



Culturing Mysids (*Mysidopsis bahia*)

Supplemental Report
For Saltwater
Video Training Tape

CULTURING *MYSIDOPSIS BAHIA*
SUPPLEMENTAL REPORT

**U.S. Environmental Protection Agency
Office of Water Enforcement and Permits
Permits Division
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FOREWORD

This report is a supplement to the video training tape "Culturing *Mysidopsis bahia*" (EPA, 1990a). The techniques illustrated in the tape and described in this report are based on the methods used at EPA's Environmental Research Laboratory (ERL) in Narragansett, Rhode Island to culture *Mysidopsis bahia* (mysids) for use in marine toxicity tests. These test methods are published in the EPA manual *Short-Term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Waters to Marine and Estuarine Organisms* (EPA, 1988).

This report and the video tape it accompanies are part of a series of training tapes and reports produced by EPA's Office of Water Enforcement and Permits. The tape entitled "Mysid (*Mysidopsis bahia*) Survival, Growth, and Fecundity Test" (EPA, 1990b) complements the material in this report by explaining the 7-day short-term chronic test method using mysids. These tapes are available through The National AudioVisual Center, Capitol Heights, Maryland 20743. Other available tapes include the freshwater series:

"Culturing *Ceriodaphnia dubia*"

"Culturing Fathead Minnows (*Pimephales promelas*)

"*Ceriodaphnia* Survival and Reproduction Toxicity Tests"

"Fathead Minnow Larval Survival and Growth Toxicity Test".

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INTRODUCTION

This report and the video "Culturing *Mysidopsis bahia*" (EPA, 1990a) were produced by EPA to clarify and expand on methods explained in the EPA manual *Methods for Measuring the Acute Toxicity of Effluents to Freshwater and Marine Organisms* (EPA, 1985). Healthy test animals and laboratory personnel who are familiar with the culturing and handling procedures of the test animals are critical for accurate and successful toxicity test results.

Mysidopsis bahia, *M. almyra*, *M. bigelowi*, *Metamysidopsis ilongata*, and *Neomysis americana*, called mysids or opossum shrimp, have all been used in toxicity tests. This report focuses on *Mysidopsis bahia*, the EPA-recommended species used in the mysid survival, growth, and fecundity test (Method 1007; EPA, 1988).

Mysidopsis bahia, are found in the coastal waters of the Gulf of Mexico (see Figure 1). Mysids usually appear transparent with a yellow, brown, or black tint and range from 4.4 mm to 9.4 mm in length (Molenock, 1969). *Mysidopsis bahia* differ from the other *Mysidopsis* species by the armature of the telson, the spines on the uropod, and an unsegmented antennal scale (Molenock, 1969). These morphological characteristics used to identify *Mysidopsis bahia* are presented in Figure 2.

The culturing procedures presented in this report and illustrated in the video were developed to meet the specific needs of the mysid in each of its life stages. The first section of this report covers the selection and preparation of the water for culturing and presents options for water delivery systems. The second section explains how to set up and maintain mysid cultures specifically for providing healthy test animals. The third section provides details on the food preparation methods used at ERL-Narragansett. Instructions for collecting young of the same age for testing are given in the fourth section. A glossary is provided describing some of the terms associated with culturing mysids. Additional references providing more information on culturing mysids are presented throughout the text and are summarized as Appendix A. Appendix B provides a list of the apparatus and equipment needed to culture and test mysids.

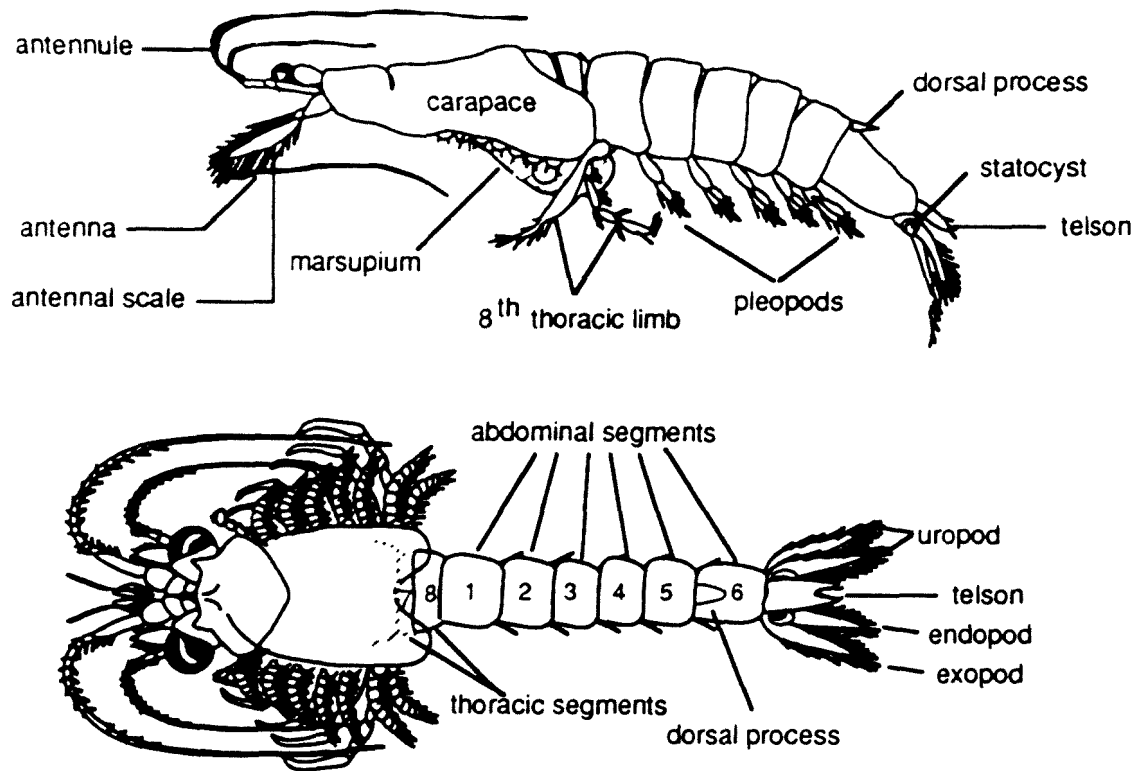


Figure 1. Lateral and dorsal view of a typical mysid. (From Stuck et al., 1979).

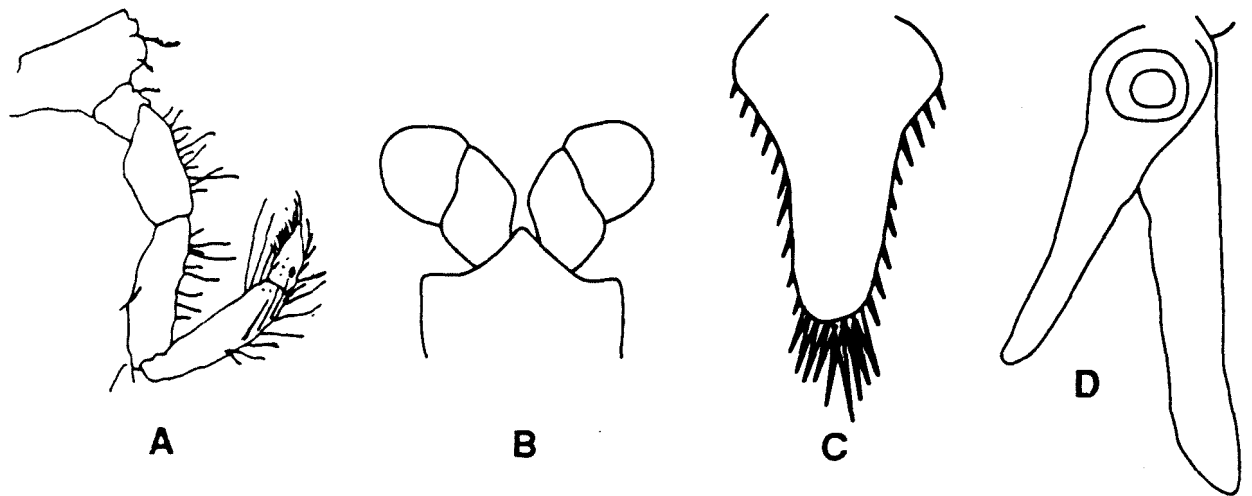


Figure 2. Morphological characteristics used in mysid identification. A, segment 6 (carpo-propodus) of second thoracic endopod with 2-3 setae on distal part of inner margin, segment 5 (merus) with 7-8 setae on proximal 2/3 of inner margin; B, anterior margin of carapace produced to form short triangular rostrum; C, apex of telson of adults usually with 3-6 pairs of long slender spines; D, uropodal endopod usually with 1-4 (normally 2-3) spines near statocyst. Scale lines 0.5 mm in length (From Molenock, 1969).

WATER AND LIGHT

Culture Water

A primary consideration when starting mysid cultures is the culture water. EPA recommends using natural seawater, although artificial sea salts also have been used. If natural seawater is used, it must be contaminant-free and filtered through a 0.45 μm screen before use. The filter removes particulates and possible predators. The source of the culture water should be uncontaminated, consistent, reliable, and periodically checked to ensure these qualities.

Although seawater is preferable, artificial seawater can be made by adding one of several recommended sea salts to distilled, deionized, or dechlorinated tap water. Satisfactory brands used for mysid cultures include Forty Fathoms®, Hawaiiin Marine Mix®, Environmental Systems Sea Salts®, Fritz Super Salts®, and Steven Spotte's GP-2® formula (Lussier et al., 1988). Reconstituted seawater must be aged approximately one month before it is used for cultures. Placing saltwater fish in the seawater helps hasten the aging process. The aged water should be tested with a small population of mysids before using it for cultures. More specific instructions for the preparation of artificial seawater are listed in EPA's manual (EPA, 1985) or by commercial suppliers.

The optimum water conditions for culturing mysids are presented in Table 1.

Photoperiod

For optimum growth and fecundity, the photoperiod for mysid cultures should be 16 hours light and 8 hours dark. ERL-Narragansett uses a system that turns the lights on and off gradually so as not to startle the mysids. The light intensity should be 50-100 foot-candles.

Table 1. Recommended Culture Conditions for *Mysidopsis bahia*¹

Parameter	Culture Conditions
Salinity	25 g/l
Temperature	25°C
pH	7.8-8.2
Dissolved oxygen	7.1 mg/l
Ammonia	0.1-0.3 mg/l
Nitrite	<0.05 mg/l
Nitrate	<20 mg/l
Alkalinity	150 mg/l
Photoperiod	12-hr light:12-hr dark to 16-hr light:8-hr dark
Filtration	20 µm
Tank Size	10-55 gal
Substrate	Dolomite, oyster shells, coral
Biological filter/algal mat	<i>Spirulina subsalsa</i>

¹ Source: Lussier et al., 1988.

Culture Vessels

Mysids can be cultured in tanks of various sizes. The most commonly used are the 20 and 29 gallon aquaria. Wider tanks are more suited for culturing than taller ones since a large surface area to volume ratio provides good oxygen exchange. Tanks, as with all culturing equipment, should be cured in the culture water for approximately 3-5 days before being used for animals.

Water Delivery Systems

Mysids can be cultured in flow-through, recirculating, or static systems. The preferred system is the flow-through arrangement where water is delivered to the tanks at a measured rate and the run off is discharged out of the system (see Figure 3). The flow rate through the culture tanks should be no less than 4-5 liters per hour or 2 turn overs per day. Non-toxic materials such as glass, fiberglass, teflon, and polyvinylchloride (PVC) pipe are recommended for the water delivery system. Materials such as rubber, copper, brass, or plastic could become a source of toxicity to the system and should be avoided.

Recirculating systems can also be used to culture mysids and should be designed to provide the same flow rate as the flow-through system. However, recirculating systems must also provide a biofiltering system that can be constructed out of any non-toxic, high-surface-area material such as crushed coral, pea gravel, or dolomite. A sand filter may also be added to the system.

Static systems are made of a series of tanks that are independently filtered and supplied with water. The advantage of this type of system is that problems such as disease are confined to one tank and complete culture crashes are less common. Each tank in a static system should be supplied with an undergravel filter and water changes should be made by replacing one-half of the tank's volume of water with fresh culture water every other day. Static systems are harder to maintain than flow-through or recirculating systems due to evaporation. Tanks should be covered and care must be taken to avoid the concentration of salts as the water evaporates.

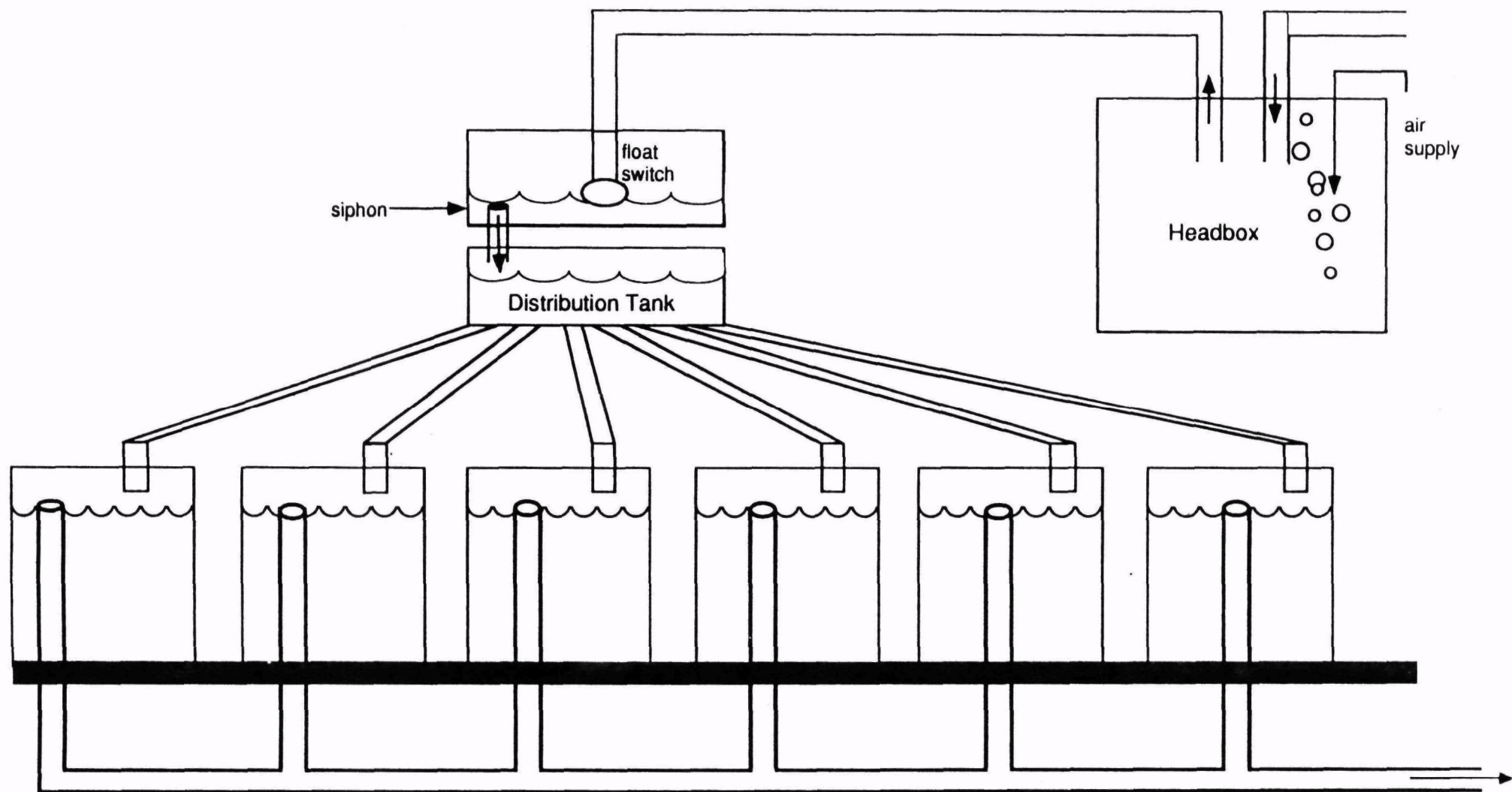


Figure 3. Intermittent Flow-through Water Delivery System

CULTURE START UP AND MAINTENANCE

Starting Cultures

Once the culture system and water source are designed, obtained, and seasoned, mysids can be purchased from a number of sources. A reliable supplier will certify that the correct species has been shipped. EPA laboratories also will supply mysids depending on demand and availability. If test animals are not needed immediately, cultures should be started with juveniles to allow laboratory personnel to become familiar with mysid handling and maintenance requirements before learning to collect the young.

Mysids should be shipped in Nalgene® containers packed inside coolers or polyfoam boxes within cardboard shipping cartons. The shipping density should be 100 mysids per liter and the container should have adequate airspace to ensure a supply of oxygen throughout the shipping period. No food should be added to the containers. A reliable overnight delivery service should be used for shipment.

After the shipment is received the mysids must be acclimated to the receiving laboratory's culture water and conditions. The temperature and salinity of the water used for shipping must be measured. Slow adjustment of the water temperature can be accomplished by placing the container in a water bath. The salinity can be adjusted by adding new culture water to the water used for shipment. Increases or decreases in temperature and salinity should not exceed 2-3°C or 2-3 ‰, respectively, per day.

For optimum growth and reproduction the stocking density for adult mysids should be between 200-300 per 20 gallon tank or approximately 20 mysids per liter. A healthy, unstressed culture should have at least 70% of the females carrying eggs in their brood pouch. Juveniles can be stocked at higher densities than adults.

Taxonomy

Figure 4 shows the life cycle of a mysid. Mysids produce live young called early juveniles. These juveniles are planktonic for the first 24 hours post release and then settle to the bottom where they orient to the current in the tank and begin to feed. Depending on water temperature and diet, females reach sexual maturity in 12-20 days. Brood pouches appear at the age of 12-15 days and young are released at approximately 17-20 days. A gravid female is identified by an enlarged and darkened brood pouch containing the developing embryos. The female is ready to release the young when the eyespots can be identified in the brood pouch. Females average 5-7 young per brood, but can produce as many as 20 in one brood. Multiple broods are produced for several months at a rate of one approximately every 4-6 days.

Collecting Test Animals

To conduct toxicity tests using mysids, animals of the same age must be collected and pooled together. To accomplish this, gravid females are separated and their young are collected and held until the proper age for starting tests. For testing needs, assume a reproduction rate of two juveniles per female per day because not all females will release their young on the same day.

At ERL-Narragansett brood chambers such as the one illustrated in Figure 5 are used to collect test animals. Gravid females are collected from a minimum of three culture tanks and placed in a 4 liter Nalgene® beaker that is placed inside a separatory funnel containing culture water. The solid plastic bottom of the Nalgene® beaker is replaced by 1 mm mesh screen. The screen allows the newly released young to pass through while preventing the adults from leaving the beaker.

Once the females are placed in the brood chamber, provide food and gentle aeration or water flow by placing an airstone in the neck of the separatory funnel or by providing water inflow and outflow to the funnel. The females should be left overnight and the young collected the next day.

To harvest the young, remove the airstone and drain the separatory funnel into a 300 μ m mesh cup placed in a culture dish. The mesh cup should be partially submerged in water to prevent injury to the young. While the water is draining, gently lift and dunk the beaker

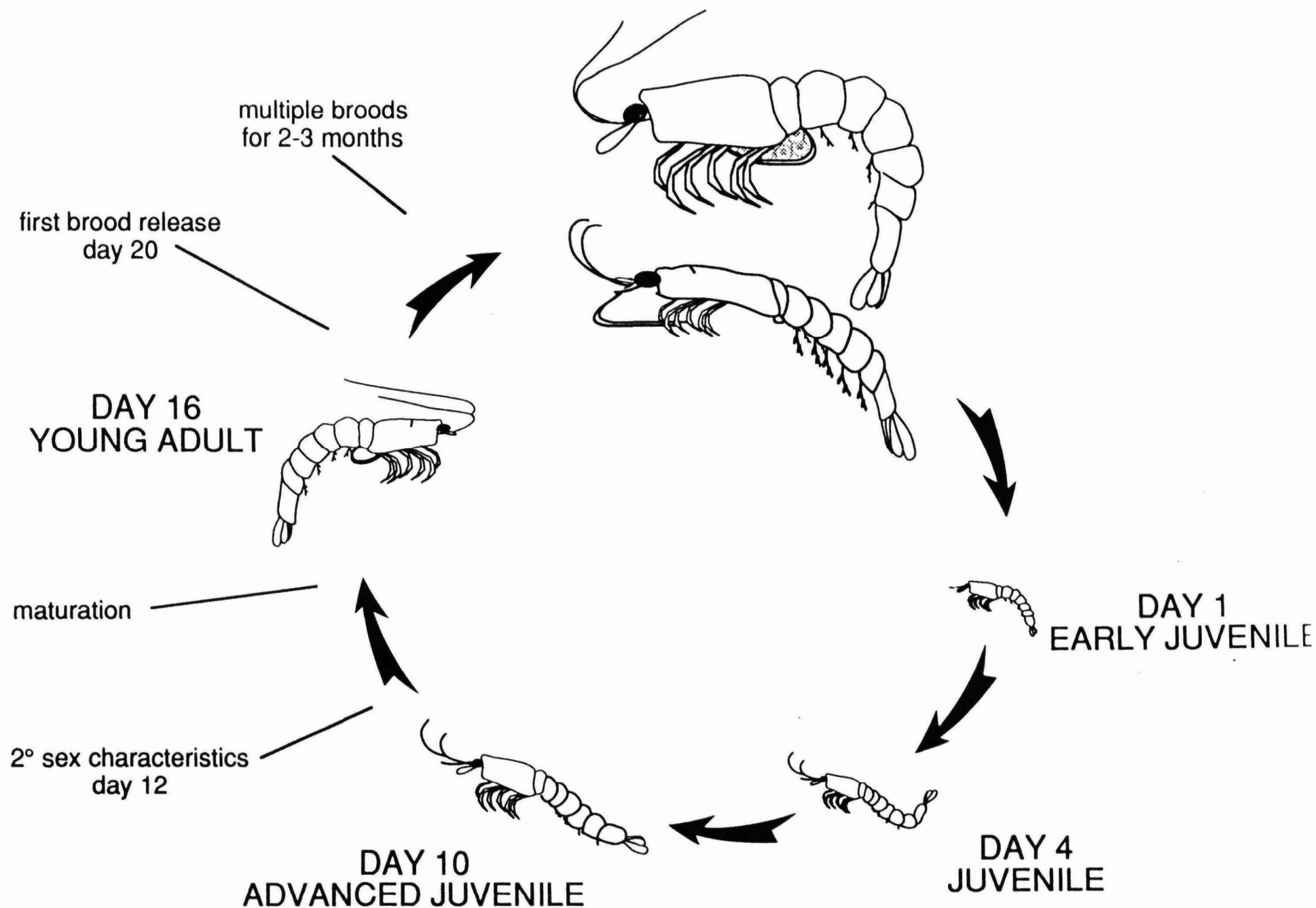


Figure 4. Life Cycle Of *Mysidopsis bahia*

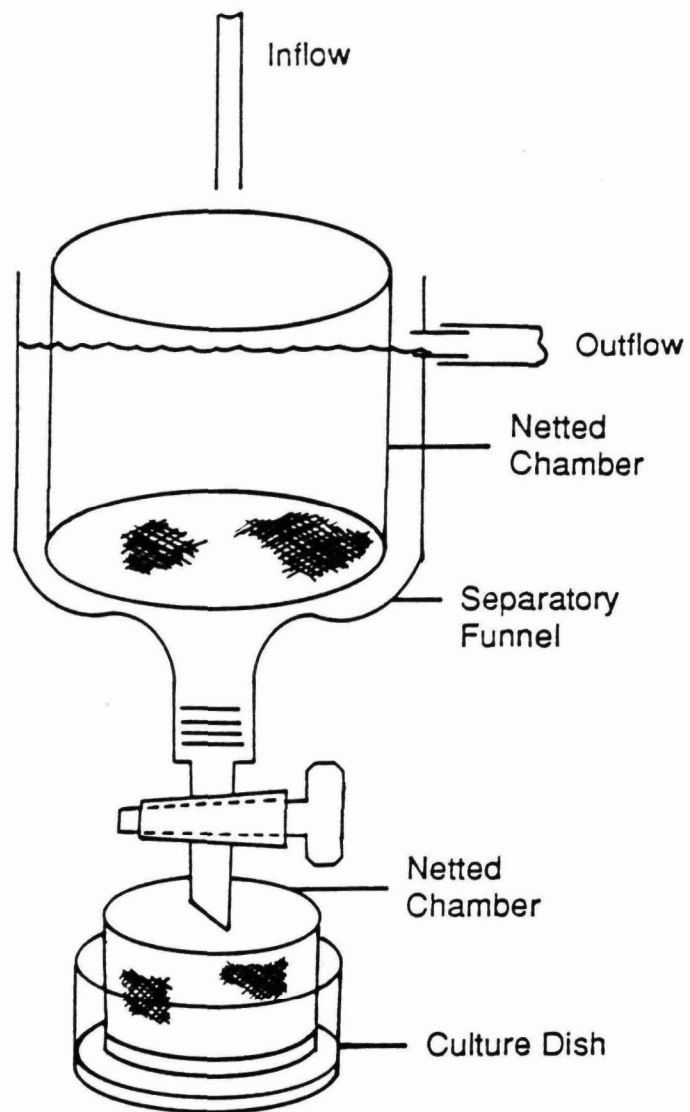


Figure 2. Apparatus for collection of postlarval mysids from gravid females.
From Lussier et al., 1988.

containing the females to wash any remaining young out through the screen. As the water drains from the funnel, gently rinse the sides 2-3 times with clean seawater to wash out any mysids that may stick to the sides. The females should be placed back into the culture tanks. The young can be used immediately for testing or grown out in a separate tank to the desired age for test start up. The harvested young should be maintained at conditions similar to the regular cultures.

An alternative system for collecting young is a siphon entrapment system, or a "mysid generator" (see Figure 6). The siphon inlet is covered by a 750 μm screen that excludes adults and allows juveniles to pass through to a collection vessel. In the collection vessel juveniles are deposited into a 350-370 μm Nitex® screen cup. The juveniles in the screen cup are collected daily for test use. When using mysid generators, care must be taken to siphon all of the juveniles out of the tank each day. Otherwise, the collected juveniles' ages may not be within 24 hours of each other as test methods require.

Tank Cleaning

Culture tanks should be cleaned at least once each month. The sides of each tank should be scraped to remove any algal growth and the gravel should be stirred to dislodge the accumulated debris. This will clear the dolomite filter.

Approximately twice each year, the tanks should be completely emptied and scrubbed. At this time the gravel should also be replaced. It is important to cure any new materials as described under Starting Cultures, above, before using them in culture tanks.

Record Keeping

Culture tanks should be monitored and all conditions recorded on data sheets that are kept in a permanent file. These record sheets can then be used to assess any problems that may occur with the cultures and assist in the elimination of possible causes.

Figure 7 is a data sheet adapted from the one used by ERL-Narragansett for mysid cultures. Each of the conditions is checked daily and initialed by the technician taking the reading, checking the condition, or performing the task. Daily tasks performed are temperature, pH, salinity, and dissolved oxygen measurements, seawater flow and air flow checks, and feeding (twice daily).

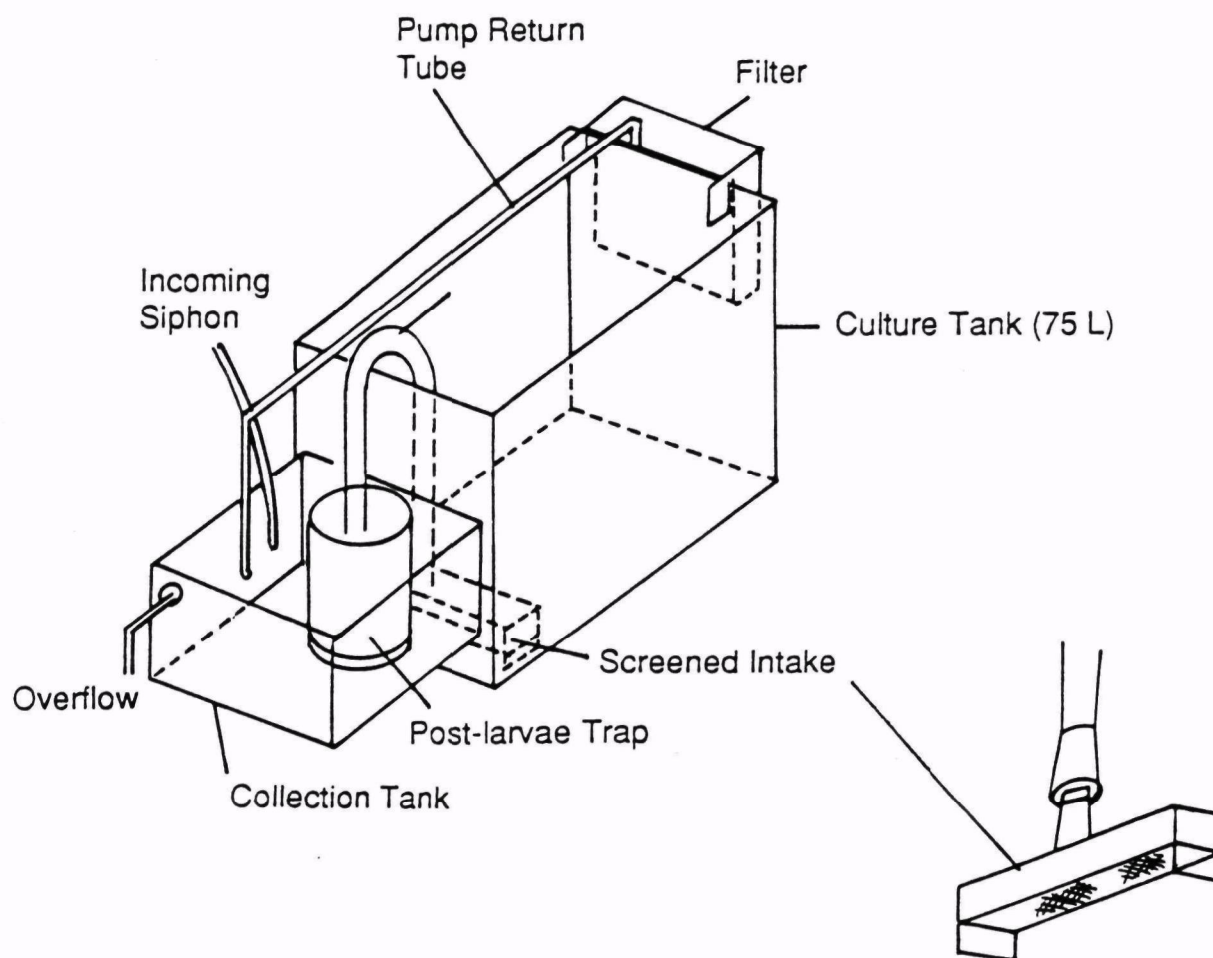


Figure 6. A mysid generator. From Lussier et al., 1988.

MYSID CULTURE TANK CONDITIONS

DATE	TEMP °C	pH	SALINITY ‰	D.O. mg/l	SW FLOW	AIR FLOW	MYSIDS FED am/pm	COMMENTS

Figure 7. Data sheet for mysid cultures.

MYSID CULTURE TANK CONDITIONS

DATE	TEMP °C	pH	SALINITY ‰	D.O. mg/l	SW FLOW	AIR FLOW	MYSIDS FED am/pm	COMMENTS

Figure 7. Data sheet for mysid cultures.

should be drained through a 150 μm screen and rinsed with clean seawater to remove any chemicals released during hatching.

To determine the correct amount of *Artemia* for feeding, an aliquot of the hatched *Artemia* should be counted under a microscope to determine the density of the culture. This density will serve as a reference to ensure that future cultures are hatching at the same rate.

Once the *Artemia* are rinsed, the volume of clean seawater that is added determines the volume of food provided to each tank. From the calculated and adjusted density of the diluted food supply, determine the volume of food needed for each tank by estimating a feeding rate of 150 *Artemia* per mysid per day. This should be supplied over two feedings and should be adjusted so that there are always live *Artemia* available for the mysids to feed on.

GLOSSARY

Artemia - Brine shrimp used as food for mysid cultures; Brazilian or Columbian strains are preferable.

Cyst - The life stage of unhatched *Artemia*.

Fecundity - Productivity or fertility.

Flow-through water delivery system - An open water flow system that delivers fresh water or seawater to culture tanks and is disposed of after it leaves those tanks.

Mysid (*Mysidopsis bahia*) - An estuarine crustacean ranging 4.4 mm to 9.4 mm in length found from the Gulf of Mexico and along the Atlantic coast used in test procedures as an indicator species for aquatic toxicity.

Nauplii - The first stage of newly-hatched *Artemia*.

Recirculating water delivery system - A water flow system that treats water after it passes through the culture tanks (usually with sand and biofilters) and delivers the same treated water back to the tanks.

Static water system - An enclosed system contained within one culture tank. The water is filtered through an underground or charcoal filter and is delivered back to the same tank.

APPENDIX A

REFERENCES

- American Society for Testing and Materials. 1987. Practice for using brine shrimp nauplii as food for testing animals in aquatic toxicology. ASTM Designation E-1203-87. Philadelphia, PA.
- Blaxter, J.H.S., F.S. Russell, and M. Yonge, eds. 1980. The biology of mysids and euphausiids. Part 1. The biology of the mysids. Adv. Mar. Biol. 18:1-319.
- Brattegard, T. 1969. Marine Biological Investigations in the Bahamas 10. Mysidacea from shallow water in the Bahamas and southern Florida, Part 1. Sarsia 39:17-106.
- Davey, E.W., J.H. Gentile, S.J. Erickson, and P. Betzer. 1970. Removal of trace metals from marine culture media. Limnol. Oceanogr. 15:486-488.
- Farrell, D.H. 1979. Guide to the Shallow-water mysids from Florida. Fla. Dept. Environ. Reg., Techn. Ser. 4(1):1-69.
- Fotheringham, N., and S.L. Brunenmeister. 1975. Common marine invertebrates of the northwestern Gulf coast. Gulf. Publ. Co., Houston, TX.
- Heard, R.W. 1982. Guide to the common tidal marsh invertebrates of the northeastern Gulf of Mexico. Publ. No. MASGP-79-004, Mississippi-Alabama Sea Grant Consortium, Ocean Springs, MS.
- Johns, D.M., W.J. Berry, and W. Walton. 1981. International study on *Artemia*. XVI. Survival, growth, and reproductive potential of the mysid *Mysidopsis bahia* Molenock fed various geographical strains of the brine shrimp *Artemia*. J. Exp. Mar. Biol. Ecol. 53:209-219.
- Lawler, A.R. and S.L. Shepard. 1978. Procedures for eradication of hydrozoan pests in closed-system mysid culture. Gulf Res. Rept.
- Lussier, S.M., A. Kuhn, M.J. Chammas, and J. Sewall. 1988. Techniques for the laboratory culture of *Mysidopsis* species (Crustacea: Mysidacea). Environ. Tox. Chem. 7:969-977.
- Molenock, J. 1969. *Mysidopsis bahia*, a new species of mysid (Crustacea: Mysidacea) from Galveston Bay, Texas. Tulane Stud. Zool. Bot. 15(3):113-116.
- Nimmo, D.R. and T.L. Hamaker. 1982. Mysids in toxicity testing - a review. Hydrobiol. 93:171-178.
- Nimmo, D.R., T.L. Hamaker, E. Matthews, and W.T. Young. 1982. The long-term effects of suspended particulates on survival and reproduction of the mysid shrimp, *Mysidopsis bahia*, in the laboratory. In: G.F. Mayer, ed., Ecological Stress and the New York Bight: Science and Management. Estuarine Res. Found., Columbia, S. Carolina. pp. 41-50.

- Nimmo, D.R., T.L. Hamaker, C.A. Sommers. 1978. Culturing the mysid (*Mysidopsis bahia*) in flowing sea water or a static system. In: Bioassay Procedures for the Ocean Disposal Permit Program, U.S. Environmental Protection Agency, Environmental Research Laboratory, Gulf Breeze, Florida. EPA-600/9-78-010. pp. 59-60.
- Price, W.W. 1982. Key to the shallow water Mysidacea of the Texas coast with notes on their ecology. Hydrobiol. 93(1/2):9-21.
- Stuck, K.C., H.M. Perry, and R.W. Heard. 1979a. An annotated key to the Mysidacea of the North Central Gulf of Mexico. Gulf Res. Rept. 6(3):225-238.
- Stuck, K.C., H.M. Perry, and R.W. Heard. 1979b. Records and range extensions of Mysidacea from coastal and shelf water of the Eastern Gulf of Mexico. Gulf Res. Rept. 6(3):239-248.
- U.S. EPA. 1985. Methods for Measuring the Acute Toxicity of Effluents to Freshwater and Marine Organisms. Third Edition. Environ. Monit. and Supp. Lab., Cincinnati, OH. EPA/600/4-85/013.
- U.S. EPA. 1988. Short-term Methods of Estimating the Chronic Toxicity of Effluents and Receiving Waters to Marine and Estuarine Organisms. Environ. Monit. and Supp. Lab., Cincinnati, OH. EPA/600/4-87/028.
- U.S. EPA. 1990a. Culturing *Mysidopsis bahia*. Videotape. Produced by the Office of Water Enforcement and Permits. 19:13 min.
- U.S. EPA. 1990b. Mysid (*Mysidopsis bahia*) Survival, Growth, and Fecundity Test. Videotape and Supplemental Report. Office of Water Enforcement and Permits. 31:19 min.; 26 pp. EPA 505/8-90-006a.
- Venables, B. 1986. *Mysidopsis* sp.: Life history and culture workshop report. Gulf Breeze, FL. October 15-16, 1986, Institute of Applied Sciences, North Texas State University, Denton, TX.
- Ward, S.H. 1984. A system for laboratory rearing of the mysid, *Mysidopsis bahia* Molenock. Progr. Fish-Cult. 46(3):170-175.
- Wigley, R.L. and B.R. Burns. 1971. Distribution and biology of mysids (Crustacea: Mysidacea) from the Atlantic Coast of the United States in the NMFS Woods Hole Collection. Fish Bull. 69:717-746.

APPENDIX B
APPARATUS AND EQUIPMENT

- Facilities for holding and acclimating test organisms.
- Brine shrimp culture unit -- see Food Preparation above.
- Mysid culture unit -- see Culture Start Up and Maintenance above.
- Samplers -- automatic sampler, preferably with sample cooling capability, that can collect a 24-hour composite sample of 5 liters.
- Environmental chamber or equivalent facility with temperature control ($26 \pm 1^{\circ}\text{C}$).
- Water purification system -- Millipore Super-Q®, deionized water or equivalent.
- Balance -- capable of accurately weighing to 0.000001 g.
- Reference weights, Class S -- for checking performance of balance.
- Drying oven -- 105°C , for drying organisms.
- Desiccator -- for holding dried organisms.
- Air pump -- for supplying air.
- Air line, and air stones -- for aerating cultures, brood chambers, and holding tanks, and supplying air to test solutions with low DO.
- pH and DO meters -- for routine physical and chemical measurements.
- Tray -- for test vessels; large enough to hold 8 vessels at one time.
- Standard or micro-Winkler apparatus -- for determining DO and checking DO meters.
- Dissecting microscope (240-400X magnification) -- for examining organisms in the test vessels to determine their sex and to check for the presence of eggs in the oviducts of the females.
- Light box -- for illuminating organisms during examination.
- Refractometer or other method -- for determining salinity.
- Thermometers, glass or electronic, laboratory grade -- for measuring water temperatures.

Thermometer, National Bureau of Standards Certified (see USEPA Method 170.1, USEPA, 1979) -- to calibrate laboratory thermometers.

Test vessels -- 200 ml borosilicate glass beakers or 8 oz disposable plastic cups (manufactured by Falcon Division of Becton, Dickinson Co., 1950 Williams Dr., Oxnard, CA 93030) or other similar containers. Cups must be rinsed thoroughly in distilled or deionized water and then pre-soaked (conditioned) overnight in dilution water before use. Forty-eight (48) test vessels are required for each test (eight replicates at each of five effluent concentrations and a control).

Beakers or flasks -- six, borosilicate glass or non-toxic plasticware, 2000 ml for making test solutions.

Wash bottles -- for deionized water, for washing organisms from containers and for rinsing small glassware and instrument electrodes and probes.

Volumetric flasks and graduate cylinders -- Class A, borosilicate glass or non-toxic plastic labware, 50-2000 ml for making test solutions.

Separatory funnels, 2-liters -- 2 - 4 for culturing *Artemia*.

Pipets, volumetric -- Class A, 1-100 ml.

Pipets, automatic -- adjustable, 1-100 ml.

Pipets, serological -- 1-10 ml, graduated.

Pipet bulbs and filters -- PROPIPET®, or equivalent.

Droppers, and glass tubing with fire polished edges, 4 mm ID -- for transferring organisms.

Forceps (fine tips such as jewelers' forceps) -- for transferring organisms to weighing boats.

NITEX® mesh sieves (150 μ m and 100 μ m) -- for concentrating organisms.

Depression glass slides or depression spot plates -- two, for observing organisms.