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Culturing the Estuarine Mysid *Mysidopsis bahia*: A Synopsis of Three Case Studies[1]

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Abstract.—Since the late 1970s, the estuarine mysid *Mysidopsis bahia* has been used to test toxic conditions of saltwater environments, and several test and culture methods involving natural salt water have been published. This paper presents the results of three investigations that successfully cultured and maintained *M. bahia* in artificial salt water. The efforts had elements in common, among them a basic diet of artemia larvae, the use of recirculating biofilters for removal of nitrogenous materials produced by the cultures, and similar lighting regimes. Two of the investigations augmented the diet by feeding mysids nematodes, *Panagrellus redivivus*. Major differences among the three approaches were the volume of the tanks used to culture the organisms, materials used as substrates, dilution water and brand or mixture of sea salts used to prepare salt water, and various foods to augment diet. One group of investigators attempted to quantify various combinations of food, light, and other factors needed to sustain production. All investigators agreed that additional research was needed on dietary requirements of mysids.

Within the past two decades, interest in testing toxic conditions in both fresh and salt surface waters has resulted in the use of a variety of species and procedures in evaluation processes. In 1975, investigators at the Environmental Research Laboratory, U.S. Environmental Protection Agency (EPA) in Gulf Breeze, Florida, began to use an estuarine mysid, *Mysidopsis bahia*, in acute and chronic tests (Nimmo et al. 1977). Within a few years, methods of culturing and toxicity data for a variety of substances began to be published; some of them were summarized by Nimmo and Hamaker (1982). A more recent compilation of marine toxicological data, including that on mysids, was completed by Suter and Rosen (1988), and techniques for culturing mysids with natural salt water were published by Ward (1984) and Lussier et al. (1988). Early mysid culturing emphasized natural saltwater media and, for food, hatched larvae of the brine shrimp *Artemia salina*. Increased testing needs and new procedures for addressing effluent quality, industrial chemical and pesticide toxicity, and effects of drilling fluids led EPA to provide matching funds via cooperative agreements to develop methods for using artificial rather than natural salt water and to investigate use of various food combinations rather than brine shrimp exclusively. Research was completed at Environmental Research and Technology (ERT)[4] and Colorado State University (CSU), both located in Fort Collins, Colorado. Parallel research on culture methods was begun at the Environmental Research Laboratory (ERL) at the University of Arizona, Tucson. In this paper we present three procedures that have been successfully used to culture and maintain *M. bahia* in artificial salt water, and we describe the effects of different diets. Although elements in the procedures are similar, they differ enough to warrant discussions of key aspects of each approach.

Methods

Culture methods generally followed those published by Nimmo et al. (1978), but there were substantial differences in tank sizes, substrates, and dilution waters (Table 1). In all cases, the

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TABLE 1.—Comparison of basic methods for culturing the mysid *Mysidopsis bahia* under artificial conditions.

Component	Laboratory		
	ERT	CSU	ERL
References for basic method	Nimmo et al. (1978); Reitsema and Neff (1980)	Nimmo et al. (1978); Reitsema and Neff (1980)	This report
Volumes (L) of culture aquaria	75 and 850 L	75 and 189 L	75 and 208 L
Substrate type and depth	Crushed dolomite in separate aquaria; 8–10-cm depth	50:50, volume:volume crushed oyster shell:dolomite; 10-cm depth	66:33, volume:volume crushed oyster shell:dolomite; 7.6–9.0-cm depth
Dilution water	Tap	18 MΩ deionized	Tap
Salt water brand or mix	Tri-S®[a]	Marinemix®[b]	Marinemix®[b]
Basic diet (brand or type)	24-h-old artemia larvae (San Francisco Bay Brand®)	48-h-old artemia[c] larvae supplemented with nematodes (*Panagrellus redivivus*) and a modified shrimp diet (Zeigler Brothers, Inc.)	48-h-old artemia[d] larvae added daily with nematodes (*Panagrellus redivivus*)

[a]No longer manufactured. The symbol ® is a registered trademark.
[b]Other brands were used in the research; details are discussed in this report.
[c]The feeding regime was designed to test the efficacy of various foods singly and in combinations (artemia available from Aquarium Products®, Glen Burnie, Maryland).
[d]The feeding regime was designed to test combinations of artemia (available from Great Salt Lake strain—Sander's® brand) and nematodes needed to maximize production of mysids.

source of mysids was the original culture at the EPA Laboratory at Gulf Breeze, Florida. A subset of animals was transported to the EPA Laboratory at Narragansett, Rhode Island. A stock was then transported to ERT, then to ERL, and finally to CSU. During the initial phases of the CSU project, multiple stocks were received from Rhode Island to augment cultures.

ERT culture methods.—Initial cultures were kept in four 75-L aquaria; later, two circular 850-L fiberglass tanks were constructed with undergravel filters, following methods discussed by Spotte (1979). Major components of the larger aquaria included filter plates used to suspend crushed oyster shell (8–10 cm deep) above the floor of the tank and an airlift system (Figure 1). The filter plates consisted of corrugated fiberglass roofing panels with slits cut crosswise at right angles to the ribs. Spacers were 1.9-cm inside-diameter (ID) sections of polyvinyl chloride (PVC) pipe distributed evenly to support the weight of the filter material. Fiberglass window screen was placed over the corrugated base and sealed in place with silicone cement to prevent oyster shell from falling to the tank bottom. Along the edge of the tank, four airlift tubes with right-angle outlets moved water through the system (Spotte 1979). Airlift tubes were sections of PVC pipe, 2.54 cm ID and 0.69 m long. Inside were airstones (15 cm long) used to lift the water to outlets positioned at 90°. This arrangement provided a slow counterclockwise current in the circular tank. Two large banks of fluorescent cool-white bulbs provided continuous light.

Aquaria were maintained essentially as described by Nimmo et al. (1978) and Reitsema and Neff (1980). To reduce time for nitrifying bacteria to establish a biological filter, repeated additions of newly hatched *Artemia salina* were added for 2–3 weeks. Weekly, 20% of the saltwater volume was replaced after "aging" of the biological filter, a condition usually associated with a nitrite-N concentration less than 0.1 mg/L. Salinity was maintained between 21 and 30‰ and temperature between 22 and 29°C. All mysid cultures were fed artemia larvae ad libitum daily.

CSU culture methods.—During the study at CSU, two sizes of static-recirculating undergravel culture systems were used and subsequently modified to maximize production. Biofilters in four 75-L aquaria consisted of oyster shell washed with hot tap water, mixed with an equal volume of washed crushed dolomite (available from local tropical fish retailers), and spread over a plastic grid platform of the undergravel filter system to a depth of 10 cm. Salt water was prepared by mixing Marinemix® artificial sea salt with 18-MΩ deionized water to provide a salinity of 28‰. We attempted to hasten formation of biofilters by (1) adding 48-h-old artemia nauplii daily, (2) adding an inoculum from a local pet store's "established" saltwater aquarium, and (3) introducing salinity-acclimated Amazon mollies *Poecilia formosa* for several days. Later, an additional set of

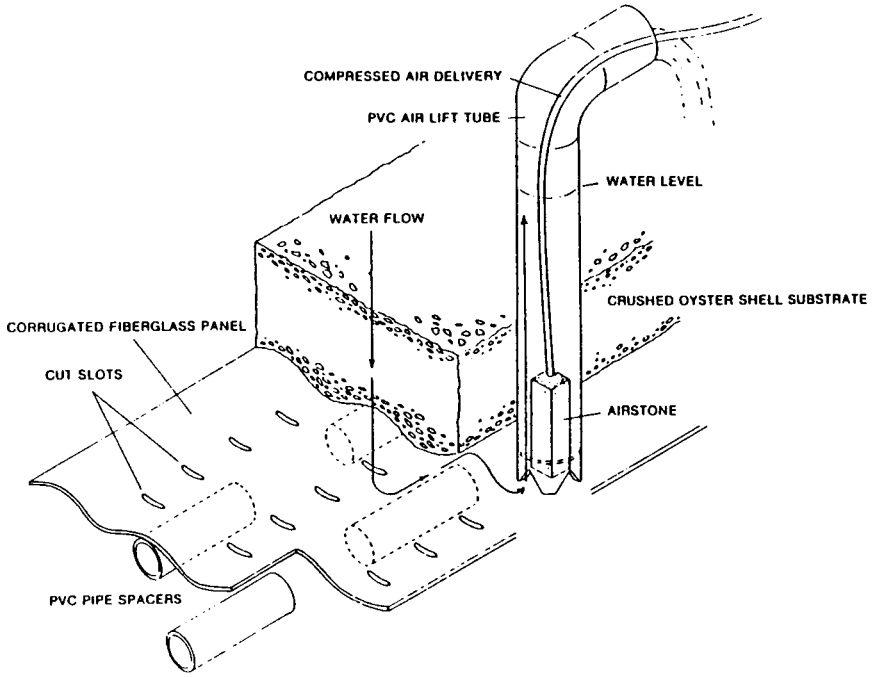

FIGURE 1.—Undergravel filter system for 850-L culture tanks used at ERT.

five 38-L static, recirculating aquaria was prepared in a similar manner and used to compare establishment of mysids in various sea salts.

A second design consisted of two 189-L aquaria with 121-L biofilters separated from and mounted on the side of each. The biofilters were constructed of two plastic buckets, one nested inside the other. Each bucket had been continuously flushed with water for about a year and was believed to be free of plasticizers. The smaller (76 L) of the two was filled with several hundred 2.5-cm polypropylene ballast rings and covered with a nylon mesh to prevent the ballast rings from floating and moving into the larger bucket. A 3.78-L polypropylene bottle, filled with water, was used as a weight to keep the ballast rings and inner bucket submerged. One-centimeter slits were cut in the 76-L bucket, and compressed air was bubbled through the biofilter. Curved plastic tubes supplied with compressed air were used to move water from the biofilter into the aquarium, and back siphons covered with Nitex® bags to prevent escape of mysids were used to move water from aquaria to biofilters. Exchange rate of water was approximately 3.2 L/min or about 1 aquarium-volume/h. No substrate was placed in the aquarium except for a weighted, plastic grid platform, which was placed on the bottom to provide refuge for newly released mysids. Vigorous aeration was supplied to the biofilters and to the bucket of ballast rings; modest aeration was supplied to each aquarium to provide currents to aid in mysid orientation. Salt water prepared from Marinemix was also used in these systems. A commercial nitrification accelerator, Fritz-Zyme No. 9® liquid formulation (Bower and Turner 1984), was used to decrease the time for nitrifying bacteria to colonize the solid surfaces of the biofilter (Table 2).

During the midpoint of culturing with the 189-L systems, a charcoal filter was added to each aquarium to aid in nitrification. A 19-L plastic bucket was filled with 7.5 cm of ballast rings separated by a nylon screen from a cover of 25 cm of coarse activated charcoal. Water exchange at a rate of 1 L/min was accomplished by siphon tubes fitted with airlifts.

Salinity was maintained at 26–29‰, temperature at 26–29°C, pH at 8.2–8.4, and alkalinity at 100–300 mg/L as $CaCO_3$ (Wickins 1983). Cool-white fluorescent bulbs augmented with two 100-W incandescent bulbs (to promote algal growth) provided a photoperiod of 12 h:12 h light:dark (L:D).

TABLE 2.—Frequency of ammonia additions and resultant biofilter nitrification after a single inoculum of Fritz Zyme® No. 9 at CSU.[a]

Day	Addition of		Concentration (mg/L)	
	Fritz Zyme No. 9 (mg/L)	Ammonia-N as ammonia chloride (mg/L)	Total ammonia-N	Nitrite-N
0	6.22	3		
8		3	16.0	4.5
12		3	12.0	19.1
15		3	16.0	32.8
18		3		
19		Additions stopped	17.0	20.5
22			14.0	32.4
26			9.8	14.2
34			3.0	0.04
37			1.3	0.11

[a]A similar procedure was used at ERL, except Mozambique tilapia from Taiwan were introduced to accelerate formation of a biofilter. To enhance establishment of *Nitrosomonas*, *Nitrocystis*, or *Nitrobacter* spp., additions of ammonium chloride (Siddall 1974; Srna 1975) were made to attain a concentration of 1.0 mg/L of total ammonia-N. After ammonia was reduced to below 0.1 mg/L, a second and sometimes a third addition of ammonium chloride was made. Mysids were added to the aquaria only after total ammonia or nitrite concentrations were less than 0.1 mg/L.

At CSU, a third approach to culturing involved artificial salt water in flow-through conditions. Following the methods of Peltier and Weber (1985), flowing salt waters were provided by storing salt water at 27‰ in 20-L polyethylene carboys and metering the water through holding chambers containing mysids. Salt waters tested (Table 3) were 40-Fathoms®, Ocean-50®, Marinemix®, and a reference salt water prepared by diluting natural saltwater brine from Narragansett (supplied by Environmental Research Laboratory, Office of Research and Development, U.S. EPA, Narragansett, Rhode Island). Flows were provided with a variable-speed peristaltic pump. Each holding chamber consisted of a glass jar (8 cm diameter × 7 cm high) with a 1-cm hole drilled 3 cm from the bottom. Nylon screen (200 μm mesh) was cemented over the hole to prevent escape of mysids. Temperature of the flowing water was regulated at 25 ± 2°C by a thermostatically controlled water bath. All tests were conducted under 12 h:12 h L:D at approximately 100 lx. Dissolved oxygen was maintained at 80% saturation by compressed air delivered by 23-gauge surgical tubing (two per chamber) at about 10 bubbles/s. Mysids were placed in the holding chambers as 24-h-old juveniles. Four replicate chambers contained five mysids each for each saltwater test. Mysids were fed live artemia nauplii ad libitum; the diet was supplemented about twice a week with nematodes (*Panagrellus redivivus*). Three series of tests lasting 3, 4, and 7 weeks were conducted. Success of these tests was evaluated on the basis of survival, growth, sexual maturation, and reproduction of mysids.

Acute (96-h) toxicity tests were conducted at CSU with various salt waters and with copper nitrate as a reference toxicant. Static-renewal techniques of Peltier and Weber (1985) were used, and concentrations of copper in the dilutions were measured by atomic absorption spectrophotometry. The end point used in these tests was survival of mysids.

ERL culture methods.—Salt water for culture at ERL was prepared from Marinemix, although 40-Fathoms, Instant Ocean®, Tri-S®, and Fritz® brands were also used successfully. To prepare salt water with a salinity of 25‰, Marinemix was dissolved in tap water with vigorous stirring and aeration in two 473-L fiberglass tanks. After 24 h, aeration was decreased and the water was pumped through a 5-m cartridge filter to remove particulates. The filtrate in the second tank was aerated gently and held until used. Both tanks were covered with black plastic to prevent growth of algae.

At ERL, three culture systems were used: eight

TABLE 3.—Results of testing various sea salts or brines.

Variable	Sea salts or brines at 28‰				
	40-Fathoms[a]	Ocean-50[b]	Marinemix[c]	Kester's[d]	Narragansett Brine[e]
Solubility clearing	Excellent	Fair	Excellent	Poor	Excellent
Precipitation on standing	Little	Severe	Some	Severe	None
pH	8.3	8.3	8.2		7.8
Relative success in culturing mysids	Fair	Fair	Good	Poor (acutely toxic)	Fair

[a]40-Fathoms Salt Mix (Marine Enterprises, 8755 Mylander Lane, Baltimore, Maryland 21204).
[b]Ocean–50 (Jungle Laboratories Corporation, P.O. Box 630, Cibolo, Texas 78108).
[c]Hw Marinemix (Hawaiian Marine Imports, 16810 Barker Springs Road, Houston, Texas 77084).
[d]Kester's formula available in Gentile and Lussier-Sosnowski (1978).
[e]Salt brine (90–100‰ salinity, shipped from the ERL/ORD, U.S. EPA, Narragansett, Rhode Island 02882).

75-L aquaria equipped with undergravel filters, two 75-L aquaria (each connected by siphons to a third 36-L aquarium equipped with a 6-cm-deep biofilter), and one 208-L aquarium equipped with an undergravel biofilter. In the 75-L aquaria, water was transferred from the biofilter to the culture aquaria; back siphons, fitted with Nitex screens (145–45 μm) to prevent transport of mysids, moved the water back to the biofilter. Crushed oyster shell and dolomite (66:33, volume: volume) were used as substrate.

Lighting was provided by 20-W fluorescent cool-white lamps that supplied 139–186 lx to the aquarium glass. Algal growth was kept to a minimum with a 12 h:12 h L:D regime.

Nitrification was accelerated in aquaria as follows. The oyster shell and dolomite mix, placed in 18.9-L plastic buckets, was suspended in a 1,000-L fiberglass tank containing artificial salt water at 20–25‰ salinity and 4–6 kg of Mozambique tilapia *Tilapia mossambica* (=*Oreochromis mossambicus*) from Taiwan. Eventually, substrate containing the bacteria was ready for inoculation. To prepare the substrates, pea- to grape-size gravel was positioned in the bottoms of the buckets around a 2.5-cm-diameter PVC standpipe to prevent oyster shell and dolomite from being pulled up the standpipe by the airlift. The standpipe, slotted at the bottom, contained an airline and airstone that moved water through the mix. A square of fiberglass window screen, with a hole in the center to accommodate the standpipe, was placed over the top of the bucket and held in place by a large rubber band. The screen kept fish out of the bucket and the oyster shell and dolomite in the bucket. Each bucket was numbered and cycled into a mysid culture tank, where it was allowed at least 6 weeks residence time. Each week, the buckets to be cycled were removed, washed with tap water to eliminate most organic debris, and replaced in the tilapia aquarium. To begin an active filter in a culture tank, washed oyster shell was mixed with washed dolomite (66:33, volume: volume), placed in the aquarium, and smoothed. Salt water from the conical holding tank was pumped into the aquarium, airlift lines were connected, and the system was aged for 3–5 d. In emergencies, if conditioned water was not available, animals were placed in aquaria as soon as the water cleared (after 12–18 h).

Common elements in the methods.—In all studies reported here, artemia cysts were hatched in salt water and used as the basic diet for culturing mysids. At ERT, cysts were hatched in 28‰ salt water prepared from Tri-S sea salts in 2-L glass separatory funnels agitated vigorously with compressed air. Hatching funnels were maintained at 25°C in a temperature-controlled room. After 48 h, aeration was stopped, and living nauplii settled to the conical bottom where they were separated from unhatched cysts and debris above and fed to the mysids daily. At CSU, cysts were hatched in 28‰ salt water prepared from Marinemix and vigorously aerated by compressed air in 1-L separatory funnels. Funnels were placed in a 25°C water bath for 36 h, and nauplii were harvested by separating the lower layer of hatched nauplii from overlying debris. Nauplii were transferred to a glass tray, separated by a siphon after movement toward light, washed with freshly prepared salt water in separatory funnels, resuspended in salt water, and fed to mysids twice daily ad libitum. Although artemia were fed to mysids at various times during daylight hours, near the end of the project it was discovered that adding the nauplii at "dusk" increased mysid predation. At ERL, nauplii were hatched in 28‰ Marinemix salt water in glass jars maintained in a water bath at 25–28°C. After hatching, the cysts–nauplii mixture was filtered through Nitex screen, washed with freshly prepared salt water, and separated in a funnel by drawing living nauplii off the bottom. Separation was enhanced by increasing salinity to about 100‰ before drawing the organisms through a Nitex screen and placing them in clean salt water. The process was repeated several times until nauplii were free from debris. Finally, living nauplii were resuspended in clean 25‰ salt water and aerated for an additional 24 h. The nauplii were divided into two batches. The first half was fed to mysids on the afternoon of harvest. The other half was refrigerated overnight at 15°C and fed to mysids the next morning.

At CSU and ERL, the artemia diet was augmented with the nematode *Panagrellus redivivus*. Nematodes were originally sent by C. T. Fontaine of the National Marine Fisheries Service Laboratory (NMFS), Galveston, Texas, to R. R. Williams and then to D. R. Nimmo. At CSU and ERL, yellow cornmeal purchased at a grocery store was made into a slurry and placed in either 275-mL wide-mouth glass canning jars or 500-mL polypropylene jars with screw-on caps. The jars were autoclaved or (at CSU) heated in boiling water for 20 min and allowed to cool. A sterile spatula was then used to inoculate nematodes into each new jar of cornmeal from an existing culture. About 3 mL of water were added to the culture to

maintain moistness. Nematodes could be harvested after 3–6 d, but a superior harvest resulted after incubation for 10 d to 2 months. Maximum harvest was obtained when several sets of cultures were rotated, with nematodes harvested from each set every third day. Harvesting was accomplished by washing the sides of jars with distilled water from a plastic squeeze bottle, thereby flooding the cornmeal surface. A few gentle swirls of the container loosened the nematodes along with some cornmeal and yeast. To separate the nematodes and yeast from the cornmeal at CSU, the slurry was poured from culture jars and allowed to settle for 5 min; then the supernatant was added to mysid cultures. At ERL, moistness of cornmeal was maintained by adding a few milliliters of tap water to the cultures. Another finding at ERL was that nematodes ate bacteria and yeast associated with cornmeal; therefore, nematodes were fed a suspension of baker's yeast.

Hardware used to light cultures varied greatly among the investigations. At ERT, two large banks of fluorescent white bulbs provided continuous light for cultures; the amount of illumination was not recorded. At CSU, where a 12 h:12 h L:D photoperiod was maintained, lighting regimes were changed from Growlux® fluorescent to cool-white bulbs, with additional lighting from 100-W incandescent bulbs placed 2 m above and at 45° angles to the surface of each aquarium. The resulting light was 88–175 lx at the surface and 0.7 lx at the bottom. The aquaria with side-mounted biofilters had 110 lx at the surface and 0.7 lx at the bottom. At ERL, lighting relied on 20-W fluorescent bulbs that supplied 139–186 lx to the sides of the aquaria; the photoperiod regime was 12 h:12 h L:D.

Results and Discussion

Among the three investigations, all successful culture of *M. bahia* relied on

- an efficient, continuously functioning biofilter that maintained the nitrite-N concentration below 0.1 mg/L;
- artemia nauplii fed ad libitum (but all investigators recognized the apparent need for supplemental foods);
- continuous low-level light regime or one with a 12 h:12 h L:D cycle with incident light intensity of 88–186 lx; and
- a systematic replacement procedure for culture water to prevent waste buildup.

Two common problems encountered by all investigators were the appearance of colonial hydrozoan coelenterates that entrapped young mysids or competed with mysids by removing artemia nauplii and periodic loss of biofilter efficiency in removing nitrogen compounds.

At CSU, the hydrozoans were of two types. One was a colonial form that grew via a hyphal network at the tank sides, with polyps directed toward the center of the tank; the other appeared to be a spongelike benthic form. Both had characteristics of hydrozoans described by Lawler and Shepard (1978), who reported that they were members of the family Eirenidae. At ERT and CSU, eradication with liquid bleach as proposed by Lawler and Shepard (1978) was attempted. At ERL, eradication involved removal of all adult mysids, treatment of mysids in 50-mg/L formalin baths for 4 h, and several rinses in clean salt water, followed by transfer to sterilized aquaria. After treatment, contaminated and noncontaminated aquaria were isolated to prevent cross-contamination.

Different approaches were used to maintain functioning biofilters. At ERT, loss of efficient biofilters (monitored by concentration of nitrite-N) was believed to be caused by overpopulation of mysids and accumulation of waste. To minimize the problem, populations were cropped each week by selectively removing about 100 adults per aquarium. In addition, 20% of the culture water was replaced weekly with fresh salt water.

At CSU, increased nitrite-N was associated with lowered pH (Wickins 1983) and a concurrent loss of denitrifying bacteria. As a preventative, additional oyster shell was added to the biofilters. When this failed to raise the pH, $NaHCO_3$ was added weekly until the pH stabilized between 8.2 and 8.4 and alkalinity above 100 mg/L as $CaCO_3$ (Wickins 1983).

Investigators at ERL used fish to establish biofilters and NH_4Cl additions to enhance bacterial populations, but biofilters become less efficient in 6–8 months (depending on addition of food and production of mysids). More added food led to more mysid production and a shorter biofilter life. Consequently, aquaria were dated and a schedule was developed for installing and terminating biofilters, thus, diminishing production in one aquarium did not endanger the total laboratory supply of mysids.

Benefits of algae in culture tanks are still questionable. Unicellular algae such as species of

Selenastrum and *Chlamydomonas* (both freshwater genera) were used as supplemental food at ERT, and an alga identified as *Schizothrix* sp. appeared to enhance mysid reproduction. Ward (1984) suggested that *Spirulina subsalsa* may provide some essential component in the mysid diet, and Odum and Heald (1972) noted that laboratory-held populations of *Mysidopsis alymra* were provided extra nutrition by microfauna diatoms and detritus in aquarium substrate. At ERL, subdued light conditions prevented luxuriant blooms of algae in culture aquaria, and less algal production appeared more conducive to mysid production. However, at CSU, where algae were believed to be beneficial, aquaria became dominated by a toxic blue-green alga identified as *Oscillatoria* sp. (P. Kugrens, CSU Department of Biology, personal communication). One source of this organism was artemia cysts from Colombia. Growth studies in artificial saline media revealed that the alga was euryhaline, thriving in salinities ranging from fresh water to 36‰. Adult mysids placed in aquaria infested with *Oscillatoria* sp. died in 4–48 h; in aquaria with minor infestations of this blue-green alga, however, the mysids survived but showed reduced reproduction.

A review of the literature revealed other problems with toxic algae in crustacean culture. Blooms of the marine blue-green algae *Spirulina subsalsa* and, more frequently, *Spirulina calcicola* caused a disease called hemolytic enteritis in tank- or raceway-reared shrimp *Penaeus stylirostris* (Lightner 1978). Furthermore, shrimp reared in tanks in which *S. subsalsa* was the dominant alga showed reduced growth rates, reduced biomass, and reduced survival, compared with shrimp reared in tanks in which *Enteromorpha* sp. and various diatoms were dominant. Lightner et al. (1978) also said that blooms of certain oscillatorians and perhaps other blue-green algae may have produced diseases in raceway-reared shrimp. Hemolytic enteritis of the gastrointestinal tract of marine penaeid shrimp *Penaeus* spp. and of the freshwater prawn *Macrobrachium rosenbergii* was caused by the oscillatorian *Schizothrix calcicola* (Lightner et al. 1982). Similarly, in laboratory studies Infante and Abella (1985) found that increasing concentrations of *Oscillatoria aghardhii* reduced growth and reproduction of *Daphnia pulicaria* and *D. thorata* from 1 to 400 filaments/mL, and they suggested these results were consistent with the scarcity of daphnids in Lake Washington, Washington, during the 10-year period before 1976.

At ERL, Instant Ocean, Tri-S, Fritz, and Marinemix mixes were used to culture mysids; Marinemix appeared to be an especially good product. At ERT, Tri-S was used for culturing mysids and in testing silver chloride in flow-through tests (Nimmo and Iley 1982). Unfortunately, Tri-S is no longer produced commercially. At CSU, several preliminary tests were conducted on various attributes of salts or brines before selection of a single source for culturing. Variables studied were elapsed time for clearing (solubility), amount of precipitation on standing, initial pH, and relative success in culturing mysids. For comparison, a modified Kester's formula was used (Gentile and Lussier-Sosnowski 1978), as was a reconstituted brine from Narragansett Bay, Rhode Island. The latter was produced by evaporation and sent from the EPA laboratory in Narragansett. Marinemix was superior to all others for culturing mysids (Table 3). Ocean 50, 40 Fathoms, and the reconstituted Narragansett brine did not produce such robust populations, although reproduction occurred in all three. Ocean 50 ranked poorest for solubility, and Narragansett brine produced the least precipitation. The salt water made from Kester's formula was acutely toxic to mysids even after several months of circulating through the biofilter, and attempts to culture mysids in it failed in spite of repeated introductions. Marinemix has been used for culturing penaeid shrimp (Wilkenfield et al. 1983, 1984) and to culture and test mysids in flow-through tests (Horne et al. 1983).

At CSU, three attempts to culture mysids in flow-through conditions were unsuccessful. None of the mysids reproduced, but the animals appeared to grow to normal size (length). Sexes were distinguishable after about 15 d, but microscopic examination of females revealed no developing embryos in oviducts or brood pouches. In the study lasting 3 weeks, survival of mysids was grater in Narragansett brine (13%) than in 40 Fathoms (7%) or in Marinemix or Ocean 50 (0%). In 4-week tests, mysids survived longer in Marinemix (47%) than in Narragansett brine (22%), Ocean 50 (19%), or 40 Fathoms (19%). In tests lasting 7 weeks, Marinemix conditioned by cycling through a biofilter did not appear to be notably superior to freshly prepared Marinemix. Survival of mysids in conditioned Marinemix was 10%, versus 5% in freshly prepared Marinemix.

In 12 static-renewal tests with various salt waters (Table 3), no test was successful beyond 72 h because of mortality of the controls exceeding

10%; but 6 tests were successfully continued beyond 24 h, and 2 went beyond 48 h. One test in which the 10% control mortality criterion was exceeded at 92 h was continued to full term (96 h).

Several reasons exist for our inability to culture mysids or to complete successful testing in artificial salt water with flow-through conditions. First, while testing reference toxicants with copper nitrate, we discovered that copper concentrations ranged from 42 to 68 μg/L in freshly prepared Marinemix. These concentrations were a substantial fraction of the 96-h LC50 (lethal concentration to 50% of the mysids) reported as 181 μg/L (Lussier et al. 1985). In a subsequent test conducted with conditioned Marinemix, copper was below detection limits, indicating that it was removed by the biofilter (perhaps by adsorption to surfaces). Ammonia and nitrite may also have been toxic in the flow-through culture or in reference-toxicity testing. Total ammonia concentrations at the beginning of each test or in successive renewals of test solution ranged from 0.25 to 1.5 mg/L. Nitrite, not measured at the beginning of each test, increased to about 0.5 mg/L during each 24-h interval. Finally, an improper balance of major ions could have existed in freshly prepared salt water. Salt water introduced to the stabilizing conditions in the cultures, presumably due to activity of the biofilters, apparently corrected this situation.

Conclusions And Further Research

Only at CSU did we attempt to quantify various combinations of food, light, and other factors needed to sustain production of *M. bahia*. Based largely on the CSU experiments, we concluded that maximum production of mysids in the laboratory under artificial conditions is possible with

- suspension of live nematodes, *Panagrellus redivivus*, at a rate of 5 mL/75 L aquarium water per day;
- suspension of 24-h-old artemia nauplii hatched from 2.5 mL of cysts/75 L aquarium volume per day fed only at (perceived) dusk;
- a light regime of 12 h light (700 lx at the water surface) and 12 h dusk (an average of 100 lx at the water surface and 0.7 lx at the bottom) produced by incandescent 100-W bulbs placed 2 m above and at 45° angles to the aquarium tank; and
- pH 8.2–8.4, with alkalinity above 100 mg/L as $CaCO_3$ (Wickins 1983).

During late phases of culture, a larval shrimp diet fed to juvenile mysids was used to enhance production. This diet involved a reduction of food particle size (≤150 μm). Optimal feeding rates were 0.25 g/75-L aquarium per day and 0.50 g/189-L aquarium per day. Production of young in two 75-L aquaria with undergravel filters averaged 104 juvenile mysids per day for 10 d and 140 juveniles per 189-L aquarium per day for 10 d.

Culturing of mysids has slowly evolved, and many factors such as trace nutrients, ionic balance, and diets need further research. Most salt water made from artificial sea salts and not conditioned by biological filters will not support growth and production of test organisms. The study at ERT with Tri-S brand sea salts, in which juvenile mysids were produced in salt water that was not conditioned by biological filters, was an exception.

Finally, an artemia nauplii diet for *M. bahia* must be supplemented by other foods. Nematodes used at ERL and CSU increased the quality of the diet, but the reason for benefit from the larval shrimp used at CSU as a diet supplement is still unknown. As Ward (1984) suggested, introduction of *Spirulina subsalsa* or related alga, such as *Schizothrix* sp. used at ERT, may provide a necessary component of mysid diets in laboratory cultures.

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