DRUM *and* CROAKER

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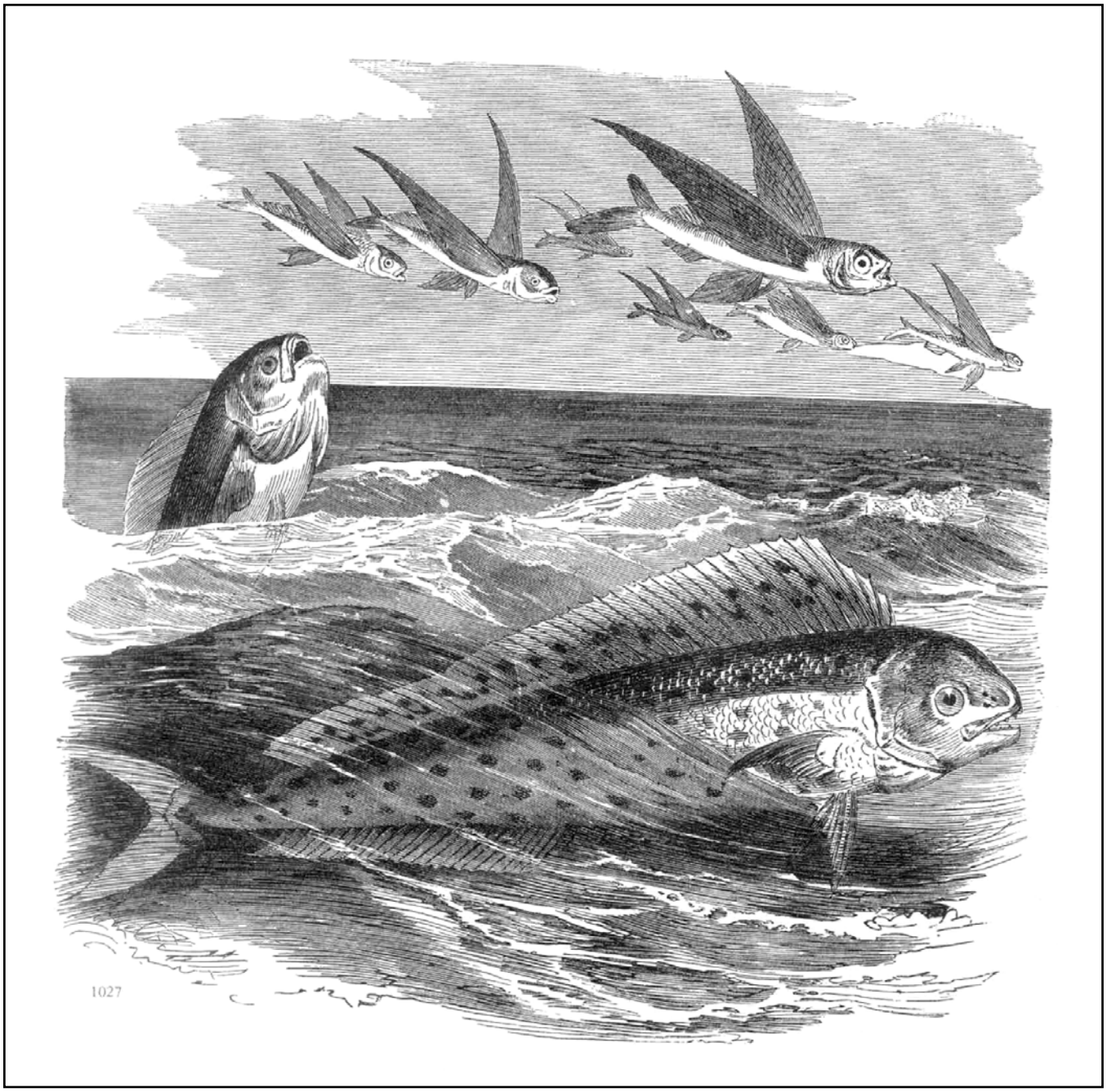

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DRUM AND CROAKER 30 YEARS AGO

Richard M. Segedi

(From D. & C. Volume 13 (72), Number 2 (7/72). Edited by John G. Shedd Aquarium)

New Tidepool Display at Sea World, San Diego. Dave Powell, Sea World, San Diego

In recent years, exhibits which allow physical contact between the visiting public and the animals have become quite popular and successful in both zoos and aquariums. The first aquarium to use this approach to display marine invertebrates was the Point Defiance Aquarium in Tacoma, Washington. On the basis of their success in Tacoma, Sea World designed and installed five open "tidepools" containing invertebrates and fishes of Southern California. Although no tidal fluctuation occurs, these displays have proved to be one of our most popular exhibits.

A Program For Envenomation - Do You Have One? Alan K. Levitt, Nat'l Fish. Ctr. & Aq.

I have seen lionfish sold to children with the sole warning, "better not let it sting you." Some fish are advertised and bought just because they are venomous or dangerous. Moreover, on at least two occasions this aquarium received phone calls from people who had bought "venomous" fish from pet stores in the area. The fish were described to us over the phone and we were queried about their identities since the stores which had actually sold them did not know!

On the Preservation of the Manatee. Craig Phillips, National Fisheries Center and Aquarium

It is difficult now to find a large Florida manatee that does not bear at least one scar obviously inflicted by a boat keel or propeller, and numerous individuals are killed outright when struck by boats. Lacking the dolphin's ability to echolocate, being virtually invisible from a short distance as it surfaces to breathe, and relatively sluggish in its reflexes, the manatee is in imminent danger of having its final numbers reduced to "the point of no return" in its last two strongholds in the U.S. — the Miami River and the western portion of Everglades National Park.

Can you identify the mystery guest? Who is Olaf's friend? Ten pounds of whipped cream and clams to the winner.

The photo at left appeared in this issue in 1972. As the caption reads, you were supposed to guess who the man was. Does anyone now (in 2002) remember him or guess his identity?

Hint: He was a famous aquarium director of the '40s and '50s.

COLLECTION AND HUSBANDRY OF DOLPHINFISH AT MONTEREY BAY AQUARIUM, MONTEREY, CALIFORNIA

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Monterey Bay Aquarium

Over the last two years efforts have been made to add dolphinfish (*Coryphaena hippurus* and *C. equiselis*) to the Outer Bay Waters exhibit at the Monterey Bay Aquarium. The common dolphinfish (*C. hippurus*) has only been displayed at a few public aquariums. Typically, due to the high activity level and the pelagic nature of dolphinfish, small juveniles (3-5 cm) are collected offshore, transported to the facilities and then reared to a size suitable for exhibit. This paper describes the methods used by the staff at the Monterey Bay Aquarium to successfully collect, transport and display dolphinfish.

Grow-out of wild-collected or aquacultured juvenile dolphinfish (*C. hippurus*) has been used at Waikiki Aquarium, Aquarium of the Americas (AOA) and Sea World San Diego. At Waikiki Aquarium, the wild-collected juveniles become broodstock for a captive-breeding program that produces successive generations for exhibit (Kraul 1993). AOA attempted to grow-out dolphinfish in 1992 and 2000. The largest fish from these two attempts reached 35 centimeters. Water quality, a small grow-out tank and the tendency of the fish to jump out of the grow-out tank were all factors that contributed to these less than successful attempts (J.D. Hewitt, pers. comm.). In 1978, Sea World San Diego exhibited two adult common dolphinfish reared from a batch of wild-collected larvae. These adults were on exhibit for approximately a year.

The grow-out method has its drawbacks as larval and juvenile dolphinfish are cannibalistic, often collected from the wild a few at a time and require a large tank (6 m diameter or greater) for grow out (Kraul 1993). During the grow-out period, the dolphinfish may also suffer deformities or damage due to the confines of the grow-out tank. Due to the limitation of this method of obtaining dolphinfish for display, the aquarist staff at MBA decided to try and collect small adult dolphinfish in a manner similar to the methods used to collect tuna. Even though collection of adult dolphinfish is described in Szyper (1991), many have thought that adult dolphinfish would be too skittish to be held in a transport tank or livewell.

During October 2000, 13 small (50 cm FL) pompano dolphinfish (*C. equiselis*) were collected. These fish were collected with rod and reel using baited barbless hooks and lures. The fish were placed in a 12,000 L livewell on board the *F/V Shogun*. All of the fish immediately settled into a calm swimming pattern and navigated the well without any problems. The fish stayed calm and exhibited no abnormal behavior for the duration of the five-day trip back to San Diego. In San Diego, the fish were off-loaded in the same stretchers used to move tuna. These vinyl stretchers completely enclose the fish along with several gallons of water. Even after the water level was lowered and staff climbed into the well and began capturing the fish, they remained calm. After off-loading from the boat, six of the dolphinfish went directly into the trailer mounted, 12,000 L tuna transport tank, with an oceanic white-tip shark for the ten-hour drive back to Monterey Bay Aquarium. The tuna transport tank is capsule-shaped (3.96

m X 2.28 m X 1.37 m) with all the necessary hardware to maintain circulation and oxygen levels. The other seven dolphinfish went into a five-meter diameter holding pool on the dock in San Diego. Again, both the fish in the transport tank and the fish in the holding pool behaved in a calm manner. The fish in the holding pool were transported to Monterey Bay Aquarium the following day in the tuna transport tank.

At the Aquarium, six pompano dolphinfish were put directly in the four-million- liter Outer Bay Waters (OBW) exhibit. The other seven pompano dolphinfish were put in a connected holding pool by themselves. This 106,000 L uncovered, outdoor holding pool is 6.7 meters in diameter and 3 meters deep. Both groups of fish were presented chopped squid and smelt. Several fish, in both the OBW exhibit and the holding tank, quickly became accustomed to the dead food and began to feed regularly. Unfortunately, several of the fish put directly into the OBW exhibit died within the first few days. Based on evidence collected during the necropsies, it was concluded the length of time the fish spent on the boat without food compromised the fish to a point that even once they were offered food, they did not recover. The aggressiveness of the other fish on exhibit may have also played a part, as a higher percentage of the fish in the holding pool survived. After four weeks, the dolphinfish in the holding pool were moved into the exhibit. For approximately two months, the dolphinfish were target fed halved squid and smelt. Within the two months following introduction of the exhibit, all of the dolphinfish were actively feeding during the regular exhibit feeding sessions. The OBW exhibit is fed 100-115 kg of squid and smelt four times a week.

The growth rate of the dolphinfish is rapid. At collection, the fish were between 35-42 cm FL and 560-615 grams. Three months following collection, they were approximately 47-52 cm FL and 1.5-1.7 kilograms. Eight months following collection they were approximately 2.5 kilograms and 59 cm FL. At this time, the pompano dolphinfish are also exhibiting spawning behavior on a regular basis.

During October 2001, 12 common dolphinfish (100 cm FL and 3 kg) were collected offshore Southern California. During the three days at sea, live sardines were fed to these fish. Handled in the same manner as the pompano dolphinfish, the common dolphinfish were transported back to Monterey Bay Aquarium. Seven of these fish were put directly into the Outer Bay Waters exhibit and five were put in the connected holding pool. The initial feeding attempt was with live anchovies, and a few fish in each tank started feeding within a day or two following introduction into the respective tanks. Following the initial feeding attempts, halved squid was offered to the dolphinfish. All four fish in the holding pool started feeding within two weeks. After three weeks, all of the fish in the Outer Bay Waters exhibit began feeding on the halved squid. In this time period, four of the dolphinfish began to feed on whole squid and smelt during the general exhibit feeding. Three dolphinfish from the holding pool were introduced into the Outer Bay Waters exhibit five weeks following collection. Prior to moving the dolphinfish from the holding pool to the exhibit, one died from a collision with the side of the holding pool and one was euthanized due to the extensive injuries sustained during collection. Eight weeks following arrival at the aquarium, all of the common dolphinfish began feeding on whole squid and smelt during the general exhibit feeding.

Both species of dolphinfish associate very closely to the surface of the water. This makes it easy to broadcast food to them if they are either too small to take the food offered during the general feeding or they are too skittish to feed during the commotion of the general feeding. The dolphinfish were fed daily until they began regularly feeding during the general feeding. As they become accustomed to the exhibit and the other fish, they spend some time in the water column, but still spend most of their time near the surface.

While some fish were lost due to wall collisions or jumping, this mainly occurred in the smaller holding pool where some of the fish were first introduced. Kraul (1993) recommends the use of the large diameter raceway style tank to prevent the fish from being able to swim directly across the tank and colliding with the opposite wall. With the smaller holding pool, the tradeoff is the potential of mortality due to impact or jumping with the shorter amount of time it takes the fish to become acclimated to feeding on dead food. The observations at MBA are that more fish are lost due to not beginning to feed rather than collisions. The collision mortalities that have occurred in the Outer Bay Waters exhibit have been due to fish-to-fish collisions during feedings. Food is widely distributed over the surface of the exhibit to minimize collisions, but occasional collisions do occur.

While it is still too early to tell how well the common dolphinfish will do on exhibit long term, based on the experience with the pompano dolphinfish the expectations are high. Most of the problems associated with the grow-out method were bypassed by collecting small adult dolphinfish. By collecting small adult dolphinfish, MBA was able to introduce small schools of two new species with a unique body shape and coloration from the yellowfin and bluefin tuna that are presently on exhibit.

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DINOFLAGELLATE CULTURE AND NOTES: A POSSIBLE FOOD SOURCE FOR CTENOPHORE, *PLEUROBRACHIA BACHERI*, HATCHLINGS?

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Introduction and Purpose:

For the past few years the culturing of ctenophores for exhibition has become a topic of interest for a couple of reasons. First, the availability of wild caught animals is based on natural cycles or a surplus from another institution/personal contact. This basically means a large component of a major exhibit comes from nature and this creates many challenges (i.e. Are they present in local waters? Are conditions such that we can go out on the boat?). Second, it would also be an exceptional achievement for an aquarium to break ground on something that has had minimal success. Therefore, culturing ctenophores would help to assure a more consistent and spectacular display.

The missing link in the process of ctenophore culture, specifically *Pleurobrachia bachei,* has been a food source for juvenile ctenophore size 0.10 mm to 1.0 mm. According to Strathmann et al. (1987), prey size is critical to growth and survival. Size is recommended to be 1/3 to 1/5 the size of the larvae (Hirota, 1972). It has also been suggested that relative availability and vulnerability are characteristics to look for in a food source (Greene et al. 1986). These suggestions along with the fact that the Northern Anchovy, *Engrauilis mordax*, will feed on the locally abundant unarmored dinoflagellate *Gymnodinium splendens* (Lasker et al. 1970; Hunter, 1976) led to the culture of this dinoflagellate as a possible food for *P. bachei* hatchlings. Another dinoflagellate *Prorocentrum micans*, was suggested by Suzanne Stromm, at Friday Harbor Marine Laboratories, as another easily cultured potential food source. Both dinoflagellates are between 50 to 80 micrometers. A wild caught and a laboratory cultured strain of *Gymnodinium splendens* were cultured as well as a laboratory strain of *P. micans*. Neither of these species has been found to be suitable food sources for *P. bacheri*, even after several feeding attempts. This may be due to size, lack of vulnerability, or construction of the of the dinoflagellates. However, the purpose of this paper is to outline the protocol for general dinoflagellate culture.

Maintenance of a Pure Starter Culture:

Once you have obtained a pure culture from a reputable source or isolated the dinoflagellate desired, set them in a 78^{o} F incubator with a 12:12 photo period until ready to use. I ordered *P. micana* and *G. splendens* fron the University of Texas, in Austin, and I obtained a locally isolated culture of *G. splendens* from a researcher at UC Santa Cruz.

Preparation of Microalgae Media:

Micro Algae Grow Mass Pack (Florida Aqua Farms) was used with a few exceptions and additions to the directions. First, you do not need silicates. Second, check the pH using a meter

and if necessary adjust it.

Once the four liters of media have been made it needs to be filtered through a sterile 47 mm microanalysis filtration system with a fritted glass support and bottled for later use. To filter/sterilize the media several steps need to be taken. First, autoclave four one liter brown glass bottles, two 1000 ml filter flasks with the openings covered in aluminum foil, two filter bases with rubber stoppers covered in foil, two graduated glass funnels covered in foil, and one funnel covered in foil for 15 minutes at 15 psi on fast exhaust. Next obtain one suction pump, an inline filter, some tubing that fits the filter and pump, a stand if you have it, clamps, and paper filters. When setting up, place a .45 micron filter (Milipro) between the filter base and the graduated glass funnel. Use tweezers that have been sterilized in alcohol to place the filter. The paper filters consist of two sheets and when placed properly should have the fat filter placed first and the slender paper filter (with grid side up) placed on top. Secure with a clamp. Pore media into graduated glass funnel and let it get sucked through the filter. Generally one filter is used per 250 ml of media. This part of the procedure takes almost 4-5 hours to fill all four bottles. Lastly, leave enough space in the bottles for the expansion of liquids because three out of the four bottles will be frozen for later use.

Inoculation of Dinoflagellates:

To prepare media solution, sterilize all the equipment needed and follow a step by step process. The items needed are a sterile glass filtration system (as described above), sterile flasks, filter paper, a calibrated pipette, and media. Let everything cool after being autoclaved. Next, collect the appropriate amount of filtered seawater and filter it through a .22 micron filter. For every 2 L of filtered seawater add 1ml media. Take the pH with a meter and if necessary add enough HCL for the media solution to be at around 7.5.

To culture dinoflagellates add the 10 ml vial of animals to about 30 to 50 mls of media solution in a clean, sterile flask. Cover the opening with aluminum foil and put into an incubator at 78^{o} F (12:12 photo period) without aeration. When culturing dinoflagellates aeration is not necessary, and actually harmful to these animals. Also, when picking a container, choose a flask that is appropriate for the volume of the culture. In a culture with too much surface area they seem to expire at a faster rate than in a medium size container. This is also true of a small container, but smaller seems to work better. Lastly, it is wise to check on your culture every week to see if the batch is dense. A dense culture is best described by a cloud of golden individuals that cluster towards the top of the water column. A cell count can also be very helpful and a “mature” culture will have numbers ranging from 3500 to 4000 per 1ml.

Culture Splitting:

To split a culture you again need to sterilize all equipment, make new media solution, and make sure there is a proper animal solution ratio. The best ratio I have found for splitting a culture is 1:1. For example, I have mixed 50 mls of media with 50 mls of “mature” culture and had good success. It varies a bit between different dinoflagellates, but this is generally a good ratio to begin with.

Notes:

Dinoflagellates can be temperamental and several things can lead to a potential crash in culture. For example, if you check your flasks and the color of the fluid is bright green, another protozoan (blue-green algae) most-likely has taken over the flask. This may be due to dirty glassware, air contamination, water quality issues, old media, or a poor starter culture (Hoff et al. 1987). If the culture fails or produces a creamy sludge the cause may be poor water quality or a sudden temperature change (Hoff et al. 1987). In some cases, the culture can be saved by decanting the live dinoflagellates and putting them in fresh media solution. If the culture has not grown at a normal rate or is light this may be due to poor lighting or unbalanced nutrients (Hoff et al. 1987). Lastly, it is good to know that if there are dinoflagellates on the bottom for a long period of time they are probably dead.

Acknowledgments:

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CAPTIVE REARING OF GARIBALDI

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Natural History of Garibaldi

Garibaldi (Hypsypops rubicunda) are the largest of the damselfish (Pomacentridae) reaching a length of 14 inches (30 cm). Adults are golden-orange. Juveniles look like miniature adults but possess iridescent blue spots and stripes all over their bright orange bodies. They are found on the west coast of North America from Monterey, California in the north to the Gulf of California in the south where they inhabit rocky reefs and kelp beds from subtidal to 100 feet (30 meters) (Goodson, 1988; Love, 1999; Miller and Lea, 1972; Moser, 1996; Snyderman, 1998). Stratton (1999) states, "Its popular name is taken from Italian revolutionary, Giuseppe Garibaldi. He was famous for wearing a bright orange shirt and was best known for trying to unify Italy in the 19th century. He had a reputation for reckless abandon and attacking out of seemingly nowhere. The fish named for him has similar qualities."

Garibaldi are very territorial, aggressively defending their chosen reef spots. They charge intruders and make loud thumping sounds by grinding their pharyngeal teeth. They are able to chase away surprisingly large intruders. Despite their territoriality, garibaldi are very curious and will often approach divers to investigate. They have been protected in California since the early 1970's and in 1993 were recognized as the California state marine fish.

They remain solitary until spawning season (March through August) at which time males culture a nest of low-lying red algae in the hopes of attracting a female to lay her eggs. Females produce 15,000 to 88,000 eggs. Once the eggs are laid, the male chases the female away and guards the nest.

Egg Collection

Garibaldi do not lay eggs in Cabrillo Marine Aquarium (CMA) exhibit tanks. Eggs are collected from a nest on the LA breakwater under the auspices of a Scientific Collector's Permit issued to the co-author by the CA Department of Fish and Game.

The nest is carefully observed to note when new eggs were laid. The eggs are collected on the 12th day of development. Eggs attached by mucous strings are caught in between the tines of a comb as it is passed through a small area (approximately 5cm^2) of the nest. The comb and eggs are put into a bag with seawater, sealed and brought back to CMA. Most of the time the eggs hatch during the 20 minute trip back to CMA.

Egg Development

Newly spawned eggs are clear with a small light orange inner ball (oil droplet) giving the batch an overall orange appearance easily distinguishable on the red algae carpet that makes up the nest. From day one to day three, the eggs darken due to development of the embryo's body. By day four, the eggs darken further and eye spots become visible. On days five and six, eye spots develop a silver pigmentation. From day seven to nine, the embryo is well defined with

well developed eyes and chromatophores. By days ten and eleven, the oil droplet is adsorbed and the egg mass takes on a silver/blue hue. On day twelve, eggs begin to hatch. The larvae are about 3.3 mm at hatching (Table 1).

Rearing Tank

Newly hatched larvae are slowly acclimated from a temperature of 58 F (14 C) to a temperature of 70 F (22 C) in a 10 gallon (38 L) glass tank with flat black sides. The sides are made black with flat black Plexiglas taped to the sides. Any covering that reflects light acts as a mirror and larvae are drawn to the sides. The larvae have eyes on the side of their heads, and seem to have trouble locating prey with light coming from all sides at once. In the ocean sunlight is overhead and at slight angles. By darkening the sides and concentrating the light source from above, the larvae are able to locate prey. Hoff (1996) states painting a tank black yields the best results.

The bottom of the tank is clear and set on a tan surface. This makes it easier to see and siphon detritus and dead larvae off the bottom.

Lighting

A small fluorescent light (60 watts) is suspended six inches above the tank with egg crate to reduce the light intensity. The light is on a timer with a 9 hour on and 15 hour off cycle. The fluorescent light does not create excess heat and is cheaper to run. If the lighting is too intense the larvae are driven to the bottom of the tank and stand on their heads. We find that a lux of 500, kept the larvae in the middle of the water column. As the larvae developed we were able to increase the strength of the lighting by removing the egg crate.

Aeration

Two airstones are positioned on opposite sides of the tank. Care must be taken to ensure that suction cups with clips are used to lift the airline away from the sides. Any small crevice can become a trap for larvae. The most important issue with aeration is to create sufficient oxygen, without creating turbulence that damage larvae. Oxygen demands are taxed heavily due to the high numbers of larvae and live food required. If air bubbles are too large, newly hatched larvae may become damaged and die. On the other hand, if the air bubbles are too small, they stick to the larval bodies causing death. We also found that if the air bubbles are too fine, the larvae eat them resulting in death. Blue ceramic airstones are used so we can easily adjust the size of the bubbles, creating sufficient flow to keep the larvae in the water column and keep sufficiently high oxygen levels. As the larvae grow and became stronger, the intensity of the bubbles can be increased.

Temperature

A heater is set to maintain 70 F (22 C). At a lower temperature the larvae grow more slowly. By increasing the temperature, larval development is quicker. The heater should be placed near one of the airstones to ensure the tank is heated evenly.

Filtration

No filtration was used in the interest of not filtering out live food (see Feeding below) or entraining larvae. Pristine water quality is necessary. Daily water changes and bottom cleaning are done from day three onwards.

Once the larvae complete metamorphosis (approximately 40 days) filtration can be incorporated into grow out tanks.

Water Quality

The rearing tank is monitored daily for salinity, pH, ammonia, nitrites and nitrates, and temperature. Water quality parameters are kept at the following levels: salinity 33ppt (1.024 specific gravity), 7.8 to 8.4 pH, ammonia 0.0 to 0.1ppm, nitrites and nitrates at 0.0ppm. Greenwater (microalgae *Nannochloropsis*) is added (just enough to show a green tinge to the water) to "sop up" ammonia that may begin to accumulate.

From days one to three, no water changes are performed. Starting on day four, we do daily water changes of 20%. Daily water changes also incorporate bottom siphoning and removal of any dead larvae. After testing makeup seawater to assure the best water quality, it is dripped into the tank at a rate of 3 drops per second. Live food and reverse osmosis or dechlorinated tap water (if needed to maintain salinity) is added to makeup water to maintain water quality parameters. One drop of iodine, recognized as an important element for metamorphosis in developing fish larvae (Frakes, Hoff and Hoff, 1983), is also added with makeup water.

Food

Food is chosen for different developmental stages of the larvae and juvenile garibaldi (Table 2). Rotifers (*Branchionus sp*.) are the primary food source for the first 25 days. Rotifers are harvested, then set to feed (1 hour) on Roti-rich™ formula (Florida Aqua Farms, Inc.) every other day. On the opposite days, the rotifers are fed microalgae (*Isochrysis* or *Nannochloropsis*). After feeding on Roti-rich™ or microalgae, rotifers take on an orange or green color, respectfully, and are easily seen by the larvae. Concentrations of rotifers are kept at about 5-7 per ml in the 10 gallon tank (for the first 3 weeks). Another way to judge levels is to look through the water – as long as a larva only has to swim a body length (or 1.5 body lengths) in any direction to find food, levels are sufficient.

About 16 oz (470 ml) of microalgae is added to the tank to provide rotifers with a food source, which promotes their reproduction thus helping to replace some rotifers eaten by the larvae. The microalgae also helps maintain water quality (see Water Quality above).

Newly hatched *Artemia* nauplii are introduced together with rotifers on day 25. The nauplii are enriched with Super Selco™ (containing essential fatty acids: 20:5 omega 3, 22:6 omega 3 and vitamins B^{12}, C and A) overnight and then fed to the larvae.

Hoff, 1996 states that *Artemia* nauplii are 2/3 larger than new hatched rotifers and equivalent to approximately 10 adult rotifers in volume and to 30 rotifers in weight. Therefore, there is no need for as many new hatched nauplii to achieve proper feeding levels. We would

harvest our nauplii, stand them next to a light and then draw out 1 concentrated ml from the area where they congregated (the senior author once counted 364 Artemia nauplii in one milliliter). Personal judgment is best here. Sometimes 1 ml is sufficient other times 2 ml is required. The late flexion/post flexion larvae tend to eat the nauplii as soon as they are introduced. We break the nauplii feedings into 3 times a day. A caution here: do not overfeed – the larvae will eat continuously and can become so bloated that they sink to the bottom and die. When food is located, the larvae form their body and tail into an "S" shape, and in a burst of speed lunge forward, open their mouths and capture their prey.

We originally used Artemia that hatched from cysts, but found larvae dead with cyst shells in their mouths. Larval fish can choke on partially hatched cysts. We find decysted nauplii to be cleaner and virtually all digestible.

Conclusion

With the protocols listed above we have successfully raised four batches of garibaldi over the last three years totaling 5, 24, 7, and 7 juveniles, respectively. We have shared the surviving juvenile garibaldi with aquariums all over the nation.

We are working with researchers at California State University, Long Beach investigating the DNA of the garibaldi we have raised. All of the eggs have been collected from the same nest. We are interested in determining how many different females have laid eggs in the nest and if the same male is responsible for the nest each of the last three years.

We have plans to continue our captive rearing program for garibaldi. We will continue to share the 'fruits of our labor' with other aquariums and the data we accumulate with research institutions. This program serves as a training ground for future scientists (many young aspiring researchers help with this program) and may supply a popular tank resident for public aquariums with North American West Coast galleries.

Acknowledgements

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Table 1. Size and Metamorphosis

Newly Hatched approx. 3.3 mm	Body clear with dark pigmentation spot halfway down body region, speckled chromatophores around head and yolk-sac region, underslung lower jaw
Day 7 – 14	preflexion: increase in size, main growth area head region
Day 14	late flexion: color change, greenish/golden-yellow head region, pectorals very defined and large, clear body region (wider), diminished dark pigmentation spot half way down body region
Day 16	pectorals rimmed with light yellow
Day 20 – 37	postflexion: approx. 8.5 mm, dorsal, anal, and caudal fins all well defined
Day 39	juveniles: darker body coloration, all fins defined, mouth terminal, eye further forward
Day 44	orange coloration evident
Day 49	iridescent blue markings appear
Day 60	approx. 18 mm

Table 2. Feeding Regime

Days 1 – 25	Rotifers
Days 25-30	Rotifers and Artemia naupli
Days 30-53	Artemia nauplii
Days 53+	Artemia adults/flake food/amphipod/mysid shrimp/gel food

DIVING FOR CANDY BASS, LIOPROPOMA CARMABI, OFF OF CURACAO

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The candy bass is a beautiful and extremely interesting aquarium fish. Combining this with the ease of maintaining it in captivity, make the candy bass an excellent selection for the marine aquarium.

This fish inhabits a wide range of habitats in the Caribbean Sea and is found not only on vertical walls in relatively deep water, but also on deep water coral heads and rubble piles as well. The typical depth range is from 60 to 100m, though they can be found both deeper and shallower. While this fish is rarely or occasionally seen off of Florida and in the northern Caribbean, it is much more common in the southern Caribbean.

This fish was only recently discovered in the 1960's by Dr. Jack Randall off the island of Curacao, Netherlands Antilles. The species name, "carmabi" was used in honor of the famed Antillean marine biological research facility, Caribbean Marine Biology Station (Car-Ma-Bi).

The candy bass is a relatively secretive species and it usually lives around coral heads and coral ledges. Sometimes they live alone and but often they can be found together living as a mated pair. They prey on small fishes and crustaceans that live in these areas. It typically stays close to the coral and does not venture very far from the bottom.

They are quite an intelligent species (as far as fish are intelligent) and are more difficult to capture than almost all other reef fish species. The only other small reef fish here, that is more difficult to catch, is a swissguard bass in a very deep crevice. We catch all of them in a small hand net that we make out of very fine plastic netting and an aluminum handle.

A typical dive for candy bass is prepared one or two days in advance by mixing the gasses needed for both the penetration to the bottom and for the long decompression that follows. Since we are diving relatively deep, we have to use a lot of care and select our gas mixes according to the dive plan that we establish well in advance of the dive. We will use a minimum of four different gas blends in this dive, and often we add a fifth gas for additional safety. This means that for every gas blend, we will need a separate tank and regulator, plus secondary back up regulators should one of the primary regulators fail. The author often dives deep with as many as five regulators and five tanks carried all at the same time on a special rig and harness.

Each day of deep diving is preceded by a full day of preparation. That includes gas mixing, gas analysis, rigging the boat with all the gas mixtures stored in separate diving cylinders and planning the operations in advance

In deep helium-mix diving, we have what is known as a "virtual ceiling", one that we cannot penetrate without serious risk of severe decompression illness that include, death,

complete paralysis or partial paralysis. We cannot surface to breath normal air until all the decompression stops are finished. For this reason, we have to approach the diving with much training and much preparation; this is part of the reason that deepwater fishes are expensive. Further there is a significant amount of mental preparation, as all of us are completely aware of the substantial risks that we are taking.

For dives in the 50 to 60 m range we will use air as the bottom mix, but for dives in excess of 60 m, which are the usual for candy bass collections, we will use tri-mix which is a blend of helium, nitrogen and oxygen. The usual candy bass dive is to 70 or 80 m and we use a mix of 17% oxygen, 33% nitrogen and 50% helium for these dives Beyond 67m, the partial pressure of oxygen in air (under this pressure) makes air dangerous to breath, as it becomes "toxic"at this level. Many people (the author included) have dove this deep and deeper breathing air, but it is strongly discouraged. We have had several near-accidents using air at depths in excess of 67m and we do not do these dives any more.

There is an American saying that goes something like this: " there are bold divers and there are old divers, but there are no old BOLD divers!"

We typically follow a dive profile of 30 minutes on the bottom, or at a specific depth if we are on a wall or a sloping area, and spend about 120 minutes decompressing to allow the excess dissolved nitrogen and helium escape our body tissue gradually, with out harm to us. Please see table #1. for a dive table that we commonly use with tri-mix as calculated by Dr. Bill Hamilton. Please do not use these tables for diving without the supervision of an expert. This kind of diving is for trained professionals and should NEVER be attempted by recreational divers.

Our normal procedure is to descend to the collecting area in 60 to 80 m. During the descent, we run a line with a large capacity, cave diving reel from directly under the boat to the collecting area in the depths that we are working. We then work a small distance away from the reel/line until we have used one half of our bottom time or one third of our gas supply (leaving 2/3 still in our tanks), whichever is reached first. This rule of thirds (developed by the late Mr. Sheck Exley, cave diver extraordinaire) states that one uses: one third of the gas for the dive, one third for your dive buddy and one third for emergencies. It has saved many lives in both deep diving and cave diving.

At the turning point, we head back in the direction of the reel and line and once the line is reached, we slowly ascend at about 10m per minute until we reach the first decompression stop at 34m or 31m (depending upon actual bottom time). The safety diver meets us at 34m to be sure that our decompression bottles and regulators are functioning properly. Further, the safety diver carries a spare set of decompression bottle(s) and regulators with him or her at all times.

At 34m we switch from breathing the helium mix to a mix of 36% oxygen and 64% nitrogen. This gas mix is breathed through all the stops until a depth of 70 feet(21.5m) is reached.

At 70 feet, we begin breathing a gas mixture of 50% oxygen and 50% nitrogen. This gas is breathed at the 70, 60, 50, 40 and 30 foot stops. Also at the 70 foot stop, we use a very small hypodermic needle to deflate the gas bladder of the fish and allow it to survive the transition from living at 5 to 7 times (5 to 7 BAR) normal atmospheric pressure to sea level atmospheric pressure (1BAR). Once the fish have had the excess gas removed, we put them into individual small holding containers so they do not fight during the balance of their decompression.

At 20 feet (6.15m) we switch gasses again and for the rest of the decompression, we breath pure oxygen. By keeping our lung tissue exposure to a partial pressure of oxygen at the highest level that the body can stand safely, we greatly accelerate the rate of dissipation of inert gasses out of our body tissue. The use of these many gas blends reduces our decompression times by as much as 300%. The real safety issue in technical diving is managing our body's exposure to the toxic effects of oxygen. The balance of that discussion is well beyond the scope of this article.

After we have finished our decompression at 6.15m, we slowly ascend to the surface, while leaving the candy bass at the last stop depth. We let the fish there for about 1/2 hour more and raise them to about 3.1m for another 1/2 hour. After this time (total approximate time, 3 hours), we bring the fish to the surface.

If any of the candy bass have excess gas in their bladders at this time, it is again carefully removed by insertion of the needle into the gas bladder. The small wound that is caused by this action, heals quite quickly and most of the fish (98%) experience no problems with this procedure. During the first week of captive husbandry, we administer antibiotics (furacin is the most commonly used antibiotic) to reduce the chances of infection of this small wound and any other small capture nicks and scrapes. After a week or two, the candy bass are ready to be shipped to their new homes all over the world.

Most of the collection of other species of deepwater fishes is done in a manner similar to this. The real difference is usually in different gas mixes for shallower or deeper penetration and in examining the other types of habitats that are searched for the different fish species.

PRELIMINARY EVALUATION OF SELECTED NUTRIENT COMPOSITION OF TWO LIFE STAGES OF *ARTEMIA SALINA* BEFORE AND AFTER FEEDING AN ENRICHED TORULA YEAST PRODUCT

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Abstract

Aquarists commonly feed brine shrimp, *Artemia salina*-variation San Francisco, to invertebrates and both larval and adult fishes. We analyzed both larval and adult stages of *Artemia*, before and after being fed an enriched torula yeast diet (Microfeast Plus® L-10 Larval Diet). Moisture, ash, crude fat, crude protein, water soluble carbohydrates, neutral and acid detergent fiber, lignin, thiamin, ascorbic acid, choline, vitamin E and A activity, carotenoids and 12 minerals (Ca, K, Mg, Na, P, Cr, Co, Cu, Fe, Mn, Mo, and Zn) were analyzed. There were 5 groups of *Artemia* each with n=2: 1) newly hatched unfed nauplii, 2) 24 hr post-hatch unfed nauplii, 3) 72 hour post-hatch fed nauplii, 4) unfed adults, and 5) fed adults. *Artemia* fed the yeast diet had increased vitamin E activity (nauplii: 418.1 versus 618.6 IU/kg, adults: 75.9 vs. 155.6 IU/kg; unfed vs. fed, respectively), increased ash in adults (16.1 vs. 27.5%; unfed vs. fed, respectively), and decreased fiber fractions in nauplii. Results of water soluble vitamin and mineral analyses were inconsistent, making it difficult to determine trends. Vitamin A and carotenoids were detected in only adult life stages of *Artemia*. While literature suggests that yeast enrichment may be useful for altering fatty acids, amino acids, and many water soluble vitamins, we could find few references to the nutrients assayed in our study. Overall, nauplii and adult *Artemia* (both enriched and not) met the 1993 NRC recommended nutritional requirements of fishes for crude protein and crude fat, vitamin E, and all minerals measured, with the exception of Ca. Based on these preliminary data, we cannot determine whether feeding *Artemia* with Microfeast Plus® L-10 enhances its nutritional value.

Introduction

Appropriate nutrition is a major factor in improving the longevity and fecundity of fish and invertebrate species in captivity. Deficiencies or excesses of individual nutrients can lead to circumstances where the life of the fish is severely shortened or its ability to reproduce compromised (Halver, 1972; Post, 1987; Noga, 1996). In fish fry or larvae, nutrition plays an even more important role, and improper or inadequate diets during the early stages of development can lead to morphological malformations and/or an inability to successfully reproduce later in life (Halver, 1972; Post, 1987; Verreth, et al., 1987; Noga, 1996). We have observed that fishes of the genera *Hippocampus* (sea horses) and *Syngnathus* (pipefish), commonly kept at the New york Aquarium and fed *Artemia salina* (brine shrimp) as a major portion of their diet, exhibit shortened lifespans, decalcification of their exoskeleton, and poor survival rate amongst their fry.

As public aquariums become more involved with the captive breeding and the long term husbandry of endangered or threatened fish and invertebrate species, it is imperative that a better understanding of the nutritional needs of these animals be utilized when choosing diets. *Artemia salina* (brine shrimp) are a common food source for invertebrates and both larval and adult fishes such as juvenile butterfly fish and larval anemonefish. The ease of hatching cysts and the commercial availability of the adult stage makes it a reliable food source. The quick recognition of *Artemia* as a food organism by both larval and adult fishes and invertebrates also makes it an ideal food choice. Previous studies have shown that enriching the diets of *Artemia* can increase their nutritional value as a food item (Wantanabe, et al., 1982; Clawson and Lovell, 1992; Ako, et al., 1994), but most studies concentrate on the fatty acid composition of *Artemia*. Few studies report the proximate, vitamin and mineral levels of *Artemia* life stages (Wantanabe, et al., 1983; Verreth, et al., 1987; Webster and Lovell, 1990).

Therefore, this study was performed to determine whether juvenile and adult *Artemia* were a nutritious food source with regard to proximate, vitamin and mineral components both before and after being fed a torula yeast product. Five groups of *Artemia* n=2: 1) newly hatched unfed nauplii, 2) 24 hr post-hatch unfed nauplii, 3) 72 hour post-hatch fed nauplii, 4) unfed adults, and 5) fed adults were analyzed for moisture, ash, fat, crude protein, water soluble carbohydrates, neutral (NDF) and acid (ADF) detergent fiber, lignin, thiamin, ascorbic acid, choline, vitamin E activity, retinol, and eleven minerals (Ca, Co, Mo, K, Mg, Na, P, Cu, Fe, Mn, Zn). Sample sizes were limited due to the cost of analysis.

***Artemia* life history**

The brine shrimp (*Artemia salina*) is in the phylum Arthropoda, class Crustacea and is closely related to zooplankton like copepods and *Daphnia*. *Artemia* life cycle begins by the hatching of dormant cysts which are encased embryos that are metabolically inactive. The cysts can remain dormant for many years as long as they are kept dry. When the cysts are placed into salt water, they are re-hydrated and resume their development.

After 15 to 20 hours at 77^{o}F (25^{o}C) the cyst bursts and the embryo leaves the shell. For the first few hours, the embryo hangs beneath the cyst shell, still enclosed in a hatching membrane. This is called the umbrella stage, and it is during this stage the nauplius completes its development and emerges as a free swimming nauplii. In the first larval stage, the nauplii is a brownish orange color because of its yolk reserves, and does not feed because its mouth and anus are not fully developed. Approximately 12 hours after hatch they molt into the second larval stage and they start filter feeding on various microalgae, bacteria, and detritus. The nauplii will grow and progress through 15 molts before reaching adulthood in about 8 days. Adult Artemia average about 0.32" (8mm) long, but can reach lengths up to 0.78" (20mm). An adult is a 20 times increase in length, and a 500 times increase in biomass from the nauplii stage.

In low salinity and optimal food levels, fertilized females usually produce free swimming nauplii at a rate of up to 75 nauplii per day. They will produce 10-11 broods over an average life cycle of 50 days. Under super ideal conditions, adult *Artemia* can live as long as three months and produce up to 300 nauplii or cysts every 4 days. Cyst production is induced by conditions of high salinity, and chronic food shortages with high oxygen fluctuations between day and night.

Adults can tolerate brief exposures to temperatures as extreme as 0^o to 104^o F (-18^o to 40^o C). Optimal temperature for cyst hatching and adult grow out is 77^o to 86^o F (25^o to 30^oC), but there are differences between strains. Artemia prefer a salinity of 30-35 ppt (1.0222-1.0260 density) and can live in fresh water for about 5 hours before they die.

MATERIALS AND METHODS

Enrichment media

Provesta Micro-Feast® L-10 larval diet, an enriched dehydrated torula yeast, was used in this study. This diet claims to have benefits over other enrichment media for several reasons: 1) It contains fatty acids, various macro- and micro- minerals, amino acids and vitamins. 2) Whereas other supplements tend to have a usable life of seven to ten days, Micro-Feast L-10 tends to have a shelf life measured in weeks, longer if kept frozen. 3) It tends not to foul the water as quickly as many other supplements, thereby reducing the mortality of *Artemia* due to degrading water quality. 4) It does not have to be cultured by the end user. 5) The nutritional makeup of the diet can be augmented to meet the specific need of the culturist.

Obtaining *Artemia salina* var. San Francisco nauplii

A 132.5 l Cal-Wal container was filled with 94.6 l of filtered seawater buffered with calcium hydroxide to pH 8.2, and heated to a temperature of approximately 27.7 ^{o}C utilizing a 250 watt Visi-Therm aquarium heater. *Artemia salina* var. San Francisco cysts (200 g) were added and aeration sufficient to fully suspend the cysts was provided by three 4.5 mm air lines. Full spectrum illumination (20 watt DuroTest Vita-lite™), placed directly next to the hatching container, was provided constantly to enhance hatching (Hoff and Snell, 1987).

After 22 hours, the heater was removed from the container, and aeration stopped, allowing the newly hatched nauplii to separate from the cysts. After 30 minutes, the nauplii were run through a 200 micron mesh to further separate them from any remaining cysts. Nauplii were subjected to one of three treatments:

1) The first batch of nauplii (newly hatched), were collected and concentrated using a cheesecloth net, and rinsed with reverse osmosis (RO) water to remove any traces of salt water. They were then placed in clean high density polyethylene (HDPE) containers and frozen to -20^oC.

2) The second batch of nauplii, (24 hour post-hatch), were collected and concentrated using a cheesecloth net, then divided between two containers of approximately 75.7 l each. The nauplii were allowed to remain in these containers for an additional 24 hours before being harvested, rinsed in RO water, and frozen at -20^oC.

3) The third batch of nauplii (fed nauplii), were collected and concentrated using a cheesecloth net, and were divided between two containers of approximately 75.7 l, each filled with 73.8 l of filtered seawater. Water temperature was kept at 23.9 ^{o}C, and aeration was moderate. The nauplii remained unfed for the next 24 hours. In a Waring blender, 1.89 l of filtered seawater and 10 grams of Microfeast ® L-10 Larval Diet were added. The enriched yeast was blended on high speed for 30 seconds, and the resulting suspension was added to each container, bringing the total volume to 75.7 l. The nauplii were then allowed to feed for 24 hours

Table 1. Proximate composition of Two Life Stages of *Artemia salina* Before and After Feeding with Microfeast Plus® L-10 as Compared to the Nutritional Requirements of Fishes.

Nutrient % DM basis	Requirement[a]	Newly hatched nauplii	24 hour post-hatch nauplii	Fed nauplii	Unfed adult	Fed adult	Microfeast Plus® L-10
Water (as fed)	n/a	89.09 ± 5.58	90.86 ± 0.37	90.58 ± 0.22	92.41 ± 0.71	93.07 ± 0.13	1.73
Ash	n/a	9.34 ± 3.23	10.84 ± 1.22	11.15 ± 0.12	16.13 ± 1.62	27.45 ± 5.05	12.01
Crude Protein	32-38	57.20 ± 0.69	60.37 ± 1.65	57.59 ± 0.41	61.33 ± 1.56	58.64 ± 4.45	42.65
Fat	R	12.85 ± 6.40	12.89 ± 2.23	14.00 ± 1.26	4.63 ± 0.77	5.65 ± 0.18	20.22
Carbohydrate	R	6.46 ± 3.94	5.61 ± 1.06	6.03 ± 0.10	3.77 ± 0.21	4.71 ± 0.43	11.72
NDF	n/a	7.65 ± 0.61	6.90 ± 2.06	2.15 ± 0.46	7.14 ± 1.91	5.72 ± 0.23	0
ADF	n/a	4.23 ± 0.70	3.76 ± 0.71	1.48 ± 0.33	5.09 ± 1.40	3.77 ± 0.22	0
Lignin	n/a	2.10 ± 0.39	2.14 ± 0.31	0.61 ± 0.42	2.64 ± 1.12	1.76 ± 0.11	0

n/a = not available, R = required, [a] Nutrient Requirements of Fish, NRC, 1993.

Table 2. Vitamins in Two Life Stages of *Artemia salina* Before and After Feeding with Microfeast Plus® L-10 as Compared to the Nutritional Requirements of Fishes.

Nutrient (DM basis)	Requirement[a]	Newly hatched nauplii	24 hour post-hatch nauplii	Fed nauplii	Unfed adult	Fed adult	Microfeast Plus® L-10
Water Soluble Vitamins							
Choline mg/100g	40 - 100	102	97.6	76.9	79.6	62.4	268.7
Thiamine mg/100g	0.05 - 0.10	0.141	0.13	0.178	0.017	0.037	19.5
Ascorbic Acid mg/100g	2.5 - 5.0	3.11	1.45	0.65	<0.44	<0.44	11.32
Fat Soluble Vitamins							
Vitamin A Activity IU/kgretinol + retinyl palmitate	1,000-4,000	ND	ND	ND	6911.5 ± 917.5	5116.4 ± 1521.6	7470.7*
Vitamin E Activity IU/kg	50 - 100	454.5 ± 30.1	381.7 ± 8.2	618.6 ± 6.8	75.9 ± 1.2	155.6 ± 11.4	250.3
Lutein ng/g	n/a	ND	ND	ND	4845.2 ± 3248.2	4806.3 ± 549.6	NT
Cryptoxanthin ng/g	n/a	ND	ND	ND	88.4 ± 63.9	145.2 ± 112.9	NT
Lycopene ng/g	n/a	ND	ND	ND	859.1 ± 289.6	457.3 ± 130.6	NT
□-carotene ng/g	n/a	ND	ND	ND	1646.2 ± 300.7	1402.5 ± 344.6	NT
□-carotene ng/g	n/a	ND	ND	ND	13408.9 ± 900.9	11482.8 ± 4498.7	NT

ND = not detected, NT = not tested, n/a = not available, [a] Nutrient Requirements of Fish, NRC 1993, * Retinol only tested.

before they were harvested, rinsed in RO water, placed in clean HDPE containers and frozen at -20 ^{o}C.

Obtaining *Artemia salina* var. San Francisco adult stage

Adult *Artemia* salina var. San Francisco were obtained through Zimmer and Son, Brooklyn, NY. Due to unfavorable harvesting conditions in San Francisco Bay brought on by El Nino, it was impossible to obtain sufficient amounts adult *Artemia* in one shipment. The resulting data is based upon five separate shipments, all originating from the same collection locale. The first batch of adult *Artemia* (unfed adult) was collected and concentrated using a cheesecloth net, rinsed in RO water, and frozen in clean HDPE jars at -20^{o} C. The second batch (fed adult) was collected in the same way, and then divided between two containers of approximately 75.7 l each filled with 73.8 l of filtered seawater. Temperature was kept at 23.9^{o} C, and aeration was moderate. The adult *Artemia* remained unfed for the next 24 hours, then allowed to feed for 24 hours on the same dietary suspension fed to the nauplii before they were harvested, rinsed in RO water, placed in clean HDPE containers and frozen at -20^{o} F.

Analyses

Upon arrival at the Wildlife Conservation Society Nutrition Lab, Bronx, N.Y., all samples were thawed overnight in a refrigerator at 4° C. Samples were mixed thoroughly and sub-sampled. Vitamin A and E assays were performed immediately. An 80 g sub-sample was frozen at -30° C for choline, thiamin and vitamin C assays, and the remaining sample was freeze-dried and ground using a laboratory grinder prior to fat, protein, fiber, carbohydrate and mineral analyses. Extracts from the vitamin analyses were frozen and analyzed for carotenoids.

Proximate Composition

Percent moisture, ash, crude fat, and crude protein were obtained for all samples using AOAC methodology (Ellis, 1984; AOAC,1996). Duplicate samples ($\geq$ 0.5 g) were weighed, then freeze-dried, and percent moisture calculated. These samples were then incinerated in a Thermolyne muffle furnace at 550° C overnight and total ash was calculated. Crude fat was determined by extraction with petroleum ether using AOAC Official method 991.36 (1996). Crude protein was determined using a macro-Kjeldahl method with a copper catalyst. Water soluble carbohydrates were obtained using a phenol/sulfuric acid colorimetric assay of Dubois et al. (1956) as modified by Strickland and Parsons (1972) and using sucrose as a standard. Fiber fractions (NDF, ADF, and lignin) were obtained using the methods of Van Soest (1994).

Vitamins

Vitamins A and E were analyzed using a modification of the methods of Taylor et al. (1976) as detailed in Barker, et al. (1998). Vitamin E activity was calculated as 1 mg a-tocopherol = 1.49 IU; 1 mg g-tocopherol = 0.15 IU; 1 mg d-tocopherol = 0.05 IU (Horwitt, 1993). Vitamin A activity was calculated as 0.3 μg retinol = 1 IU; 0.55 μg retinyl palmitate = 1 IU (Olson, 1984). Carotenoids were analyzed at Our Lady of Mercy Research Facility (Bronx, New York) using HPLC methodology.

Choline, thiamin and vitamin C levels were run by Woodson Tenet Laboratories (Memphis, TN).

Table 3. Minerals in Two Life Stages of *Artemia salina* Before and After Feeding with Microfeast Plus® L-10 as Compared to the Nutritional Requirements of Fishes.

Nutrient (DM basis)	Requirement[a]	Newly hatched nauplii	24 hour post-hatch nauplii	Fed nauplii	Unfed adult	Fed adult	Microfeast Plus® L-10
Macrominerals							
Ca %	1	0.07	0.08	0.10	0.13	0.28	0.75
P %	0.45 - 0.6	1.23	1.15	1.16	0.93	0.81	2.69
K %	0.7-0.8	1.37	1.49	1.38	1.12	1.02	1.86
Mg %	0.04-0.06	0.20	0.21	0.23	0.30	0.64	0.28
Na %	0.6	2.32	2.68	2.88	4.65	8.06	0.35
Ca:P	2:1	0.06	0.07	0.09	0.14	0.35	0.28
Microminerals							
Cu ppm	3-5	8.98	10.40	22.47	34.74	22.42	24.32
Fe ppm	30-150	103.37	698.63	1629.63	3100.00	2188.12	221.84
Mn ppm	2.4-13	19.68	9.57	70.47	28.93	20.29	14.96
Zn ppm	20-30	125.98	125.46	170.55	225.12	238.41	155.69
Cr ppm	n/a	<0.20	<0.20	0.42	0.73	0.48	0.53
Mo ppm	n/a	<0.30	<0.30	1.23	<0.30	0.96	10.89
Co ppm	n/a	0.25, <0.10	0.18	0.20	0.22	0.11	0.81

[a] Nutrient Requirements of Fish, NRC, 1993, n/a = not available

Elemental Composition

All mineral values were obtained by inductively coupled plasma-atomic emission spectroscopy at the Laboratory of Large Animal Pathology and Toxicology at the University of Pennsylvania.

Lipids

Lipids, in the form of fatty acids, appear to be essential to the proper growth and development of both marine and freshwater fishes. However, there is a differing need in these acids between the two, with marine species requiring eicosapentaenoic (EPA, 20: 5n-3) and docosahexaenoic (DHA, 22:6n-3) acids and freshwater species requiring fatty acids more along the line of the n-3 unsaturated fatty acids. *Artemia*, depending on which variety it is, may contain EPA in the nauplii stage thus making it suitable for marine species (marine-type *Artemia*), or n-3 unsaturated fatty acids such as 18: 3n-3 (but lacking EPA), making it suitable for freshwater organisms (freshwater-type *Artemia*) (Navarro and Amat, 1992; Navarro, Amat, and Sargent, 1993). Some *Artemia* strains contain the 20:5n-3 fatty acids, while none contain the 22:6n-3 fatty

acids (Navarro, Amat, and Sargent, 1993). The cause for this variability in fatty acids is unknown, but it may well be that the types of fatty acids found in certain strains of Artemia is influenced by the type and quality of food items (Navarro and Amat, 1992; Navarro, Amat and Sargent, 1992). *Artemia salina* var. San Francisco tend to vary greatly in their fatty acid profile between batches and location of harvest. Since this variety is most commonly utilized within the USA for a wide range of aquaculture organisms, and since fatty acid composition of this strain is so uncertain, enrichment appears to be key here. Testing the lipid content of Artemia was outside the scope of this project, but a typical lipid analysis for unenriched Artemia can be found in Table 4.

Table 4. Fatty Acid Analysis of Unenriched Fresh *Artemia salina*[a,b]

Component	Percentage	Component	Percentage
16:0	2.5 +/- 0.1	18:3(n-3)	0.8 +/- 0.01
16:1(n-7)	3.7 +/- 0.03	20:4(n-6)	0.4 +/- 0.03
18:0	0.9 +/- 0.03	20:5(n-3)	1.9 +/- 0.21
Cis 18:1(n-9)	3.7 +/- 0.05	22:6(n-3)	0.0 +/- 0.00
18:1(n-7)	2.5 +/- 0.03	Total n-3	2.7
18:2(n-6)	1.1 +/- 0.00	EPA-linolenic acid ratio[c]	2.44

[a] modified from Webster and Lovell, 1990

RESULTS AND DISCUSSION

Proximate Composition

The results of the proximate analyses are found in Table 1. Feeding had little effect on the proximate nutrient composition of the *Artemia* tested. Percent moisture was similar across all life stages of *Artemia*. Nauplii ash (mineral) content (X=10.4 %) was comparable to those previously reported (X = 10.07 %, Wantanabe, et al., 1983; 4.5 % Webster and Lovell, 1990). Feeding increased ash content only in adults. This may be attributed to the ingestion of the diet, which contained 12.01% DM ash as well as the presence of the exoskeleton in the adult shrimp. However, we cannot discount the contribution of minerals from the water as a potential source of inorganic material; the water mineral content was not analyzed in this study.

Crude protein content of *Artemia* was similar across all life stages (nauplii X = 58.4 %, adults X = 60.0 %, DM basis) and was higher than the level present in the diet (42.7%). The values reported here are like those of nauplii reported by Wantanabe et al. (1983) X=61.6% DM and Webster and Lovell (1990) 55.6% DM. Crude fat was almost two times higher in the nauplii stage than in adults. It appears that the crude fat content of the diet (20.22 % DM) did not contribute to the crude fat content of *Artemia* in any stage, or the level present was not adequate for the needs of *Artemia*. Fat values reported earlier for nauplii (X=19.4% DM, Wantanabe et

al., 1983; 20.1% DM, Webster and Lovell, 1990) are comparable to the nauplii values from this study (X = 13.2 % DM).

Soluble carbohydrates were higher in nauplii than in adults, and lower in all life stages compared to the diet. Either the diet is not a significant source of carbohydrate, or the level present in the diet is not adequate for the needs of *Artemia* in any life stage. Fiber constituents (NDF, ADF, and lignin) were diluted by feeding in both life stages, perhaps due to the lack of any fiber in the yeast diet. It should be noted that in the 24 hour post-hatch and the unfed adult *Artemia*, data indicated a downward trend for all nutrients tested. It is therefore essential that *Artemia* be utilized as quickly as possible in order to avoid nutrient degradation.

Vitamin composition

There was no straight-forward relationship between dietary concentrations of any of the vitamins measured and their presence in *Artemia* (Table 2). Although the fish choline requirement (NRC, 1993) was met by all nauplii and adult *Artemia*, levels decreased over time for both life stages despite the high dietary concentration of this nutrient (268.7 mg/100g). This is perhaps due to inadequate uptake of the diet, or the use of choline directly by the *Artemia*. Feeding increased thiamin levels in both fed nauplii and adult *Artemia*, although only nauplii stages met the 1993 NRC requirement of fish (NRC, 1993). Feeding did not increase vitamin C levels in any life stage, and was below detectable levels in the adult stage. The level of this nutrient may be low since the dietary concentration (11.3 mg/100g) did not meet the dietary recommendation for penaied shrimp (100-1000 mg/100g; Lim and Persyn, 1989). If this recommendation holds true for *Artemia*, it may explain the steady decrease of vitamin C in the nauplii stage and the non-detectable levels in all adults. Only newly hatched nauplii met the fish requirement for vitamin C (NRC, 1993). Vitamin A activity and carotenoids were detected only in the adult *Artemia* regardless of feeding. Therefore, if feeding carotenoids for fish coloration is important, only adult Artemia will meet those needs. Vitamin E activity increased in both fed nauplii and adults. However, even the unfed life stages met the vitamin E requirement for fishes (NRC, 1993).

Mineral composition

With the exception of calcium, all mineral requirements of fishes were met or greatly exceeded by all stages of *Artemia* analyzed in this study (Table 3). Besides the low Ca level, the Ca:P ratio provided by *Artemia* in all life stages was less than the recommended optimal 2:1 . This may be why some species, notably seahorses and pipefish, come down with a "soft plate" condition after being fed a diet consisting solely of *Artemia*. However, it is not known how important dietary mineral concentrations are since minerals can be obtained by fish directly from the water. Future studies should determine how to improve the Ca:P ratio in *Artemia*. Calcium values of nauplii (0.26 ± 0.06 % DM) reported by Wantanabe, et al. (1983) were double those reported here (0.08 ± 0.02 % DM).

Iron concentrations were exceedingly high in 24 hour post-hatch nauplii, fed nauplii, and both stages of adults. While the iron level in the diet was significant (221.84 ppm), this alone does not appear to be contributing to increasing iron concentrations. It is possible that *Artemia* may be concentrating iron found in the water. The high level found in the above mentioned life stages are of interest since iron toxicity has been reported in rainbow trout fed diets containing >1380 mg Fe/kg (Desjardins et al., 1987 in NRC, 1993). Wantanabe, et al. (1983) also reported

high levels of iron from *Artemia* nauplii from South America (3237 mg/kg DM) and Canada (2434.8 mg/kg DM), but not from San Francisco (n = 4, X = 365.4 ± 113.4 mg/kg DM). All other minerals reported here were similar to previously reported values for nauplii regardless of their geographic origin (Wantanabe, et al. 1983).

Conclusions

Based on these preliminary data, we cannot determine whether feeding *Artemia* Microfeast Plus® L-10 enhances their nutritional value. Feeding had little effect on the proximate nutrient composition of the various life stages. Ash content increased for the adult *Artemia*, but water could not be discounted as a potential source of minerals. There was also no straightforward relationship between dietary vitamin levels and their presence in the *Artemia*. One cannot assume that increasing vitamin levels in the diet will lead to higher concentrations in the feed organisms.

Acknowledgments

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WINDOWS INTO ELASMOBRANCH EMBRYONIC DEVELOPMENT: A METHOD TO INSTALL A WINDOW ON THE EGG CASE OF THE BIG SKATE, *RAJA BINOCULATA* (GIRARD 1855), BEFORE THE COMPLETION OF THE FIRST TRIMESTER

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An excellent display for public aquaria is one that demonstrates live elasmobranch embryonic development. Many aquaria have such exhibits and these are usually quite popular with the guests. These types of exhibits demonstrate an interesting physiological phenomenon, have a strong visual impact on guests, and are an excellent opportunity for a glimpse into pre-natal development.

At Aquarium of the Bay in San Francisco, several females of both big skates, *Raja binoculata*, and longnose skates, *Raja rhina*, have deposited eggs in the *Beyond the Golden Gate* exhibit, a 350,000-gallon community display with a sandy bottom and rock outcroppings. Divers recover the eggs, place them in floating baskets in an acclimation tank, and log the date and lay location. Then the data are recorded in a permanent logbook and the eggs are tagged and transferred to a permanent holding system that runs on the same system water. After a certain point in gestation, eggs are selected for display and clear plastic windows are installed to permit unobstructed views of the developing embryos. Eggs are selected based on lay date and several are mounted on acrylic stands in chronological order to demonstrate various stages of development.

The accepted timeline for installing windows on capsules of the genus *Raja* has been to wait until approximately one third of the gestation was complete. At this stage, the jelly has dissolved and there is a complete opening of the four ports allowing an embryo-assisted flow of seawater through the capsule (Long and Koob, 1997). With the temperature of incoming water ranging from 56^0 - 60^0 F, complete dissolution occurred in two to three months after deposition for the big skate capsules and three to four months for the longnose skate capsules. It seems largely dependent on temperature. It is possible that additional variance may be attributed to the predisposition of individual embryos.

To determine if the ports are open, simply lift the capsule from the water and check for an outflow of water from the ports. After the completion of the first trimester, the process of installing windows is straightforward and not difficult to complete successfully. However, the development is quite advanced by the first trimester. For an even more dramatic display, methods were researched to demonstrate earlier stages of development.

Observations on oviparous elasmobranch capsules indicate that they are a nutritionally closed system and the embryo is dependent solely on the yolk for organic materials (Read, 1968). Evidence on the role of egg jelly in the egg capsules of *Raja erinacea* presented by Koob and Straus (1998) supports Read's assessment. Their analysis of the carbohydrate concentrations in the jelly matrix at different stages of development, demonstrates that the jelly serves primarily as a cushion against mechanical stress and does not serve as a nutritional source for the developing embryos. Based on this evidence, it seemed possible that a window could be installed before the ports are opened.

One concern with replacing a significant portion of the egg case with plastic so early in development is whether the change in permeability of the egg will affect the embryos, causing harm or altering the normal development of the embryos. Based on my empirical evidence, it has not been a factor. Perhaps, squeezing out the horn jelly counteracts the loss in permeability to some extent. However, this deserves further study. It appears that the greatest dangers to the embryos are mechanical stress and biological and chemical contamination from the procedure.

Materials and Methods:

Egg cases were selected based on an approximation of current development. Eggs were examined for the presence of holes at the tips of the horns. Holes must be present in at least two horns. The appearance of holes at the tips of the horns seems to correspond with the start of lumen jelly dissolution that Ouang (1931) believes is initiated by enzymes secreted through specialized glands at the heads of the embryos. At this stage in development, the egg is permeable to seawater that begins displacing the lumen jelly. The viscosity of the egg jelly differs depending on its location within the capsule (Koob and Straus, 1998). The lumen jelly, nearest the embryos is relatively low in viscosity and dissolves first. The horn jelly is stiff, comparable to cured silicone, and takes much longer to dissolve.

To remove the jelly, first identify a suitable egg case, then support it in one hand with the highly curved dorsal side down and apply pressure with the thumb and index finger at the base of a horn with a hole. Squeeze out the horn jelly by moving the other thumb and index finger outward along the length of the horn. Figure 1 shows horn jelly coming through the open port.

Squeezing out the horn jelly is critical. When the procedure is completed the lumen can be flushed of residual glue vapors, reducing the chance of chemical contamination. Additionally, air bubbles can be burped. While jelly is present in the lumen, the embryos are not able to effectively pump out air bubbles, the presence of which may lead to biological contamination.

Once the horn jelly is cleared from all open horns (note a change in color and viscosity of the jelly emanating from the horn) a piece from the ventral side should be cut. When making the incision, always angle the egg so that the embryos shift to the opposite corner away from the scissors blade. Cut out a piece that will leave a sufficient frame to lay a bead of glue and attach the clear plastic window (Fig. 2 and 3).

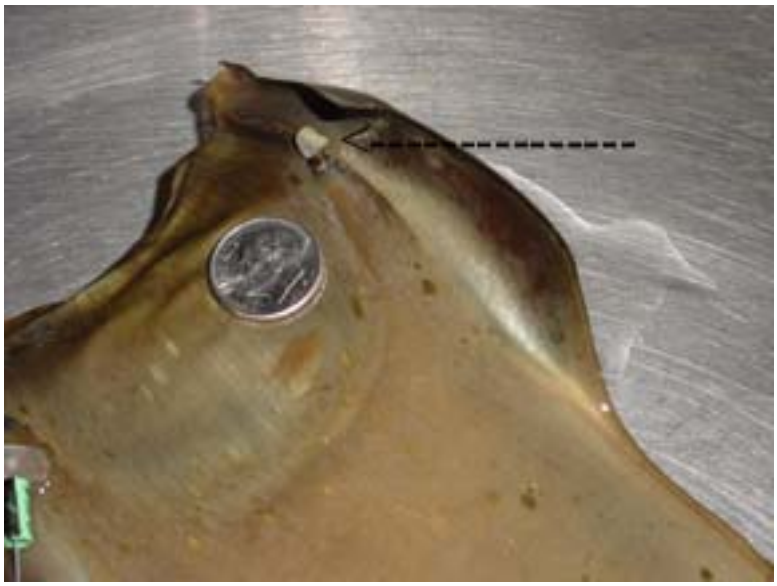

Figure 1: Horn jelly (arrow) squeezed out of an egg from *Raja binoculata*.

Figure 2: Open egg, *Raja binoculata*, embryos at 44 days

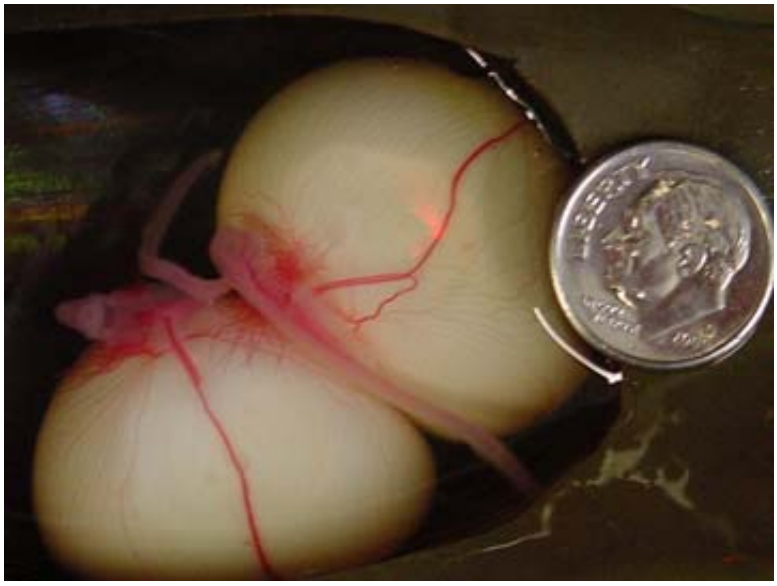

Figure 3: Close up of embryos, *Raja binoculata*, at 44 days

When the cut is complete, set the piece aside and gently dry the surface of the frame with cotton pads. Depending on the extent of liquefaction of the jelly, it may be wise to remove a portion of the jelly by drawing it into an eyedropper. This reduces the chance of contacting the jelly with glue. As the surface of the frame dries, superimpose the cut piece of egg case on a sheet of clear plastic (document protector sheets work well). Cut the clear plastic slightly larger than the egg case piece. The exact increase in size will depend on how much the frame can accommodate. If the clear plastic is cut too large it will be difficult to set it flush on the frame. If the plastic is cut too small, a greater amount of super glue will potentially contact the jelly. When the plastic is trimmed to size, dry both surfaces and lay a thin bead of super glue on the frame far enough from the edge so that when the plastic is set on top of the egg case it does not draw the glue to the edge of the egg cut. Press the plastic window in place until the surfaces hold. Then allow it to dry for several minutes. The embryos may react by vigorously beating their tails for several seconds. This is a reaction to the glue vapor. When the glue is set, return the egg to the water and burp out any air. After the bubbles are displaced, lift the egg out, allowing water to drain from the egg and refill the egg. There will not be very much water present, so repeat this several times to flush as much of the glue vapors as possible. Following this procedure leaves less room for error than if a window is installed after the first trimester. With the jelly fully dissolved, the lumen is more easily flushed after the window installation.

It will be apparent within 24-48 hours following the procedure whether or not the embryos will survive. Healthy embryos will maintain their color and continue actively wriggling their tails in the capsule, and the external yolk should remain well vascularized. The capsules are now ready to be displayed.

Acknowledgments:

I would like to thank Christina J. Slager of the Monterey Bay Aquarium for her guidance and encouragement in writing this article, and Kevin Lewand and the Husbandry staff at Aquarium of the Bay for assistance in collection of the capsules from the exhibit. Also, I would like to acknowledge Drew A. Haller, Matthew J. Tooley, and Michelle M. Chow for their help with the digital photography.

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SECOND GENERATION WHITETIP REEF SHARKS AT SEAWORLD SAN ANTONIO

Four whitetip reef sharks (*Triaenodon obsesus*) – three female and one male – were born on the evening of June 17, 2001. This is a significant event for us because the mother was born under our care back in July 1995. *T. obsesus* are viviparous and have 1 to 5 pups per litter, ranging in size from 50 – 60 cm. Adults reach sexual maturity at about 110 cm and can grow to be roughly 160 cm.

The pups, which averaged 50 cm at birth are fed daily with a variety of shrimp and cut fish. Since 1998 we have had over 30 whitetip pups, but this is the first group of second-generation whitetips at our park. Other births this year have included a pair of spotted eagle rays (*Aetobatus narinari*).

Joe Keyon, Curator of Fishes

SIZE RELATIONSHIPS BETWEEN EGG CAPSULES AND FEMALE BIG SKATES, *RAJA BINOCULATA* (GIRARD, 1855)

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Data from the fertilized egg capsules of the big skate, *Raja binoculata*, were analyzed. The data were collected from three different females that mated and laid eggs in captivity. It was determined that the size of the eggs increases with the size of the fish. This finding supports similar data for another species in the genus *Raja* (Templeman, 1982). Additionally, it was observed that individual females lay eggs with a unique, individual shape that remains more or less constant throughout the course of deposition (Figure 1). Aside from dimensional qualities, the differences in egg shape between broods are subtle but definite.

The three female big skates have been maintained in captivity for greater than three years in the *Beyond the Golden Gate* exhibit, a 350,000-gallon community exhibit with a sandy bottom and rock outcroppings. The capsules were removed from exhibit and placed in flow-through plastic bins connected to the exhibit's system water. Eggs were tagged and lay date and location were recorded. After hatchout, neonates were moved to flow-through plastic bins with a fine sand substrate.

On August 19, 2000, a pair of big skate, *Raja binoculata*, egg capsules were recovered from the exhibit. It had been over two years since the last skate egg had been laid at the aquarium. The event came as a surprised because it was not apparent that a mature female was housed in the exhibit. Based on data collected from 68 female big skates captured in Monterey Bay between January 1980 and September 1981, the total length (TL) for sexually mature females was determined to be 1300 mm (Zeiner and Wolf, 1993). The largest female on display at that time was considerably smaller (DW = 670 mm and TL = 940 mm).

The gravid individual was identified by following observations made by Luer & Gilbert (1985) on gravid clearnose skates, *Raja eglanteria*. The capsules within the gravid female were visible externally as a pair of prominent lumps anterior to the base of the tail on her dorsal side, on either side of the midline. A report on twenty egg cases from *R. binoculata* recovered from Puget Sound lists the total lengths ranging from 265 to 305 mm (DeLacey and Chapman, 1935). Eschmeyer, et al. (1983) confirm these data, reporting the egg capsules for big skates to be nearly 300mm in length. These figures contrast those recorded from the eggs recovered from exhibit at Aquarium of the Bay. Total lengths were approximately half the previously reported size, the largest measuring 179mm in TL (Table 1). It should be noted that measured length data on capsules depends on the amount of time elapsed since deposition. As capsules age, the leading edge of the posterior end wears away. This suggests the possibility that the initial lengths (< one week following deposition) on wild caught capsules might be even greater than those reported.

Templeman (1982) observed for thorny skates, *Raja radiata*, that capsule size increases with an increase in fish length. It seems likely that the capsules measured by DeLacey and Chapman and Eschmeyer were laid by females much larger than the spawning female at Aquarium of the Bay. For the little skate, *Raja erinacea*, reproductive tracts are expanded in spawning females, perhaps hormonally regulated (Koob 1981). It seems fair to assume that the skate's reproductive tract also grows larger as the female grows. This growth would provide additional volume within the tract to accommodate the production of larger eggs with each successive spawning season.

After positively identifying the spawning female and noting that her TL and her capsules' TLs were considerably smaller than previously reported for mature females, the eggs were examined more closely. In addition to being quite small, the eggs also seemed structurally weak. The horns were very pliable and the dorsal and ventral plates were not rigid. Among the first dozen eggs, some were laid with long streaming tissue extending from the posterior end. It seemed as though she was working out some "technical difficulties" on the manufacturing end. Her small size and the structural inconsistencies of the first few eggs suggested that this was possibly her first brood of eggs. Perhaps this is indicative of a smaller size limit for sexually mature females for the San Francisco Bay's big skate population.

In June of 2001 two pairs of eggs were recovered on consecutive days, indicating another female had started to lay eggs. The eggs were noticeably larger and much sturdier than the previous set. The typical swellings were observed in a larger skate (DW = 880 mm and TL = 1200 mm), confirming that she was gravid and laying the new eggs.

On June 23, 2001, the last pair of eggs from the first female was recovered from exhibit. Her deposition lasted 10 months. The second female continued to lay eggs. Then, two months later, on August 10, 2001, small eggs were again recovered from exhibit. They appeared very similar in size and shape to the original set but were slightly more rigid. The first female was again laying eggs. In the month following, three pairs of eggs were recovered from exhibit. All were deposited within three days of each other. Additionally, a new stockier, but relatively small egg shape was discovered. A third female big skate (DW = 690 mm and TL = 910 mm) was confirmed to be spawning.

Based on the observations from the big skate eggs recovered in captivity at Aquarium of the Bay, it is clear that the size of the eggs increases with the size of the fish and that individual females lay eggs with a unique, individual shape that remains more or less constant throughout the course of deposition. Also, the size at maturity for female big skates seems to be much smaller than previously reported by Zeiner and Wolf (1993). Further studies on skate reproduction are necessary to clarify these apparent discrepancies. Possible reasons for the discrepancies may include differences in population dynamics at various localities, sport and commercial fishery demands and wild versus captive environments.

Table 1: Total length for spawning females and the measured range for TL and width of corresponding capsules.

Female	TL (mm)	Egg TL (mm)	Egg Width (mm)
1	**940**	**155-179**	**75-90**
2	**1200**	**181-200**	**95-114**
3	**910**	**150-167**	**81-90**

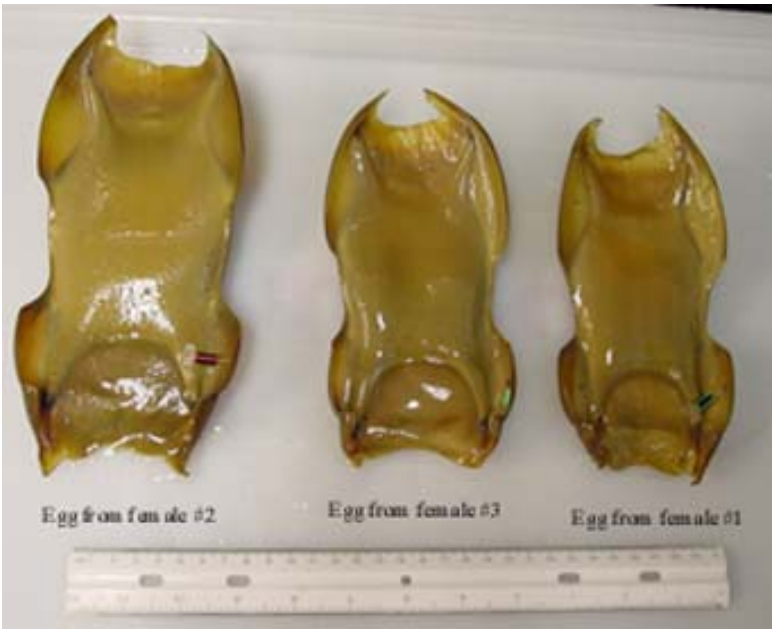

Figure 1: Egg cases, *Raja binoculata*, recovered from exhibit at Aquarium of the Bay.

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I would like to thank Christina J. Slager of the Monterey Bay Aquarium for her guidance and encouragement in writing this article and Kevin O. Lewand and the Husbandry staff at Aquarium of the Bay for assistance in collection of the capsules from exhibit.

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HUSBANDRY OF THE BIG-FIN REEF SQUID (*SEPIATEUTHIS LESSONIANA*) AT THE WAIKIKI AQUARIUM

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The Waikiki Aquarium has been a pioneer in the display of various cephalopods over the last 20 years; the first in North America to display and breed chambered *Nautilus* (two species), and the first to display flashback (*Sepia latimanus*) and flamboyant cuttles (*Metasepia tullbergi*). We have also displayed *Octopus cyanea*, *Euprymna scolopes*, paper Nautilus (*Argo* sp.), *Sepia officinalis* and *S. pharaonis*. One ceph that we have not been successful with has been the common reef squid (*Sepiateuthis* spp.). We have collected eggs on several occasions in Palau over the years and on one occasion a young adult *Sepiateuthis lessoniana* (which died a few days later despite feeding soon after arrival) from Palau but in the case of hatchlings, we were unable to keep them for more than a few weeks. To the best of my knowledge, the New England Aquarium is the only North American aquarium to successfully display *Sepiateuthis,* in the spring of 1991, which they had acquired as captive-reared specimens from the National Research Center for Cephalopods in Galveston, TX. They were able to successfully breed their population but the eggs did not prove fertile (Bailey *et al*., 1996).

Until recently, *Sepiateuthis lessoniana* were rarely seen in Hawaii, most commonly encountered in the waters off Maui, rarely, if ever, have they been seen in the waters off Oahu. All that changed in the spring of 2001 when we began to receive several phone calls each week reporting sightings of "cuttlefish" along local beaches and piers. Eventually a dead specimen was brought to the aquarium and we tentatively identified it as *S. lessoniana*. At the beginning of July Norton Chan's wife reported sighting a school of juvenile squid under a local pier. On July 4th, Dr. Bruce Carlson, Dr. Cindy Hunter and myself snorkeled underneath that pier and after about 20 minutes we came upon a small school of about 16 juvenile squid (4-6 cm mantle length) hovering just below the surface in the shadows of the pier. Using small hand nets we easily scooped up six of them and placed them into bait buckets. The squid were surprisingly easy to approach and collect, and did not display an agitated behavior in the bucket. We transported them to the aquarium by truck in a 25 gallon Rubbermaid barrel approximately half full with seawater, with a bare (no airstone) airline for aeration. Once at the aquarium they were transferred to an acrylic cylinder tank four feet wide and four feet tall. This tank had originally been setup to work with non-photosynthetic soft corals and had an alternating water current that caused the water to rotate in one direction for six hours then another for six hours. Aside from a 3" layer of live sand and a few small live rocks, the system had no filtration but did receive an input of approximately five liters per minute of new seawater, which then overflowed via a central standpipe to the sewer. Lighting was natural ambient light and no attempt was made to control temperature (78 ^{o}F- 85 ^{o}F). A small airstone was added after a few weeks to increase oxygenation. The tank was covered on 50% of the sides and top to offer some degree of shading and to prevent shocking the animals, as the tank was located in a high traffic area. The animals quickly acclimated to the tank and would react to people walking by or looking into the tank by

orienting themselves head first towards to disturbance. By the next day small (2-3 cm SL) live saltwater fish (mullet) were added to the tank and these were quickly attacked and devoured by the young squid. Each squid could easily eat between 10 and 20 of these small fish per day. Small live panaeid shrimp were, surprisingly, also readily attacked and eaten when offered. After two weeks the smallest of the squid was found on the bottom partially eaten, presumably the larger squid had attacked it in the night.

After about three weeks in holding the squid, now about 10 cm in mantle length, were transferred to our largest cephalopod exhibit. This tank holds approximately 1200 gallons of water and is eight feet long, five feet wide and four feet tall. There is a 200-gallon sump that holds a thin layer of bioballs over which returning water is passed. An ETSS 1800 skimmer provides the only filtration. The tank has a plenum covered with four inches of live sand and several low mounds of live rock. Water is pumped from the sump to a 2 HP chiller on the roof of the building and then returned to the tank via two perforated PVC pipes that run to the bottom in each of the two front corners of the tank, another return line goes to the sump. Two surface overflows carry water from the tank to the sump. Finally, there is a slow trickle of new seawater that is added on a continuous basis. Using wooden planks and shade cloth we shaded the rear half of the tank to give the squid a greater sense of security. Two, four foot double VHO fluorescent fixtures on timers are used to light exhibit. One set is controlled by a dimmer system (a SkyCast unit by Digital Oceans) and slowly comes on in the morning; the second then comes on an hour later. The cycle is reversed at night. Using this form of lighting we have not seen any light induced fright responses in cuttles or squid.

We continued to feed live saltwater fish to the squid but as they grew their food demands increased and collecting sufficient live food became a weekly chore! Eventually I was able to get them to eat frozen fish such as herring and capelin. They would also take frozen shrimp. Currently we feed each squid half a frozen fish in the morning and then in the afternoons we feed either live food or more frozen fish.

We started with five squid on display in late July and by September it became evident that one of them was a female as her nidamental glands and developing ova were distinctly visible through the body wall. The largest male would accompany her throughout the tank and would block any of the other males from approaching her. We observed several mating attempts where the male would swim upside-down above the female with his head aligned with hers. He would then reach downwards with his tentacles, grab her head and flip himself over so that he was facing her. At this time a sperm packet was injected into the female's body cavity but she would quickly expel it! Unfortunately in late September the female was found dead in the exhibit one morning and (I thought) I was now left with four males. However, by late October another of the squid began to exhibit the same anatomical differences as the previous female had, and mating attempts by the males were again observed. In late November a number of isolated egg capsules were found on the bottom of the exhibit and the female was observed lying on the bottom. I removed her off exhibit but she was dead within a few hours. At the time I write this (early December) we still have three male(?) squid on exhibit.

In retrospect, our exhibit is probably not large enough to allow the squid to grow to their full size or to exhibit completely natural behaviors. Ideally the exhibit would need to be at least 50-100% larger. It is my understanding that the Maui Ocean Center is currently planning a 25,000-gallon squid exhibit! Based on my experiences it would be better to have more females than males so that the males do not continually harass the females. In our situation, the males likely harassed the females to the point of exhaustion, which may have lead to their premature deaths. Unfortunately, our limited holding facilities at the time prevented us from removing the female to less crowded surroundings.

We have been very pleased with the exhibit potential of these animals. They exhibit a myriad of fascinating behaviors and their ability to quickly change patterns and coloring is mesmerizing! Unlike some of the open water squid genera such as *Loligo*, they had no problems navigating the tank and very few wall impacts were noted. We have received a number of favourable comments from visitors, though some have expressed a desire to see the large cuttles too! Perhaps in a future expansion we will devote a larger exhibit to squid, however, this might be a risky proposition unless *Sepiateuthis* proves to be reliably available in our area. One positive sign has been the sighting of juvenile squid under some local piers in November so we hope that they will again be available to us once our current exhibit animals live out their natural lifespan (8-10 months). It remains to be seen whether or not this springs surprise appearance was a fluke or heralded the reintroduction of these fascinating creatures to the coastal waters of Oahu.

If anyone would like further information concerning our experiences with *Sepiateuthis*, please feel free to contact me at the above email address.

Note: A small batch of eggs were brought found and brought the aquarium the week of November 25th and several juveniles have hatched and are feeding on live adult brine shrimp and mysis shrimp. We hope to be able to rear these to adulthood in the coming months.

Acknowledgements

I'd like to thank Norton Chan for alerting us to the presence of the squid and suggesting them as a possible exhibit animal. Without his enthusiasm for this project, it would never have been undertaken. I'd also like to thank the student staff and aquarists for helping to collect live food on a weekly basis.

Bailey, S., Tzinas, G, Dayton, J and S. Spina. 1996. Husbandry of one species of squid in captivity. Freshwater and Marine Aquarium 19 (1):196-197, 199, 201-202.

A DESIGN FOR A SECURABLE ISOLATION BASKET

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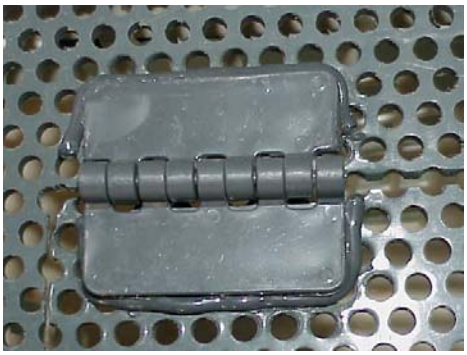
During collecting trips to the Bahamas and Florida Keys New England Aquarium often collects morays. Usually these eels are kept in small pickle barrels that are drilled out for water flow and weighted so as to sit in the bottom of the live wells. Occasionally the eels manage to escape this setup and marauding eels have been known to snack on other tank mates in the live wells much to the distress of the collectors. Back at the aquarium there are holding situations that also called for a more secure holding container than the ones we typically use (i.e.: floating baskets or barrels).

This is one of those simple designs that one day jumps out at you when you realize that you have the materials and tools to solve a problem that has bugged you for a long time. My inspiration came while learning to heat weld PVC.

Materials:
Cable ties
Laundry basket
Floatation (pipe insulation)
¼" perforated PVC sheet
PVC hinges
PVC welding rod
Either a nylon knurled head bolt or a stainless steel T bolt
Plastic mesh if the holes in the basket are too large

Tools:
Triac electric plastic welder
Jigsaw
Measuring tape
Marker
Drill & bits
Tap set

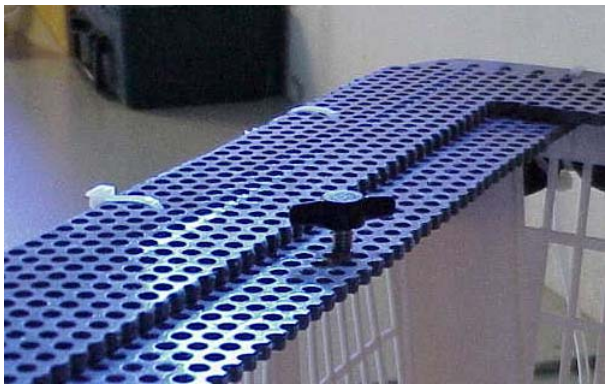
Invert the basket onto the perforated PVC and trace the outline of the top of the basket. Now cut out this shape. This will be both the frame and the door for your lid. Next determine the size of the opening that you want in the lid. Mark this out and then carefully cut it out. You may need to use a drill to widen one of the holes in the PVC sheet to start your jigsaw in unless you use a very small blade. If you cut carefully you can use the cutout for the door after a quick filing job on the edges. Lastly cut a straight strip of PVC sheet slightly longer than the opening and about 2" wide. This will become your striker plate that the lid will close against.

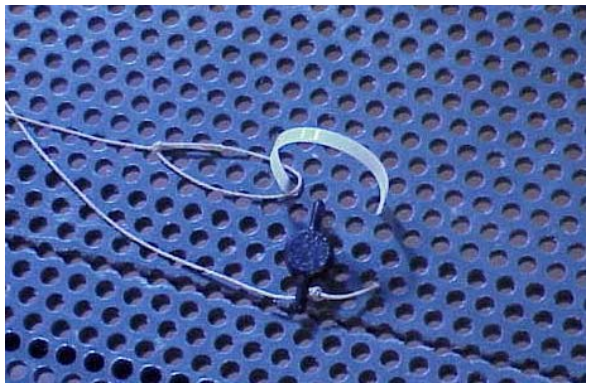


Now it is time to weld! This obviously calls for caution and some training. I would also warn against using PVC sheet thinner than ¼" due to an increased risk of melting the sheet during the welding. Align the door in its opening, then align a hinge so that its pivot is over the space between the door and the frame with one foot is on each part (Figure 1)– then weld it on to both the frame and door. Do the same for the remaining hinge(s). Once the

hinges are attached and either cooled or quenched, turn the frame and door assembly over to attach the striker. The striker attaches to the bottom of the frame and prevents the door from falling into the basket. When aligning the striker, be sure the holes in the door match up to the holes in the striker and that half of the width of the striker is under the door. Once aligned, weld the striker to the frame.

Turn the assembly back over (right side up), check to make sure the door operates smoothly and the perforation holes in the striker align with the perforation holes in the door. Select one set of holes near the center of the overlap between the door and striker. For the hole in the door, drill it out so the bolt can pass through it, for the corresponding hole in the striker, tap it out for the bolt. The bolt can now be used to secure the door. (Figures 2 & 3) To complete the assembly, loop a cable tie through to adjacent holes to make a handle.





Set the assembly on top of the basket. Mark and drill out a set of holes in the top edge of the basket to secure the lid and floatation to the basket. Once the basket is drilled, attach the lid and floatation with cable ties. If the holes in the basket are too large, be sure to attach plastic caging or netting to the inside of the basket before completing the assembly. If you used a plastic bolt you now have a corrosion proof, securable holding basket that functions easily and looks professional. (Figure 4)

Please be aware that the materials and tools used in this project are fairly expensive. New England Aquarium already had the welder ($300 - $900), hinges ($6.00 each), scrap PVC sheet ($51.00 24”x 48”x 1/4”) and bolts from other projects plus pre-existing floating baskets. So the cost to us for the prototype and the first few has basically been cable ties, welding rod and time. It might also be possible to construct this with an adhesive in place of welding. Good luck with your projects and I hope you find this design useful.

A special thank you goes out to the staff and volunteers who have served as human clamps and risked scorched fingers while these lids were developed.

BOOK REVIEW

AQUARIUM CAREERS
Jay Hemdal, 2001. 126 pp.
Writers Showcase (Lincoln, Neb.)
$12.95 US

Review by Roland C. Anderson, Puget Sound Curator, The Seattle Aquarium

This new paperback book by prolific author and colleague Jay Hemdal tells about careers in the aquarium field. He introduces us to the difference between a job, an occupation and a career, but concentrates on the latter. The interest in aquariums can consist of maintaining home aquariums or large public aquariums, which may be for profit or non-profit, but there is little difference in jobs between the two.

Hemdal starts out with the preparation for a career in aquariums. he begins as early as elementary school, recommending science classes and continues with his recommendations for high school and college courses he also recommends graduate programs, trade schools and extra-curricular activities for budding aquarists, such as computer classes and clubs, art courses and boating. A skill he highly recommends is scuba diving, not necessarily as a requisite but as a beneficial skill.

He describes several private sector jobs, with the background of having worked in the private sector before obtaining a public aquarium job. Such jobs may be working for retail pet stores, aquarium maintenance services, manufacturing and distributing, importer/wholesaler, collector, breeder or consultant. He describes each and offers the opportunities in each field.

But the main emphasis of the book is on the public aquarium sector. he describes the jobs available, what they entail, and what they pay. He even gives a typical day in the life of an aquarist and a curator, based on his personal experience. Management level positions are given short shrift, perhaps because of his lack of experience in them. Other chapters describe private sector jobs such as those in fish hatcheries and part-time careers, such as home businesses.

An important chapter deals with job acquisition, obviously based on the author's own experience in applying for jobs, and later conducting job interviews. A final chapter discusses related topics such as data management, philosophy of public aquariums, ethics of aquariums, the philosophy of problem solving, exhibit design, and public interactions. Public groups for aquarium keepers are listed and described. Three appendices include a sample resumé, a partial listing of some public aquariums around the world with a personal, somewhat biased rating of them, and acronyms used in the aquarium field.

Hemdal has the experience to write such a book, having been an avid aquarist for over 35 years. He worked in retail pet shops and at the Shedd Aquarium before settling at the Toledo Zoo in 1987, where he is currently curator of fishes and invertebrates. He has written mare than 90 articles for aquarium magazines and scientific publications.

The book is available over the internet from Amazon, Barnes and Noble or iUniverse.com.

BOOK REVIEW

A FASCINATION FOR FISH: ADVENTURES OF AN UNDERWATER PIONEER
David C. Powell
UC Press/Monterey Bay Aquarium Series in Marine Conservation
List Price: $29.95 US, Amazon.com Price: $20.96 US (as of 1/1/02)

Review by Pete Mohan, Curator of Fishes, Reptiles and Invertebrates, SFWoA

I should begin by providing newcomers to the public aquarium field with a little information on aquarium genealogy. Dave Powell was a member of the husbandry team at Marineland of the Pacific and SeaWorld during their pioneering years. Later he was Curator at Steinhart, and Director of Live Exhibit Development at Monterey. Like Dave, his contemporaries from these California institutions moved on to lead the development of important new facilities throughout the world. Dave's story is the story of our industry and profession.

While Dave's new book is clearly written to appeal to a wide audience, public aquarium professionals will find "A Fascination for Fish" to be an entertaining time capsule that reveals the origin of many devices and techniques that we all take for granted. It is amazing how technology transfers between institutions, while its origins often become obscured by time. Dave shines a bright light on the contributions of the staff of California aquariums to what we now call "aquarium science". Dave himself can claim parentage to many of these innovations. I will not spoil your enjoyment of this book by revealing the details here. You will enjoy the surprises.

The book is a series of marvelous stories that bring four decades of aquarium history together in a highly enjoyable package. Each anecdote unravels in a style that will be familiar to anyone who has shared a beer with Dave. Those who have not should seek him out at the next RAW meeting. Although the term "retired" is sometimes used when introducing Dave to audiences, this is really an honorific. He continues to actively design new exhibits and probably gets in more diving than most of us.

From the Back Cover

"This autobiography is a charming and accurate telling by a modest man whose brilliant career has allowed millions to venture into the sea and feel what Dave feels when he's underwater-without getting wet!"

-John E. McCosker, Director Emeritus, Steinhart Aquarium

"Dave Powell is one of the giants in the development of the modern public aquarium. His great talents and skill have come from years of working with the technology of public aquariums, and also from a great love of the sea. This book is a wonderful tale of his adventures (and misadventures) as he worked to capture the essence of the ocean and bring it to public view."

-Bruce Carlson, Director, Waikiki Aquarium

"Gobies, jellyfish, coelacanth, white sharks-it's all here. A delightfully written book detailing the experiences of the most innovative aquarist of our time. This book is a must read for anyone fascinated by looking through an aquarium viewing window."

-Jerry Goldsmith, Vice President, SeaWorld of California