ORP) electrodes are not consistently reliable; therefore control of ozone is not safely based on ORP alone. Aquarium LSS staff should also utilize and understand applied ozone dose (AOD), TRO, the effects that husbandry practices have on ORP, and animal behavior responses to TRO. In aquatic life support systems with typical biological loading, ozone provides effective disinfection and micro-flocculation at an AOD as low as 0.03-0.5 $mg.l^{-1,}$ and 0.01-0.05 $mg.l^{-1}$, respectively. Contact chambers should be utilized when the applied dose exceeds 0.05 mg/L to provide adequate contact time and off-gassing of excess ozone. By starting with a reasonable AOD and by closely monitoring ORP, TRO, turbidity, husbandry (feeding, cleaning activities and animal behavior), a safe ozone management regime can be attained.

Training Isn't Just for Mammals.
How Training Can Be a Useful Tool to Care for a Variety of Animals.

Stefanie Cooke, Monterey Bay Aquarium
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For decades, behavioral training among zoos and aquariums has been very useful at accomplishing a variety of tasks. It has been used to transport animals, perform medical procedures, and simply feed animals. Most familiar to us is the use of training and enrichment with mammals, but it is becoming more widely accepted in zoos and aquariums when working with a variety of other species. As aquarium exhibits become larger and more complex and community displays gain popularity, the ability to train a variety of animals and provide enriching environments becomes more valuable. From octopus and birds to fish and turtles, numerous training strategies have been implemented at the Monterey Bay Aquarium to accomplish a variety of goals. A series of short videos will demonstrate some of these techniques and how they have proved useful in our day to day care of a variety of animals.

From the Bay to Display: The *Mola mola* on Exhibit at the Monterey Bay Aquarium.

Todd Love, Monterey Bay Aquarium
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The Monterey Bay Aquarium has displayed ocean sunfish (*Mola mola*) since 1986. Since 1995 molas have been displayed in the Outer Bay Exhibit, a 1 million gallon community tank containing yellowfin and bluefin tunas, various shark species, bonito, barracuda, pelagic rays, mahi mahi and sardines. Techniques and procedures for the capture, transport, training and display of molas at the Aquarium will be discussed.

Don't Sweat It's Only Temperate

Darryl Deleske, Cabrillo Marine Aquarium
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Has your facility ever wanted to display cold or temperate water animals? Using a regular single pane fish tank can lead to condensation of the viewing panels. The Cabrillo Marine Aquarium has been making their own dual pane glass tanks for over 25 years. Double pane constructed glass panels are an economic alternative to the expensive thick acrylic panels required for low temperature tanks. You will be instructed on materials used and how to assemble your own temperate fish tank.

CMA's Aquatic Nursery Revisited: A Year of Successes!

Erik Forsman, Cabrillo Marine Aquarium
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RAW attendees were introduced to our 2004 expansion in Long Beach, CA last year. This innovative and unique exhibit puts on display not only the Aquarium's newest additions, but also the volunteer scientists that have raised them. Since opening our doors 18 months ago, 141 staff and volunteers have made attempts to raise 30 different species with varying degrees of success. Four of our major success stories will be highlighted including California grunion, Blue-banded gobies, black sea nettles, and red rock shrimp. Different aspects of the husbandry of these species will be discussed including tank designs, larval development, and food choices, as well as how each of these examples contributes to our scientific goals

Methods & Techniques for the Husbandry of *Gymnura micrura* in Closed Environments

Ashley Hartness, Adventure Aquarium
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Gymnura micrura have not been successfully raised in closed environments beyond a few months, according to my findings. Biologists from the Fish & Invertebrate team at Adventure Aquarium collected our female while seining off the Southern New Jersey coast. The pups were born a little over 10 months from the initial holding date. Both effective and ineffective force feeding techniques – free feeding, tube feeding, hand feeding, leading up to unassisted feeding - as well as food types along with wingspan and weight charts vs. age provide researchers a base knowledge of husbandry from which to develop future comprehensive and successful rearing in closed environments.

Ocean as Aquarium: VideoRay Used As an Aquarium Display

Edward Seidel, Maine State Aquarium
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Aquariums are always looking for novel ways of displaying and educating the public about aquatic animals and plants. They also explore ways of using underutilized resources at their institutions and turning them into exhibits. At the Maine State Aquarium, there is a dock that is located near the aquarium but was never used by the public. The Aquarium came upon the idea of using a Micro-ROV to educate the public about how ROVs are used in science, as well as explore the rich and vibrant aquatic life under the dock. In this presentation, Ed Seidel we will share the experiences and findings, and discuss how they plan to expand and improve the exhibit in the future.

Thursday, May 18

Disaster Preparedness, Aquatic Animal Enrichment

Proactive Inter-Facility Collaboration

Greg Whittaker, Moody Gardens
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Hurricane events over the past few years have challenged zoological facilities across the gulf coast. These disasters should serve as a wakeup call to all who manage animal care facilities with the unique nature of our responsibilities to the animals in our care. Proactive planning is necessary to prepare us for many of the known hazards that face our operations. Crisis management plans should serve to accommodate the immediate needs of on sight personnel as well as provide for communication with off site coordinators. Effective, timely communications can make the difference between tragedy and successfully weathering the storm. Make contact with neighboring facilities in advance to establish a support system that benefits our animals, staff and continued operations.

Hurricane Disaster Management

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Hurricane and other disaster planning begins well before the onset of any storms. Proper preparation and advance planning prior to storm landfall has as much to do with successful recovery as the actual execution of the recovery phases themselves.

Leadership plays a major role in disaster management, especially where recovery workers are themselves victims of storm passage.

Restoration of system services and recovery follows the outline below:
1. Hurricane preparation plan
2. Execution and riding out the storm
3. Recovery efforts immediately after storm passage
4. Long term recovery to Full Capacity

These issues will be discussed in detail from three points.
1. Reporting on the disaster of Hurricane Wilma, C. Ben Daughtry
2. Managing husbandry standards during time of diminished service capacity-Nahcole Sears
3. Organization and leadership during disaster recovery-Forrest A. Young

Evolution of Enrichment

Jill Mellen, Disney's Animal Kingdom

Enrichment for Giant Pacific Octopuses

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We should be doing enrichment for GPOs based on their intelligence, but what should the enrichment be and how can we measure its effects? How do we know if it is beneficial or harmful? Two scientific papers show the benefits of enrichment in cuttlefish and California two spot octopus but these are very preliminary.

There can be environmental as well as behavioral enrichment. Behavioral enrichment maintains healthy activity levels, alleviates space confinement and alleviates deviant behavior such as pacing. A fourth enrichment meets the expectations of the public, a form of environmental enrichment for the public, and a fifth reason for enrichment may prepare animals for

reintroduction to the wild after being held in captivity. All of these forms of enrichment should be practiced with octopuses. Such forms may include complex, suitably-sized environments for them to explore, "toys," complex food or feeding strategies including Mark Rehling's "prey puzzles," proper den or lair spaces, proper lighting and lighting regimes, and proper water quality parameters.

Purposeful Enrichment: A Practical Approach to Addressing Behavioral Situations

Margaret Whittaker, Active Environments, Lompoc
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Friday, May 19

General Interest/RAW Business Meeting

Can Saltwater Fish Exhibits Utilize Enrichment Practices?

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Throughout the field of animal husbandry, the term enrichment has been widely used. It has been defined by the AZA in 1999 as, "a process in which changes to the structures and husbandry practices are made with the goal of increasing behavioral choices available to animals and drawing out the species-appropriate behaviors and abilities, thus enhancing animal welfare."(AZA, BAG) Within the Aquarium Community, recent discussion took place trying to define and/or come up with programs that could enrich piscine enclosures. At Rainforest Café in Seattle, a program was developed to test if such enrichment was possible.

Records indicated one of the major causes of fish loss or damage at this location was due to tank-mate aggression. This held especially true when introducing new fish to the aquarium. We were faced with many challenges: our species of fish were diversified vs. being from areas they occur naturally; our coral inserts could not be altered, causing territorialism in certain areas; the amount of change in lighting duration was limited due to the restaurant being open at late hours; and the amount of space available as limited due to overly large coral structures. In order to enhance their lives, we chose a program that used the following methods: alteration of feeding programs, use of feeding stations, use of gating system, lengthening the photoperiod for darkness, use of covers for the aquariums and use of novelty items like shells and plants. Unlike many enrichment programs at other institutions, we did not have direct operant conditioning phases that we could use as a part of the enrichment program.

We found that the use of the program showed a drastic improvement in the mortality rates of all the aquaria involved. Especially noticeable was the use and interaction with the novelty items. The program also showed overall increased activity of the fish due to these interactions and the hours we increased darkness. Incidence of disease outbreak was noticeable reduced. Lastly, tank-mate damage such as fin nipping and aggressive chasing were not observed as often. Due to

the enhancement of the fish behavior and given alteration of choices in behavior, we concluded that we fit within the definitions and parameters of enrichment at this facility. We hope to share this program and discuss enrichment techniques that can be developed at other facilities within the aquarium community.

Target Training and Tactile Conditioning of Two Zebra Sharks *Stegostoma fasciatum*

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Classic operant conditioning techniques were used to train two female Zebra sharks, *Stegostoma fasciatum*, housed in a 100,000 gallon mixed species Indo-pacific exhibit. Target training and desensitization to tactile stimulation were employed via successive approximations with positive reinforcement to better facilitate their husbandry needs. These techniques allow for the easy moving of feeding stations and allow for medical procedures to be accomplished with much less stress to the animal. The target training has allowed staff to tend to their day-to-day needs in an efficient, effective, less stressful way, while increasing the safety of the animals during feeds. The tactile desensitization has made handling the sharks easier during medical procedures and is a by-product of the target conditioning. This training also has an end product of a decrease in stereotypical behaviors for these two sharks and has led to being able to accomplish relatively stress free blood draws for physicals. It is hoped that throughout the year, these blood draws can be taken regularly to obtain less-stressed baseline blood chemistry values for these two Zebra sharks.

Positive Buoyancy in Adult Leafy Sea Dragons, *Phycoduras eques*

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Leafy sea dragons, Phycoduras eques, are one of the most unique and popular animals in aquariums today. An understanding of common disorders and how to manage problems with this species can be a challenge for animal care staff. The Aquarium of the Pacific has been the home to twenty-two leafy sea dragons over the last nine years. During that time, there have been sixteen mortalities, fifteen of which were adults 3-8 years of age. 80% of the adult mortalities presented with positive buoyancy as the primary symptom and a collapse or rupture of the swim bladder was seen upon necropsy. Histology findings from each case vary, suggesting that the mortalities either had multiple predisposing factors for this condition or were unrelated to the swim bladder pathology.

Trials with Ghostpipefish

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Ghostpipefish are interesting and beautiful animals, although very difficult to keep in captivity. With the aim to understand and overcome those difficulties a trial was carried out with two species of ghostpipefish, the ornate pipefish (*Solenostomus paradoxus*) and the robust pipefish (*Solenostomus cyanopterus*). The present work gives a brief review of the husbandry, feeding techniques and captive behaviour of the animals maintained at the Oceanário de Lisboa.

Aquarium Science Program at Oregon Coast Community College

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The Aquarium Science Program at the Oregon Coast Community College, a program designed specifically to train individuals for the public aquarium, ornamental fish trade, and aquaculture professions, will soon be graduating its second cohort of AQS students. The first cohort of graduates has entered the work force in locations across the country. Developments in the AQS program include plans to break ground on a new teaching building, possibly as soon as this summer. A one-year certificate for transfer students has been added to the program in addition to the two-year Associate of Applied Sciences degree. The AQS program also welcomes a new instructor this year. As the program moves past the beginning stages and becomes more established, we again seek support and input from the industry to continue to provide new, qualified fish husbandry professionals.

POSTER ABSTRACTS

Reproduction in Captive Giant Pacific Octopus, *Enteroctopus dofleini* :
An Elusive Circle Closing?

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Challenges in rearing viable offspring of the giant Pacific octopus remain despite increased interest in public aquariums and research laboratories. In May 2004, mating occurred between a five year old male and a four year old female within an aquarium in public view. First obvious development in the resulting clutch of eggs was observed in November 2004, and hatching commenced in April 2005. The eggs developed in ambient seawater with two small groups

separated from the maternal brood to facilitate access to eggs for examination. Significant visible milestones in development were documented. Hatched paralarvae 5-7mm in length were reared in two separate aquariums which were similar to kreisel-style aquaria used to rear jellyfish. Several types of local wild and commercially-available planktonic foods were offered but it was noted the paralarvae were foraging on prey items larger than zooplankton. Shaved portions of clams and euphausid shrimp were tried which increased the feeding response (Marliave, 1981). Paralarvae were reared 3 months post-hatch, reaching 8 mm total length. Suggestions are offered for improving the survival of paralarvae by addressing abrasion in the rearing vessels and by enhancing nutrition during the pelagic larval stage.

The Use of Dental Floss in Performing Skin Scrapings on Syngnathiform Fishes, An Alternative Technique

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Rebecca Leitner, The Aquarium at Moody Gardens

The skin scraping has proven itself to be one of the most useful and important diagnostic tools in fish medicine. Skin scrapings on most fishes involve the use of a scalpel blade or microscope coverslip to remove some of the mucous and associated pathogens from the skin of fishes, though the size and armored body of Syngnathiform fishes preclude the use of these methods. The use of a sterile cotton or polypropylene swab shows better efficacy in detecting ectoparasites and other pathogens, but often leaves a number of fibers stuck to the trunk rings, creating a potential site for secondary infections if not carefully removed. Smaller protozoan and bacterial pathogens removed during the swab may also become trapped in the fibers, and thus not be transferred to the slide. The use of unflavored, waxed dental floss has shown great efficacy in removing protozoa and bacteria from the exterior of several species of seahorses, pipefishes, and sea dragons; while minimizing stress and potential damage to the animals. This method shows promise not just in the diagnosis of disease in Syngnathids, but also in smaller fishes such as certain members of the Gobiidae, Characidae, Blennidae, et cetera.

Case Study of Spinal Fracture in the Spotted Wobbegong, *Orectolobus maculatus*, with notes Regarding Rehabilitation and Husbandry

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This poster chronicles the diagnosis of spinal fracture in a spotted wobbegong, *Orectolobus maculatus*, and the specialized husbandry requirements of the specimen thereafter. On 26 May 2005 one of a group of two juvenile *O. maculatus* housed in the quarantine facility of the Aquarium at Moody Gardens was observed with an unusual “hump” on its back. The following day the specimen was taken to the office of Dr. Richard Henderson in Galveston, Texas. Dorsal and lateral radiographs were taken of the shark, from which the diagnosis of spinal fracture was obvious. The radiograph showed that the 27th trunk vertebra had fractured frontally

and lie in two pieces (dorsal and ventral). The radiograph also showed that the 43rd trunk vertebra had most likely suffered a less severe prior fracture which had healed. The break in the 27th vertebra was indicative of a compression fracture, most likely due to the specimen striking the tank wall or acrylic while striking at a live feeder shrimp. Over the course of several days the specimen lost all function of the tail below the fracture, but otherwise retained all vital functions, making euthanasia unnecessary. The specimen resumed feeding (sporadically) several weeks later, and by through the exercise of patience and extreme caution in feeding and supportive care, has regained a normal appetite and shown good growth. The most remarkable development in the ongoing convalescence is the fact that the shark has become quite proficient in swimming with its pectoral fins! This case showed that sharks which have suffered severe trauma have a remarkable ability to recuperate, and further validates the use of radiography as a diagnostic tool in cartilaginous fishes.

Studies on the Lifecycle Strategy of *Huffmanela huffmani* (Nematoda: Trichosomoididae: Huffmanelinae) in the San Marcos River, Texas.

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Huffmanela huffmani Moravec, 1987 (Trichosomoididae) is a nematode histoparasite of the gas bladders of fishes in the family Centrarchidae. While many species of centrarchids are found throughout Texas, fish infected with *H. huffmani* are known only from the headwaters of the San Marcos River (TX), which are spring fed from the Edwards Aquifer. Although prevalence of infection in this portion of the river has been reported as high as 90% and fresh eggs can be found year round, only a few adult worms have been observed, and the most obvious manifestation of infection is darkening of the gas bladder tissues caused by deposition of thousands of eggs.

The lifecycle strategy of *H. huffmani* is unknown. Twelve other histozoic species of *Huffmanela* have been described, also with unknown life cycles. But unlike the freshwater *H. huffmani*, all other species in the genus are reported from marine fishes. Consequently, it is suspected a previously unknown endemic species of invertebrate from a mostly marine taxon, perhaps a freshwater polychaete, is serving as host in the San Marcos River.

Preliminary observations from an ongoing investigation will be reported. This investigation aims to gather information on the lifecycle strategy of the nematode using controlled feeding experiments, host diet analysis, and direct observation of fish behavior in the type habitat.

THE TRAINING OF A MALE GIANT PACIFIC OCTOPUS, *Enteroctopus dofleini* TO ASSESS HEALTH AND WEIGHT

Jennifer Moffatt, Assistant Curator of Aquatic Life

Pittsburgh Zoo & PPG Aquarium

Introduction

The giant pacific octopus (GPO), *Enteroctopus dofleini*, has become known as the world's smartest invertebrate. Through investigations at public aquariums, we are able to enrich and surmise its abilities to interact and solve puzzles. To better understand the GPO's intelligence, rate of weight gain and overall health, a training plan was instilled at the Pittsburgh Zoo and PPG Aquarium.

Materials and Methods

Materials included: (1) 2'X2' Scale, (1) plastic container 3'X18"X6" D, and squid (one of many favorite food choices)

The specimen was caught in a crab trap in the Pacific northeast by local fishermen. The octopus was without water for 45 minutes before accession to the Oregon Coast Aquarium. While in Oregon, optimal care was given for the by-caught octopus. Upon receipt at the PPG Aquarium, several weeks later, the 6 lb. male GPO was non-interactive with humans and hesitant to explore his surroundings. The original holding system in which he was introduced and trained was a 200 gallon fiberglass square tank.

Training

As previously mentioned, the octopus showed no interest in human interaction. This may have been related to experience in the crab trap. With that knowledge, the keeper was slow to introduce herself. With each introduction, the keeper would slap the water surface. Once responsive, he was offered food items with a couple of minutes of tactile stimulus. After a couple of weeks, the GPO began to interact and allow longer periods of 'playtime'. Throughout this time, puzzle feeders were offered (e.g. peanut butter jars) as well as bath toys. Once the GPO seemed to 'trust' the keeper, training began.

Prior to training sessions, the keeper would smear squid juice on the inside of the tank (above the waterline). As before, the keeper would slap the water. Once the GPO moved to the top, the keeper would tug firmly on one arm, two times. The GPO was then encouraged to taste by placing his suction cups directly on the squid juice; creating interest with squid juice promoted a wanted behavior. By using successive approximation, he began to travel to the lip of the tank then eventually to a plastic container. (Before each session, the trail of squid juice was moved further from the surface of the water.) After training for several weeks, the octopus appeared comfortable enough to remain out of the water for several minutes. At this point, the keeper began to walk a few feet, at a time, with the GPO in the tray. Upon return, the keeper would place the tray on the top of tank and would allow the GPO to find the water surface. Without

assistance, he would then drop into the tank. After each successful approximation, the octopus was rewarded with tactile stimulus and food items directly.

Approximately 6 months after training began, he was transferred to a 1500 gallon exhibit. These sessions were practiced 2-3 times per month and actual weights were acquired once per month. The use of fish juice was not necessary, but allowed for a somewhat easier session. Also, during a session, the GPO would sometimes use the keeper's arm for leverage. The keeper at no point helped the octopus into the container, but only offered leverage.

Discussion

With the training of any animal, there are discussions and problems that occur. Some of these are listed as follows:

Initially, the octopus was hesitant to perform with more than one keeper present. With the presence of other keepers during the sessions, he slowly became undisturbed. (This definitely has helped with supporting the tray: his weight increased greatly.)

Additionally, during the original training time, the GPO would behave as if all interactions were a command to enter a tray; although the intent of the keeper may have been only to play or feed. In order to stop the behavior, the keeper would halt all interaction, replace the lid of the exhibit and return the next day. If it was meant to be a feeding interaction, then an alternative was found. Offering puzzle feeders, like jars, still allowed for enrichment and food consumption without neglecting his diet or encouraging unwanted behavior. After a week, the octopus no longer attempted to leave the tank unless he was cued.

After being transferred to the 1500 gallon exhibit, his reaction to the command appeared reverted. He showed little interest in human interaction, but was set on exploring his new surroundings. However, with daily enrichment offerings and attempted sessions, he quickly began to complete the command and show interest in human interaction again.

There had also been discussion regarding his behavior while in the tray. Although he was able to escape the tray easily, this behavior was not seen; not at any point did the octopus attempt to leave the tray. This was more than likely related to the reward that followed his return to the tank.

Conclusion

While the training of a Giant Pacific Octopus can be at times consuming and difficult, in this situation, it benefited both, humans and invertebrate.

The GPO became highly playful and showed no aggression with keepers and tour groups alike. This training allowed this animal to feel comfortable in the presence of humans, while offering enrichment.

In addition, this allowed for public education. With each introduction, the public was able to interact and gain knowledge regarding this species. It is our hope to promote conversation and education whenever possible through direct contact.

Disclaimer

We do not encourage the public or inexperienced keepers to attempt any interaction with a giant pacific octopus unless supervised by an experienced professional. This could be harmful for both, the octopus and the human.