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**a manual for culture of pink shrimp,
penaeus duorarum
from eggs to postlarvae suitable for stocking**

**durbin c. tabb, won t. yang,
yosuke hirono and john heinen**

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A Manual for Culture of Pink Shrimp, *Penaeus duorarum*,
from Eggs to Postlarvae Suitable for Stocking

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PREFACE

The Sea Grant Colleges Program was created by Congress in 1966 to stimulate research, instruction and extension of knowledge of marine resources of the United States. In 1969 the Sea Grant Program was established at the University of Miami.

The outstanding success of the Land Grant Colleges Program, which in 100 years has brought the United States to its current superior position in agricultural production, was the basis for the Sea Grant concept. This concept has three objectives: to promote excellence in education and training, research, and information services in the University's disciplines that relate to the sea. The successful accomplishment of these objectives will result in material contributions to marine oriented industries and will, in addition, protect and preserve the environment for the enjoyment of all people.

With these objectives, this series of Sea Grant Special Bulletins is intended to convey useful research information to marine communities interested in resource development.

While the responsibility for administration of the Sea Grant Program rests with the National Oceanic and Atmospheric Administration in the Department of Commerce, the responsibility for financing the program is shared by federal, industrial and University of Miami contributions. This study, A Manual for Culture of Pink Shrimp, *Penaeus duorarum*, from Eggs to Postlarvae Suitable for Stocking, is published as part of the Sea Grant Program.

FOREWARD

Visitors and letters from all over the world have complimented us with repeated requests for information and advice in the rapidly developing science of mariculture (farming the sea).

This provisional manual has been prepared in response to many requests for a concise description of the techniques used in rearing the pink shrimp, Penaeus duorarum Burkenroad. We encourage those for whom it was written to inform us of its application and limitations.

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INTRODUCTION

As part of the University of Miami Sea Grant Program, the Rosenstiel School of Marine and Atmospheric Science of the University of Miami has conducted research on the culture of commercial shrimp of the genus Penaeus. The facility built for this research was described by Tabb et al (1969). The aim of the research is to develop methods for profitable farming of commercial shrimp. Results of work in our laboratories and elsewhere are sufficiently far advanced that a number of commercial shrimp farming operations have been established. These include several such operations in Florida and Texas.

Meanwhile, it must be emphasized that ability to raise shrimp from eggs to marketable sizes does not guarantee a profit. So far as we know, profits have eluded all United States shrimp growers, and more research is needed on food, feeding and nutrition, and reduction of costs of land, construction and labor (Anderson and Tabb, 1971). More experience is also required in commercial-scale operations before shrimp farming becomes a profitable business in the United States.

Many difficult technical problems have been solved, and simplified procedures have been developed. This manual describes two kinds of tanks and their use in the culture of pink shrimp, Penaeus duorarum Burkenroad, from eggs to postlarvae of sizes suitable for stocking. This manual is in sufficient detail that, using it, a skilled biologist should be able to hatch shrimp and grow them to sizes suitable for stocking.

The procedures described here are not free from failure. The biologist will still face unexplained losses of larvae, and there will be .

problems in concurrent culture of diatoms for the feeding of larvae being cultured in indoor facilities. One of the most serious problems is the dependence upon wild shrimp stocks for the supply of gravid (egg-bearing) females. At some times of the year such gravid females are scarce or absent from the fishing grounds. Inclement weather can also upset trip schedules which, in turn, force changes in the diatom culture schedule. We have discarded many dense cultures of live diatoms because female shrimp could not be obtained at expected times. To overcome this difficulty, research by Drs. Charles Caillouet and J. L. Runnels is in progress at our institution and by other investigators elsewhere on reproductive physiology of shrimp, with the aim of making it possible to rear and maintain the needed females from selected captive stocks. In the meantime, it may be possible to establish a successful commercial shrimp farm using eggs obtained from wild females.

The hatchery facility should be located near a dependable source of high salinity bay water free from pollutants. The hatchery should also be as near to the source of gravid female shrimp as possible, preferably within 8 hours travel time. It is also desirable to have an abundant supply of fresh water for dilution of hatchery water should drought-induced hypersalinity occur. Adequate fresh water also makes it possible to use artificial sea salts in special cases where the main source of hatching water has become contaminated. The hatchery should also be located at an elevation, either natural or artificial, sufficient to place the essential services such as electrical system, temperature control features and compressors above the reach of most storm tides. Such storms may force evacuation of hatchery personnel for several hours. If the site is properly

selected and built at a level to be above the probable storm tides, the life support systems should function adequately in the absence of the operators even though the coastal area be inundated.

We describe two types of culture facilities, the two metric ton (about 500 gal.) and the twenty metric ton (5,000 gal.) capacity systems for a particular reason. The two metric ton tanks are especially useful for research and training purposes because of the greater number of experiments that can be conducted in a given area and because, being indoors, greater control is possible over environmental parameters. The twenty-ton capacity tanks are strictly production units. Large-scale replication of experiments in such tanks calls for a correspondingly great investment of capital.

LIFE HISTORY OF PINK SHRIMP

The pink shrimp, Penaeus duorarum, is one of the most valuable species in the decapod crustacean family Penaeidae. With two other species from the southeastern United States, P. setiferus and P. aztecus, it is being cultured on an experimental basis. The larval development of these three species is similar to that of P. japonicus of Japan. The general similarity in larval development among many penaeid species supports our belief that culture methods such as those described here may serve, with minor modifications, for many other shallow-water penaeids.

Juvenile and adult pink shrimp can tolerate a range of temperature between 11° and 40°C but individuals may be killed when exposed to a low of 10°C for 6 to 10 hours, and pink shrimp are seldom encountered in natural waters with temperatures as high as 36°C. Feeding activity is sharply curtailed in ponds when water temperatures fall below 18°C.

Juvenile pink shrimp are often found in water of 5.0 ppt salinity. They may be found in extreme south Florida estuaries during summer, in water without detectable salinity. Juveniles are generally absent in areas where salinity exceeds 65 ppt. Adults are nearly always found in 33 to 36 ppt oceanic salinity.

Spawning occurs in offshore waters from 10-20 fathoms at temperatures between 19 and 31°C, and can take place when the female pink shrimp reach about 10 cm total length or larger. The eggs are fertilized, as they are extruded, by sperm contained in a storage receptacle attached to the female by the male during copulation. This sperm receptacle, called a spermatophore, is attached sometime prior to spawning while the female is in a freshly moulted condition.

During spawning in the open sea the eggs are cast free in the water where they drift freely for half an hour or so and then become demersal for about 14 to 16 hours prior to hatching. The eggs hatch into nauplius larvae. This first nauplius stage is followed by four more nauplius stages before metamorphosis into the first of three zoeal stages, which are followed in turn by three mysis stages and a number of postlarval stages (Figures 1-13).

During the nauplius stages the larvae derive their nutrition from their own egg yolk. They begin to feed on microscopic plants called phytoplankton at the beginning of the first zoeal stage. This is the most crucial point in larval culture because the source of nutrition changes from internal (egg yolk) to external (phytoplankton). The larvae must have abundant plant food of proper size in order to make this important transition. If such food is not made available they will starve. If carried past this crucial point, the developing larvae continue to feed on phytoplankton well into the postlarval stages, but begin feeding on zooplankton, during the first mysis stage, or even earlier, as well.

In culture operations newly hatched brine shrimp (nauplii) are provided during the first mysis stage as a substitute for natural zooplankton. Brine shrimp constitute the major part of the diet of the mysis and early postlarval stages in indoor culture. In the outdoor tanks of twenty metric ton capacity, the mysis and postlarval stages undoubtedly consume zooplankton originating from the bay water as well as cultured brine shrimp larvae. Ground squid can also be used as a food beginning with the early postlarval stages.

CULTURE FACILITIES

Shrimp hatching operations have been conducted successfully at the University of Miami Sea Grant aquaculture research facility using indoor and outdoor facilities (Figures 14 and 15). The indoor facility is most useful in training technical personnel; the outdoor facility is most useful for prototype commercial production. The indoor facility permits year round production and research; the outdoor facility can be used only during the warmer months unless water heating devices are installed.

The Indoor Hatchery and Its Equipment

A substantial building was required to house the hatching tanks and to culture food organisms (Figures 14 and 16). Adequate indoor temperature control has been achieved at our Turkey Point facility by use of wall-mounted reverse-cycle air conditioners. Differing temperature requirements of the developing larvae and their diatom food imposes a need for partitioning the culture room. Fluorescent lights provide adequate illumination for all phases of larval and diatom culture.

The fluorescent light fixtures should be suspended from the ceiling by adjustable lines so the fixtures can be raised or lowered. When larvae are actively growing the fixtures are lowered to within a foot of the tank top. They can be raised out of the way when tanks are being cleaned. All lights are turned off during spawning.

Large volumes of oil-free compressed air are essential for hatching operations. Air supply should be duplicated in case one system fails or is shut down for maintenance. At Turkey Point, 5 round air stones are placed

in each indoor hatching tank. At maximum flow on all stones (0.4 cubic feet per minute per stone) about 50 cfm compressor output is required. The Turkey Point system is operated with a line pressure of 15 pounds per square inch (psi), which is handled by schedule 40 PVC (polyvinyl chloride) pipe. An in-line oil filter traps oil contamination that can originate in the compressor. A dessicant chamber and condensation loop remove moisture. A compressor with a 50 gallon storage tank is used. This compressor produces 24 cfm of air under intermittent operating conditions. Two high-volume, low pressure compressors augment the larger compressor, and two diver compressors powered by gas engines are used as emergency standbys. These can be hooked into the hatchery air line using quick-couple fittings.

Because of the critically important nature of the air supply it is desirable to provide a pressure-activated alarm in the main line to alert the hatchery crew if the air supply cuts off. Automatic switching should be installed to put the auxiliary compressor into operation immediately if the main compressor fails.

To circumvent power failures and damage to motors from power surges, auxiliary power should be provided by electrical generators powered by diesel or gasoline motors. If internal combustion units are to be used as the power supply, they should be housed away from the hatchery and compressor air intake to avoid contamination of the air by exhaust fumes.

A rigorous maintenance schedule for all machinery is essential, but it is of critical importance for the compressors. For compressors which have graphite compressor blades it is necessary to replace the blades before wear reduces the rate of compression significantly. Wear can be detected by careful observation of the line pressure and it can be anticipated by

recording running hours. For piston types it is necessary to check lubrication carefully, and to be particularly alert to bearing and piston ring wear. Wear can be accelerated by overheating, and the compressor room should have ample forced draft ventilation to cool the compressor.

Large quantities of filtered water are also required. A single electric or gasoline powered centrifugal pump can lift the water, force it through the necessary filters, and fill all tanks. A duplicate pump should be available, and both pumps should be kept in good condition by constant maintenance. Small inexpensive pumps, of about 100 gallons per minute, powered by 2.5 hp gasoline motors, serve well but care must be exercised that the motors are cleaned regularly since salt water rapidly corrodes the motor-cooling vanes and muffler assembly.

The Two-Ton Tanks

Shrimp often spawn in the plastic garbage cans, plastic shipping bags and other receptacles used to transport them from the fishing grounds to the hatchery. With suitable aeration and clean seawater, the eggs will hatch and develop in such small containers. Biologists have obtained successful hatches in 10- and 15-gallon aquaria and in 5-gallon carboys. If only small quantities of larvae are required, such techniques are suitable, but for mass culture larger containers must be used. The indoor, 2 metric ton hatching tanks at Turkey Point are of 1-inch marine plywood; the inner dimensions of the tanks are 1 m wide x 1.2 m deep x 2 m in length (Figure 14), and the insides are coated with white epoxy paint. We believe that non-toxic polyethylene or fiberglass tanks of similar volume would be superior to the wooden tanks we have used.

The Twenty-Ton Tanks

The outdoor tanks are of pre-cast reinforced concrete, the inside dimensions being 5 m long x 2 m wide x 2.1 m deep. They are sloped toward a 4-inch PVC valved drain at one end. A colorless semi-translucent fiberglass roof covers the tanks (Figure 15).

PINK SHRIMP CULTURE

Recognizing, Collecting and Transporting Gravid Females

Although it has been said that Japanese biologists have reared several generations of Penaeus japonicus in captivity, and P. occidentalis females are also reported to mature and spawn in captivity (Dr. Eric J. Heald, personal communication), this is not yet applicable on a commercial scale. For this reason shrimp (Penaeus) culture in the United States and elsewhere will have to rely for some time on wild stocks as a source of gravid females.

With pink shrimp, as with some other species in the genus Penaeus, it is necessary to obtain females only. The males have the convenient (for the culturist) trait of attaching spermatophores to the ventral surface of the females some time prior to the actual spawning (the females can become impregnated before the ova are fully mature). The sexual appendages of the males are called the petasmata (Figure 17) and are easily seen attached to the base of the first pair of swimmerettes or pleopods. In females, the external organ of reproduction is called the chelycum (Figure 17). Separation of "ripe" females from immature females and males can often be made more quickly by observing ovary condition through the dorsal surface of the abdomen. Female pink shrimp collected on the spawning grounds are usually impregnated, therefore, only females need be saved, and the males are discarded.

The nearly mature ovaries of pink shrimp are "glaucous" or gray, a color which darkens at maturity to a gray-green. Fully ripe ovaries are olive green. Other penaeids that we have seen show similar color change with ovarian maturation.

Females with the darkest and widest ovarian band should be selected. The color of the ovary is best seen in living shrimp when the abdomen is flexed slightly to expose the section of ovary under the thin muscle layer of the dorsal surface where head and tail join (Figure 18).

In south Florida, mature females may be found throughout the year, but in some months, especially December and January, only a few fully mature females can be found. In these months very few shrimp occur with green ovaries, but a number of females may have wide, pale pink to white-colored ovaries. Successful hatches can be obtained from such females, but larval survival is usually not as high, probably the result of poor yolk supply.

Females used for spawning at Turkey Point have been caught on the Sanibel and the Tortugas fishing grounds off southwest Florida by our chartered commercial shrimp trawlers. Skilled biologists accompany the crew. The charter should include the option of fishing where and for as short a drag (trawl towing time) as the biologist wishes. The best females are sometimes located outside the best grounds for commercial trawling. As a general rule, it is important that the towing time not exceed 60 minutes, because of damage done to the females if drags are longer than this. When gravid females are very abundant, sufficient numbers may be collected with the "try net," a miniature trawl used for locating shrimp before actual fishing begins. This is easier than fishing with big trawls and it results in fewer damaged shrimp.

When the shrimp are brought aboard the vessel they are quickly placed in 20 gallon plastic drums containing fresh, aerated seawater. Dead shrimp settle to the bottom of the drums, and the live shrimp swim or at least show some movement of the swimming appendages. The live shrimp are then rapidly examined for ovary development. If enough are available, about 60 to 100 gravid females would be selected for a 10-tank indoor hatching. The most lively and fully developed females are transferred to a second set of drums containing fresh seawater. These drums should be provided with close-fitting lids and dependable aerators. The water in the drums should be cooled to about 20°C during hot weather. This may be done by floating ice in plastic bags or plastic jugs in the drums, but care must be taken to monitor the water temperature frequently. This method can only be tolerated for small operations taking only a single night for fishing and a few hours for return to the hatchery. We have successfully transported up to 55 large females per drum for as long as 18 hours using this method.

When larger numbers of females are being handled, especially during warm weather, it is prudent to provide special holding tanks equipped with thermostatically controlled refrigeration coils and heavy duty aerators. We have used two such saltwater cooling and aerating units mounted on 600 l fiberglass tanks. These units operate on 110-115 volts. One such tank unit is filled with clean seawater and left on the truck at dockside with an extension cord to a local power supply. The thermostat is set to maintain 20°C. The second unit is placed aboard the trawler with a portable electric generator for power. The tank is filled upon arrival on the grounds, and aeration and cooling are begun. First sorting

to remove dead, injured and immature or male shrimp from the drums proceeds as above. In the drums, much of the excess mucous and debris is removed from the shrimp. As the best females are isolated, they are placed in the large tank. Cooling should proceed slowly enough to avoid subjecting the shrimp to thermal shock, and aeration should saturate the water. The carrying capacity of the tank is great enough to accommodate up to 500 females for periods of 8 hours or longer if power does not fail. Back-up power supplies are advisable. When the vessel returns to the dock the shrimp are quickly transferred by dipnet to the truck tank. Aeration and water temperature control must continue.

SPAWNING IN 2-TON TANKS

About one week before the expected date of arrival of a shipment of gravid females, the tanks are prepared for spawning. The inner tank surfaces are washed thoroughly with fresh water, allowed to dry, and, if slime has formed on inner tank surfaces, these may be swabbed out with 70-75% ethyl alcohol and rinsed again with tap water. No soap or detergent should be used.

The tanks are then filled to 70% of capacity with filtered seawater at 30-32 ppt salinity from the cleanest possible source. Water from Biscayne Bay, adjacent to the Turkey Point laboratory, has usually been suitable for hatching after a single pass through a 5µ fibrous filter. We accomplish filling and filtration by having a filter housing installed in the discharge hose from a 2.5 hp gasoline powered pump which lifts the water from the outdoor source. Occasionally, during rainy or windy weather, the bay water contains considerable quantities of organic materials and sediments in suspension.

To accommodate this, we have built a series of filters into the main water supply line to the hatching tanks. This filter series consists of a pressure-proof "pre-filter" containing dacron "wool" followed by a series of fibrous filters having 50, 25, 10 and 5 μ pore sizes. The dacron fibers in the "pre-filter" remove coarse material effectively and can be replaced economically; the graded fibrous filters remove fine particles. The filter holders should be transparent so that hatchery personnel can estimate when the filter elements become clogged with debris. If, after such filtration, contamination is still suspected, it may be necessary to haul suitable water in clean fiberglass tanks from a more distant source. This is very costly and emphasizes the wisdom of careful site selection before investing large sums in hatchery facilities.

Once the tanks are filled with suitable water they should be covered (e.g., with plywood sheets) and allowed to stand in the dark until the female shrimp are introduced. This aging of the water under darkened conditions seems to inhibit microbial growth and may also be used as the pre-warming period if the water happens to be below optimum temperature for spawning. We accomplish this pre-warming by setting the hatchery room temperature control at the desired level; this adjustment is usually accomplished within the week prior to spawning. Five new airstones weighted with small glass (electrical) insulators are placed in each tank.

When the female shrimp arrive at the hatchery, the lids are removed from the tanks. Aeration is then started at a very low level. If precision airline meters are used, each airstone should be set to produce about 0.1 cfm flow. If no flow meters are available each air stone should be regulated so that a very thin column of small bubbles (barely visible as they break the water surface) is emitted.

As soon as the females arrive at the hatchery, the biologist should quickly place the best of them in buckets of fresh seawater. At this time they should be examined to see if they are suitable for spawning. If they appear to be healthy and of good quality they should be rinsed again in a separate bucket of clean seawater and put in the spawning tanks. Four to six females are usually put in each 2-ton tank. In the event that a mixed lot (containing some females of high quality and some of poor quality) is obtained, it may be desirable to have 10 plastic buckets standing by. The biologist can then divide the shrimp into groups including some shrimp of both qualities in these buckets before putting them in the tanks. This gives the greatest chance for successful spawning in all tanks.

Once the tanks are stocked the hatchery room should be completely darkened. Light appears to inhibit the onset of spawning, and spawning may be interrupted by light. Light may be used to check periodically for spawning, but these observations should be made with a low-intensity narrow beam flashlight directed so that it does not shine directly on the shrimp.

Spawning is indicated by unusually active swimming behavior, often in an upright or vertical posture, accompanied by very rapid beating of the swimming appendages called pleopods. Such behavior is a reassuring sign, and the experienced operator will not disturb the shrimp again until the following day. The eggs are cast out in a trailing "cloud" behind the actively swimming females. Spawning by one shrimp seems to initiate spawning by others, and all the shrimp that will spawn often do so within an hour or two after the first spawning.

If necessary, the presence of eggs in the tank can be ascertained by examining a half-liter beaker sample from the tank. Small air bubbles can

be mistaken for eggs so one must allow time for such bubbles to rise to the surface. The whitish, newly spawned eggs will slowly settle to the bottom of the beaker. We place the beaker on a dull-finish black surface to enhance contrast. Particles thought to be eggs can be pipetted onto a depression slide and examined under a microscope. Sampling of this kind must be kept to a minimum lest the spawning be interrupted.

Females are left in the tanks for 2 to 4 hours after confirmation that eggs have been spawned. At the end of that time the females are removed to prevent them from eating the eggs which will have become concentrated on or near the bottom of the tank. The females also produce quantities of mucous and fecal matter which increases the risk of early bacterial contamination. As the females are removed, some will be found not to have spawned. If it seems desirable, these may be put in other tanks in the hope that they will spawn the following night. Some pink shrimp have spawned up to three days after arrival at the hatchery but usually those that fail to spawn the first night do not do so later. It is highly undesirable to put unspawned shrimp into tanks which already contain eggs. Delayed or prolonged spawning can result in larvae of widely different stages, and this will complicate feeding.

In the event that gravid females are scarce and every available egg is needed, these "slow starters" may be put in 5 gallon carboys, 1 per carboy. If they spawn, the eggs can be transferred to 200 l polyethylene tanks, given suitable aeration, and these can be reared in a normal fashion.

We have spawned shrimp successfully in 15 gallon aquaria and in inverted carboys; a single female placed in each carboy, or two females in each 15 gallon aquarium. With inverted carboys, a circular piece of large-mesh plastic screen must be placed in the lower part of the carboy to prevent the females from becoming wedged, head-downward, in the neck of the bottle.

There is some advantage to the use of small spawning containers for individual shrimp, since quick estimates of total egg numbers can be made, and a decision as to the adequacy of their fertility can be made before they are consigned to larger tanks for hatching. Eggs of about the same age can be combined from several jars or aquaria to achieve optimum hatching density in larger containers. If the eggs are abnormal they can be discarded without loss of the prepared hatching tank water. If these smaller containers are placed on racks slightly above the larger hatching tanks, their contents can be emptied into the larger tanks by gravity flow. We have hatched as many as 120,000 nauplii from eggs spawned in a 5 gallon carboy and transferred to a 2-ton tank. These produced nearly 70,000 postlarvae.

Each 2-ton tank can produce up to 400,000 (average 40,000) postlarvae at about three week intervals. Hence a 10-tank hatchery can be reasonably be expected to produce up to 500,000 stockable postlarvae each month, allowing an extra week for cleaning, refilling and bringing in new spawners. This production is suitable for a bait shrimp operation, but it is very demanding of the hatchery crew, since greater care is required in monitoring tank conditions and producing adequate food in separate facilities

SPAWNING IN 20-TON TANKS

A major difference between indoor and outdoor culture at Turkey Point, as elsewhere, is that the pre-filtered seawater in outdoor tanks is fertilized to produce a bloom of naturally occurring and artificially cultured phytoplankton as food for the larval shrimp.

Before the hatching operation begins the tanks are cleaned by wire brushing and high pressure water spray. No alcohol is used to disinfect the tanks. Water for hatching is taken directly from Biscayne Bay and filtered through a 25- fibrous filter. The salinity should be in the range of 28-32 ppt. Dilution may be necessary. In this event, tap water is added to achieve the above range of salinity. The tanks are filled to a depth of 1.3 m, equivalent to a volume of 13,000 liters. Ten weighted round airstones are placed to provide evenly distributed aeration from the bottom. No light-tight covers are used for the 20-ton tanks, and, except for the initial filtration, no attempt is made to minimize contamination. Female shrimp are handled as in the 2-ton tank method described above, except that larger numbers must be obtained. Between 10 and 30 females are put in each 20-ton tank. All are removed by dipnet the morning after spawning. A fine mesh dipnet is used to remove clumps of mucous. Accurate counts of eggs are virtually impossible to make in the large tanks, so the first population estimate is usually made upon the newly hatched swimming larvae. Ten 1-liter samples are taken at various places in the tank and their counts averaged and multiplied by 13,000 l to obtain an estimate of the total number of larvae.

Pre-dissolved fertilizers are added, beginning on the day of hatching, to obtain a phytoplankton bloom. We now terminate this enrichment of the growing medium when Artemia feeding is commenced during the first mysis stage in the interest of economy, but there is some evidence that fertilizer may benefit the tank environment throughout the mysis and postlarval development. For every metric ton (1,000 l) of water, 3.1 g of potassium nitrate (KNO_3) and 0.31 g of dibasic sodium phosphate ($\text{Na}_2\text{HPO}_4 \cdot 7\text{H}_2\text{O}$) are dissolved in fresh water and added to the tank.

As with indoor spawning, low aeration volumes are used during the first night when spawning takes place. However, aeration is increased to a vigorous rolling boil as soon as the eggs hatch. This requires about 0.4 cfm flow from each stone.

LARVAL DEVELOPMENT

Larval development proceeds in approximately the same fashion in indoor and outdoor tanks although low temperatures of early spring or late fall may prolong intervals between stages in outdoor tanks (Table 1).

The eggs hatch in about 14 hours into the first nauplius stage, which is the first of 11 larval stages (Table 1). Simplified drawings of each stage are presented in Figures 1 to 13. These figures are labelled to show the principal features used to distinguish each stage by microscopic examination, so that development of the larvae can be followed.

As mentioned, the larvae subsist on yolk material through the five nauplius stages, but they will quickly starve to death in the first zoea stage if adequate quantities of diatoms are not available. To prevent starvation of the larvae in indoor tanks, we carefully monitor larval development during the latter hours of the fifth nauplius stage, and when about 10% of the larvae have metamorphosed to the first zoeal stage we introduce diatoms. We are convinced that it is better to feed diatoms at densities of 10^7 - $20,000$ cells/ml in the spawning tank for maintaining better water quality in the tank and also minimizing mortality of larval shrimp after the metamorphosis into the zoeal stage. This urgent requirement for diatoms explains why preparations for this event must be started well in advance (Table 2).

On the day before the larvae are expected to metamorphose into the first mysis stage, Artemia hatching should begin. About 20 grams of Artemia eggs are hatched in facilities described below and added daily to each 2-ton tank. On an average, about 130,000 Artemia nauplii are produced from each gram of eggs. This produces a density of 2 Artemia nauplii per ml in a 2-ton tank.

Pink shrimp will metamorphose from the egg through all the larval stages to postlarvae in about 226 to 288 hours (Table 1). Four day old postlarvae (i.e., in the fourth day of the postlarval stage) can be transferred to ponds or can be shipped, but the shrimp can be held in the tanks longer than this, at least until they are 30 day old postlarvae. However, the mortality rate will increase rapidly beyond the 10th day of storage.

SHIPPING

Pink shrimp as young as 4 day old postlarvae can be confined at least 12 to 14 hours for shipping. They are packed in polystyrene foam shipping boxes (used for tropical fish), into which are placed double plastic bags (15 inches x 15 inches x 8 inches high when inflated). The day before shipping, the water level in the hatchery tanks is lowered to one-quarter full and the water is cooled to about 23°C by reducing the hatchery room temperature. Vigorous aeration is continued through the night.

The following morning as many of the shrimp as possible are dipnetted from the hatching tanks with polyethylene nets (345 μ mesh) to polyethylene tubs each containing 100 liters of clean water of the same salinity and temperature as that of the original tanks. The remaining

shrimp are collected by drawing the water from each culture tank into a seawater filled plastic tub containing a perforated basket lined with 345 μ netting. After drainage, the netting containing the postlarvae is lifted out, and the postlarvae are transferred to the 100-liter tubs. The number of shrimp in a 100 liter tub is estimated by stirring the water vigorously to get all the shrimp into suspension, and dipping up five 250 ml samples for counting. The numbers in these samples are averaged and multiplied by 100 l to estimate total numbers in a tub.

The shipping boxes are filled with desired numbers of postlarvae estimated volumetrically as described above. The shrimp being counted should be transferred by dipnet to a smaller tub or bucket of aerated water until the required number for each shipping box is obtained.

Each plastic shipping bag can be filled with 4 to 6 liters of clean (filtered if necessary) water from a 2-ton tank. The counted shrimp are dipped from the buckets with a coarse net to separate them from debris, and they are quickly placed in the plastic bags. At this time the temperature of the water in the shipping bags should be about 18-20°C.

The shrimp are chilled further, if necessary, by floating the bags in a water bath at 18°C until the bag contents equilibrate at 18°C. The bags are then placed in the shipping boxes and the bag tops gathered and loosely twisted until the air is expelled. An oxygen tube from an oxygen tank is then inserted through the twisted neck of the inner bag, and the bag is inflated until it almost fills the box.

The gathered neck of the inner plastic bag is then twisted tightly, doubled over in a loop, and wrapped with 2 rubber bands. Then the outer bag is closed and sealed in the same fashion to provide a double seal.

Two small plastic bags, each containing 150-200 g of ice cubes, are wrapped in 4 or 5 thicknesses of newsprint which prevents direct contact with the shipping bag, and they are then placed in opposite corners of the shipping box. This ice keeps the box interior cool during shipment, and this is especially important if the shipment must sit in the sun before or after arrival at destination. When the shrimp are shipped in this manner they usually arrive at their destination in water no higher than 20°C, even in the warmest weather and after travel time as long as 14 hours.

During early shipping trials, water was taken directly from the 2-ton culture tanks after filtering it through 111µ mesh nets. With this "old" water and densities of 10,000, 20,000, 30,000 and 50,000 postlarvae per box, mortalities of less than 5%, 10-50%, 70% and 80-90% respectively, occurred. Now, using fresh seawater, it is easily possible to ship 20,000 postlarvae per box with less than 5% mortality.

Upon arrival at the destination, the bags of shrimp are floated in the receiving water, preferably under shade, until the water in the bag is the same temperature as the receiving water. The postlarvae are tolerant of some change in salinity but probably should not be introduced into receiving water of salinity differing more than 5 parts per thousand (depending upon the size of postlarvae).

DIATOM CULTURE

When pink shrimp reach the sixth larval stage, the first or zoea (Figure 6), they must be given suitable diatoms as food. This stage is reached about 37-50 hours after the eggs hatch (Table 1). Hence cultures of diatoms must be started before gravid females are caught, and the two culture systems (shrimp and diatoms) coordinated carefully (Tables 1 and 2).

Diatom culture employs microbiological techniques, including great care with cleanliness. Procedures outlined in this section can be managed without formal training in microbiology; however, anyone about to undertake the cultures of diatoms for the first time is advised to consult a microbiology text and laboratory manual at the outset. A certain amount of digital skill must also be developed in the aseptic handling of glassware, pipettes, inoculating needles, syringes, microscope, counting chamber, and cultures.

Three stages are involved in diatom culture. These are (1) maintenance of stock culture, (2) growth of starter cultures and (3) growth of mass cultures.

Starter cultures of suitable diatoms can be purchased from various university laboratories or from private stocks maintained by organizations already engaged in shrimp rearing. Usually these will be growing on agar slants in test tubes. Our collection has contained as many as 20 species and varieties of diatoms but experience has shown that only a few such as Cyclotella nana and Phaeodactylum tricornutum are needed to obtain satisfactory results.

Stock cultures should be maintained permanently. This requires only a small room, but it must be kept exceptionally clean to reduce the problem of airborne contaminants such as mold spores and bacteria. The dimensions and characteristics of the room are determined by the growth requirements of the diatoms and the amount of space available. The interior of the culture room in the Turkey Point facility is 6 x 10 feet.

Lighting

Diatoms are photosynthetic plants, utilizing light to convert inorganic nutrients to cell components. The color spectrum, intensity and photoperiod of the light are important, sometimes limiting, in the growth of diatoms, but growth of most species of interest here can be obtained over a wide range of light values, and without intricate light measurements and automatic controls.

Continuous lighting is suitable. Fluorescent fixtures are satisfactory. White paint and aluminum foil on wall surfaces and shelf boards can be positioned to reflect light on the diatom cultures. The amount of light is rapidly reduced with distance, according to the inverse-square law. Hence, the intensity of light 2 feet from the source is one fourth of the intensity at 1 foot from the source.

Temperature Control

The species of diatoms cultivated in the Turkey Point facility grew well at $22 \pm 2^{\circ}\text{C}$. A reverse cycle air conditioner of 5,000 to 7,000 BTU cooling and 3,000 to 4,000 BTU heating capacity has been adequate to control temperature in the stock-culture room about 6 ft wide x 10 ft long x 12 ft high.

Sterilization Equipment

Stock cultures of diatoms must be maintained under aseptic conditions in sterile media. Starter cultures (1-liter flasks and 5-gallon carboys) should also be grown in sterile media, but pasteurization is adequate for tanks (15 to 2,000 gal.). Heat-sensitive vitamin solutions should be sterilized by filtration. Any commercial sterilizer or autoclave large

enough to enclose a 5-gallon carboy is suitable for use in the preparation of diatom media. Electrically powered upright sterilizers installed at floor level are more conveniently loaded with carboys than the common horizontal-types mounted on stands. In addition, the upright types available from several scientific supply companies, are less expensive.

Pasteurization of large volumes of seawater for growth of diatoms in tanks can be accomplished by passing the seawater through a glass-lined electrical water heater. Temperature and flow rate should be adjusted so that water emerging from the tank is at 180°F and water residence time in the heater is no less than 45 minutes.

Membrane filters are an effective means of eliminating bacterial and fungal contaminants from heat-sensitive solutions such as vitamin mixtures. The filter system includes membrane filters (0.45 μ pore size), filter holder, and a pressure or vacuum pump. Filter systems are available in a large array of sizes, quality and costs. The small inexpensive types are adequate. Consult scientific supply catalogs to determine the system best adapted to need and budget.

Stock Cultures

Approximately 25 species of diatoms were grown during the course of work on the pink shrimp. The most extensive trials were made with Cyclotella nana, Cylindrotheca sp., Chaetoceros simplex, Skeletonema costatum and Phaeodactylum tricornutum. Other species are probably suitable, but early success with these species (particularly with Cyclotella nana) eliminated the need for more extensive testing.

Maintenance

Stock cultures were maintained and grown in Guillard's F medium (see Table 3). The diatoms used for shrimp feeding should be subcultured frequently to maintain them in a vigorous state of growth so that starter cultures can be established within a reasonable time. The frequency of subculture (3 to 7 days) is determined by the growth rate. Reference cultures may also be desirable and many species can be held in an ordinary refrigerator at $6 \pm 2^{\circ}\text{C}$ for 30 to 45 days. This reduces the time and effort required to keep several stock cultures on hand.

Growth

The growth rate of a diatom culture is illustrated by the curve in Figure 19. A check of growth can be kept on diatom cultures by constructing such a curve. A convenient method of determining the numbers of diatoms at any given time is the use of a counting chamber viewed through a microscope. Chambers for counting blood cells are useful for diatoms in the size range of 15 to 30 μ . A textbook, laboratory manual, and instructions furnished with the counting chamber should be consulted for details of the technique.

Optimal growth rate and minimal growth lag can be maintained by repeated or continuous inoculation of diatoms in the logarithmic (log) phase of growth. In general, the length of the lag phase (i.e., between inoculation and beginning of logarithmic growth phase) will be extended by (1) less than the optimum number of cells in the inoculum, (2) slow-growing old cultures from the stationary or log death phase and (3) refrigerated cultures.

Cultures of Cyclotella nana started with 5% inoculum (5×10^5 cells/ml) from a culture in the log growth phase reached "full bloom" (1×10^7 cells/ml) in 3 days. Cultures started with 0.1% inoculum (1×10^4 cells/ml) required up to 14 days to reach "full bloom".

Production

Two methods of producing diatoms in quantity have been used in our facility, batch-culture and continuous flow (Figure 20). Batch culture is the simpler, but it requires much more work than continuous flow. The beginner will do well to master batch culture before attempting continuous-flow techniques. The number of batch or continuous-flow cultures (Figure 20) must be adjusted to feeding needs (Table 2).

Feeding Shrimp Larvae

The volume of diatoms to be placed in the shrimp tanks is based on the volume of water and on the larval density. The volume of diatoms fed on successive days is adjusted to maintain about the same concentration as that initially fed (about 5×10^4 cells/ml to 7×10^4 cells/ml).

Feeding of diatoms is continued by us through the third mysis stage or for about 11 days. When Artemia are introduced they consume diatoms also. Therefore, it is prudent to wait until 10-20% of the shrimp are in the mysis stage, before stopping diatom feeding. The reader should consult Table 1 for the derivation of a firm feeding schedule.

Once the hatchery operator becomes skilled, fairly accurate estimates of diatom concentration can be made from coloration of the water. However, cell counts should always be made periodically, particularly at critical stages, because waste material and other sources of water discoloration can be misleading.

BRINE SHRIMP CULTURE

At Turkey Point, brine shrimp (Artemia salina) are hatched in two sizes of containers. The smaller ones are 5-gallon glass or polypropylene carboys with the bases removed. They are set, neck down, in beveled 8 in. holes cut in a sturdy table made especially for this purpose. The necks of the carboys are plugged by a rubber stopper pierced by two short lengths of glass tubing. A hose to one of these supplies aeration from a near-by air line; a hose to the other serves as a drain. The drain hose is kept closed with a pinch clamp. About 20g of brine shrimp cysts ("eggs") are placed in 9 liters of water in each carboy (= 3.3 grams/l) and aeration is brought to a rolling "boil".

The large hatching containers are 200-liter polyethylene barrels. These are drained by a 23 mm plastic hose attached to the lower side of the tank. The barrels rest on platforms built to cause a slight lean of the tanks toward the drain pipe. Aeration is supplied by means of a weighted airstone. If the drain valves are recessed in the barrel sides, it is necessary to introduce air there also, to prevent accumulation of brine shrimp nauplii in the recess. About 200-300 g of cysts are placed in about 100 liters of water in this larger container.

The large containers are much more efficient as brine shrimp hatcheries than the carboys. The choice of which to use depends on the quantities of brine shrimp required.

Best brine shrimp hatching results are obtained when the salinity of the water is between 30-32 ppt and the temperature is maintained at 27-29°C. Aeration must be vigorous at all times during hatching to prevent the cysts from settling to the bottom of the containers.

The nauplii begin to emerge about 18 hours after the above conditions are established. In about 24 hours most of them have emerged. After this time the air is shut off for about 10 minutes to permit the shells of cysts to float to the surface. The containers are then drained into buckets. The nauplii flow out under the floating mass of empty cysts. When the dark, unhatched cysts begin to appear in the drain, most of the nauplii will have drained off. The hatching containers are then rinsed, refilled, and recharged with cysts for the next hatch.

Although Artemia will hatch if the cysts are put directly in the shrimp rearing tanks, as is commonly done, especially in outdoor tank culture, the rapid accumulation of empty cysts can lead to a deposition of a layer of decomposing material on the tank bottoms. There is also a tendency for a large portion of the unhatched cysts to cling to the wet tank walls above the level of standing water, and not hatch. This is wastefully expensive.

When outdoor temperatures permit, Artemia can be hatched in the 200 liter tanks positioned above and adjacent to the large outdoor shrimp hatching tanks. The elevated position of the Artemia tanks permits direct gravity flow of the newly hatched nauplii into the shrimp growing tanks.

Artemia nauplii can be kept alive without feeding for about 2 days in aerated water. If they are produced in excess of daily needs, they can be frozen for use as emergency food supply.

We have used Artemia from several different areas of the western United States and Canada. The best hatching results have been obtained using eggs from the San Francisco Bay area. These have about 39×10^4 cysts per gram, and at the temperature, hatching time interval and salinity used, about 50 percent will hatch. About 10 to 12 percent more nauplii can be obtained by allowing several more hours of hatching time, but the added production is not justified in large scale operations.

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While the aim of this manual is simplicity, with a format free of literature citations for work contributed by others, we are well aware of our debt to the pioneers in the field. Thus we want to express our gratitude to the "father of modern shrimp culture," Dr. M. Fujinaga, of Japan, whose 30 or more years of patient research and publication paved the way for recent world wide efforts in the field. Similarly, we acknowledge our debt to Dr. Robert R. L. Guillard, Woods Hole Oceanographic Foundation, for equally extensive work in culture of marine diatoms. Without his patient research, mass culture of marine diatoms and hence of shrimp might not be possible.

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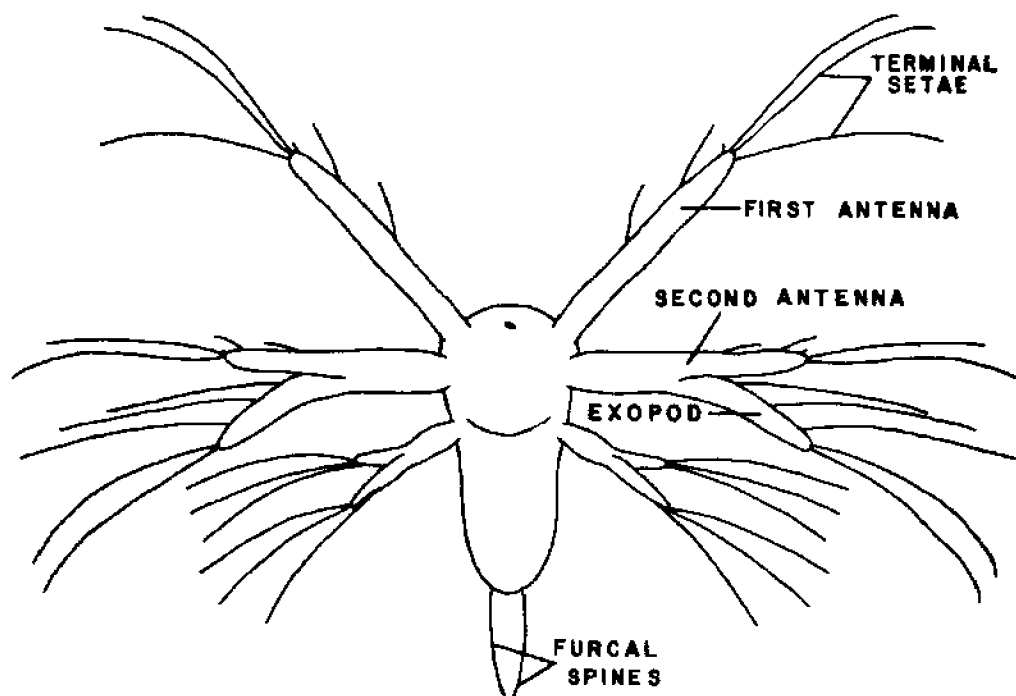
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A P P E N D I X A

FIGURES

FIRST NAUPLIUS VENTRAL VIEW



LATERAL VIEW

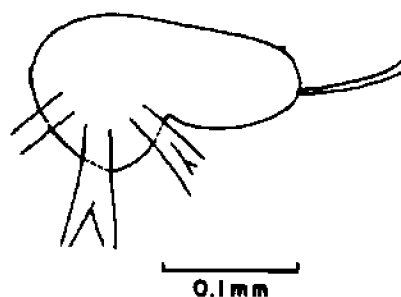
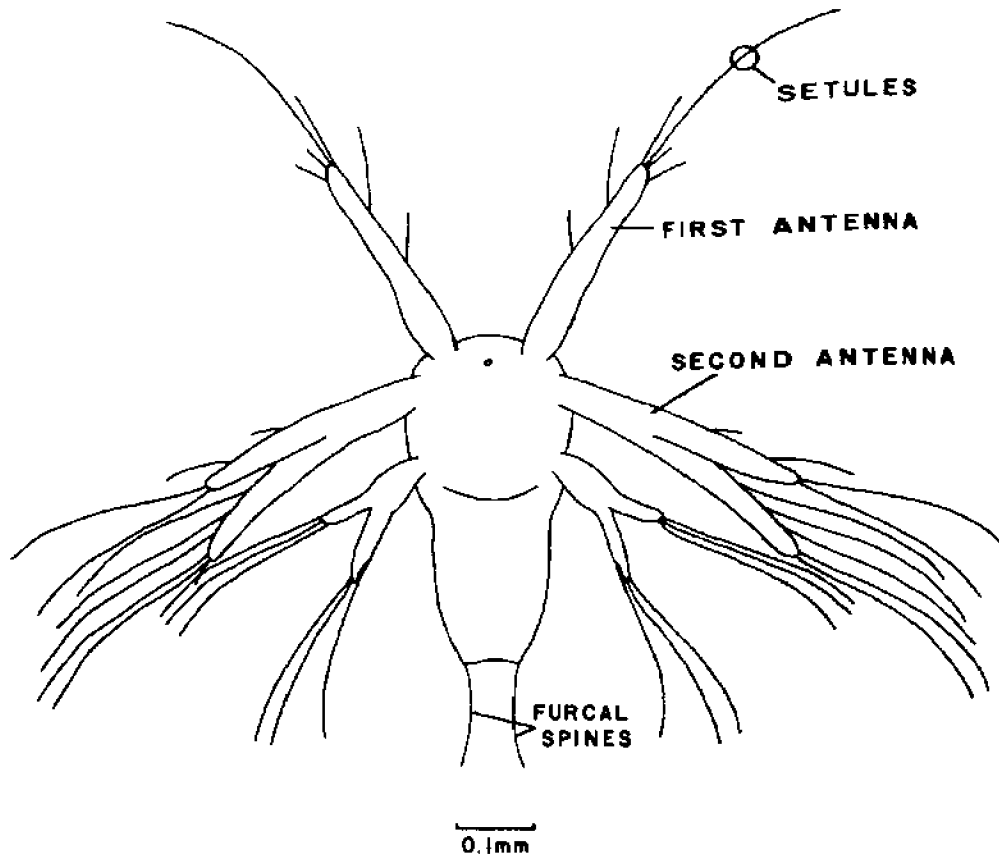


Figure 1.

Nauplius I (= N1)

- Body length, approximately 0.40 mm, exclusive of furcal spines
- Body width, approximately 0.20 mm, at widest part
- The tips of the 2 furcal spines are slightly flexed toward each other, and the body portion between is rounded (convex) toward them
- Has 3 long terminal setae on first antennae
- Has 3 long lateral and 2 long terminal setae on exopod of second antennae; this exopod adds setae at each molt, providing a useful characteristic

SECOND NAUPLIUS
VENTRAL VIEW



ENLARGED SECTION
OF SETA SHOWING SETULES

Nauplius II (= N2)

Figure 2.

- Body length, approximately 0.45 mm
- Body width, approximately 0.20 mm
- Setules appear on longer setae -- difficult to see under low (40X) magnification
- The tips of the 2 furcal spines are now flexed slightly away from each other, and the body portion between them is slightly concave toward them
- Has 1 long, 1 moderate and 1 short terminal setae on first antennae
- Exopods of 2nd antennae now have 6 setae, 3 long lateral, 2 long terminal and 1 short terminal

THIRD NAUPLIUS
VENTRAL VIEW

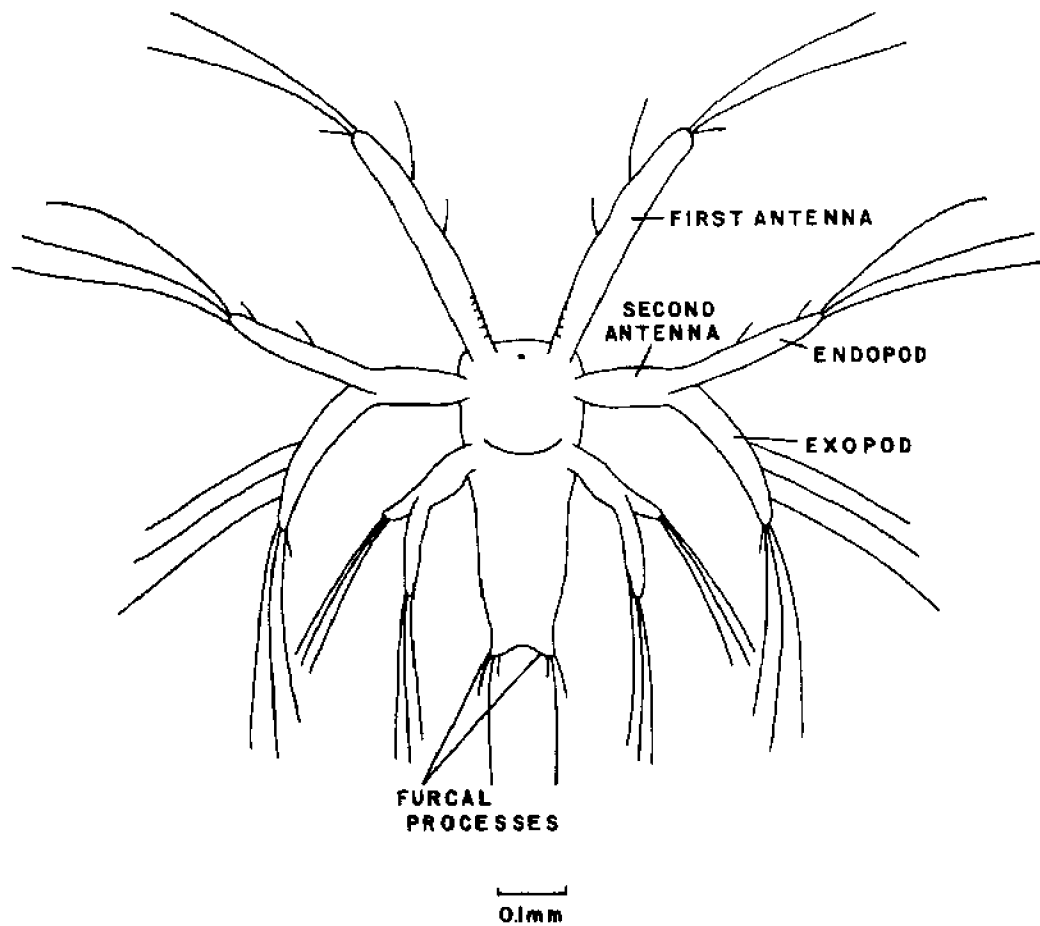


Figure 3

Nauplius III (= N3)

- Body length, approximately 0.49 mm
- Body width, approximately 0.20 mm
- 2 distinct furcal processes with 3 spines each
- First antennae have 2 long and 1 short terminal setae and bases of first antennae show trace of segmentation which is often difficult to see (experiment with lighting)
- Exopods of 2nd antennae now have 7 setae; 3 long laterals, 3 long terminals and 1 short terminal
- Endopods of second antennae have 8 long terminal setae versus 2 in N2

FOURTH NAUPLIUS
VENTRAL VIEW

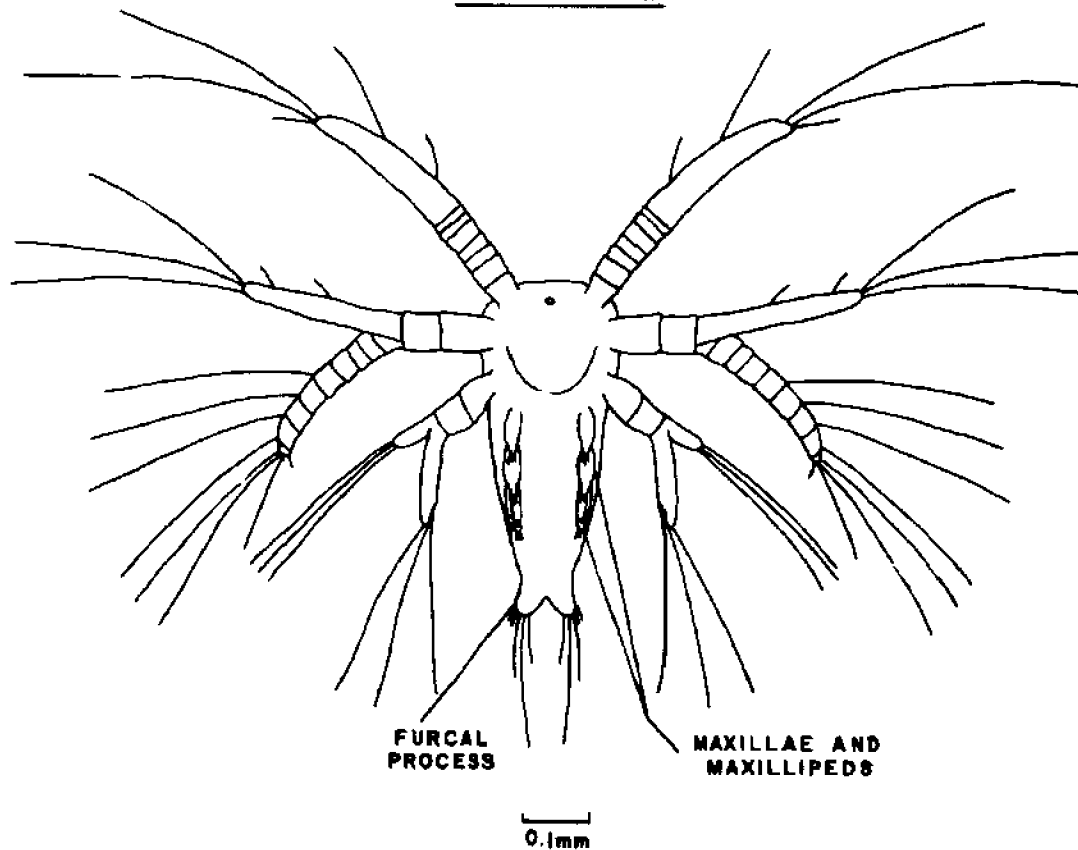


FIGURE 4

Nauplius IV (= N4)

- Body length, approximately 0.55 mm
- Body width, approximately 0.20 mm
- Each furcal process now has 5 spines
- Exopods of 2nd antennae now have 8 setae; 4 long lateral, 2 long terminal, 1 moderate terminal, 1 short terminal
- Segmentation of appendages appears; this is often indistinct; the best criterion is to look for indentations along the margins of the appendages
- The maxillae and maxillipeds (mouth parts) appear but are difficult to see clearly

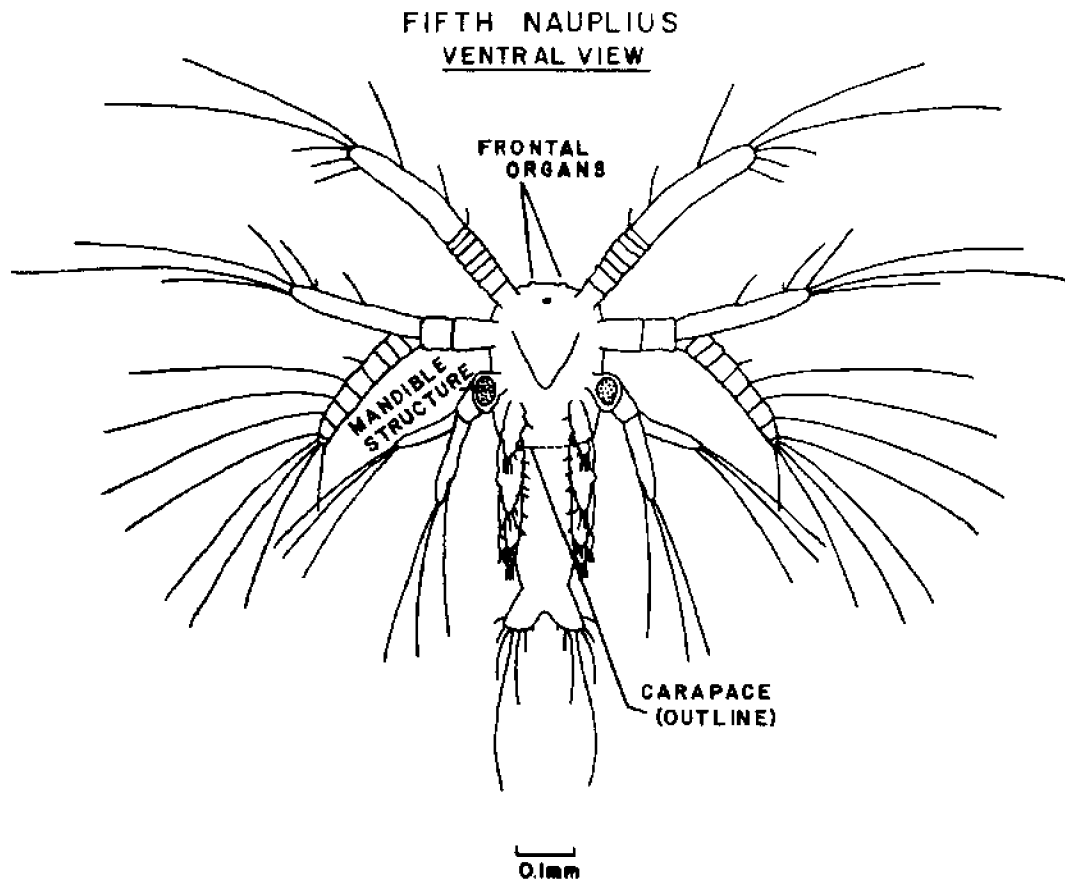


FIGURE 5

Nauplius V (= N5)

- Body length, approximately 0.61 mm
- Body width, approximately 0.20 mm
- Body more or less depressed
- Each furcal process now bears 7 spines
- There are obvious differences in setation between first and second antennae
- Swollen, knobby structures at the base of the mandibles are present
- Frontal organs present but difficult to see
- In the dorsal view, the outline of the developing carapace can be seen under the cuticle (dotted line in drawing)

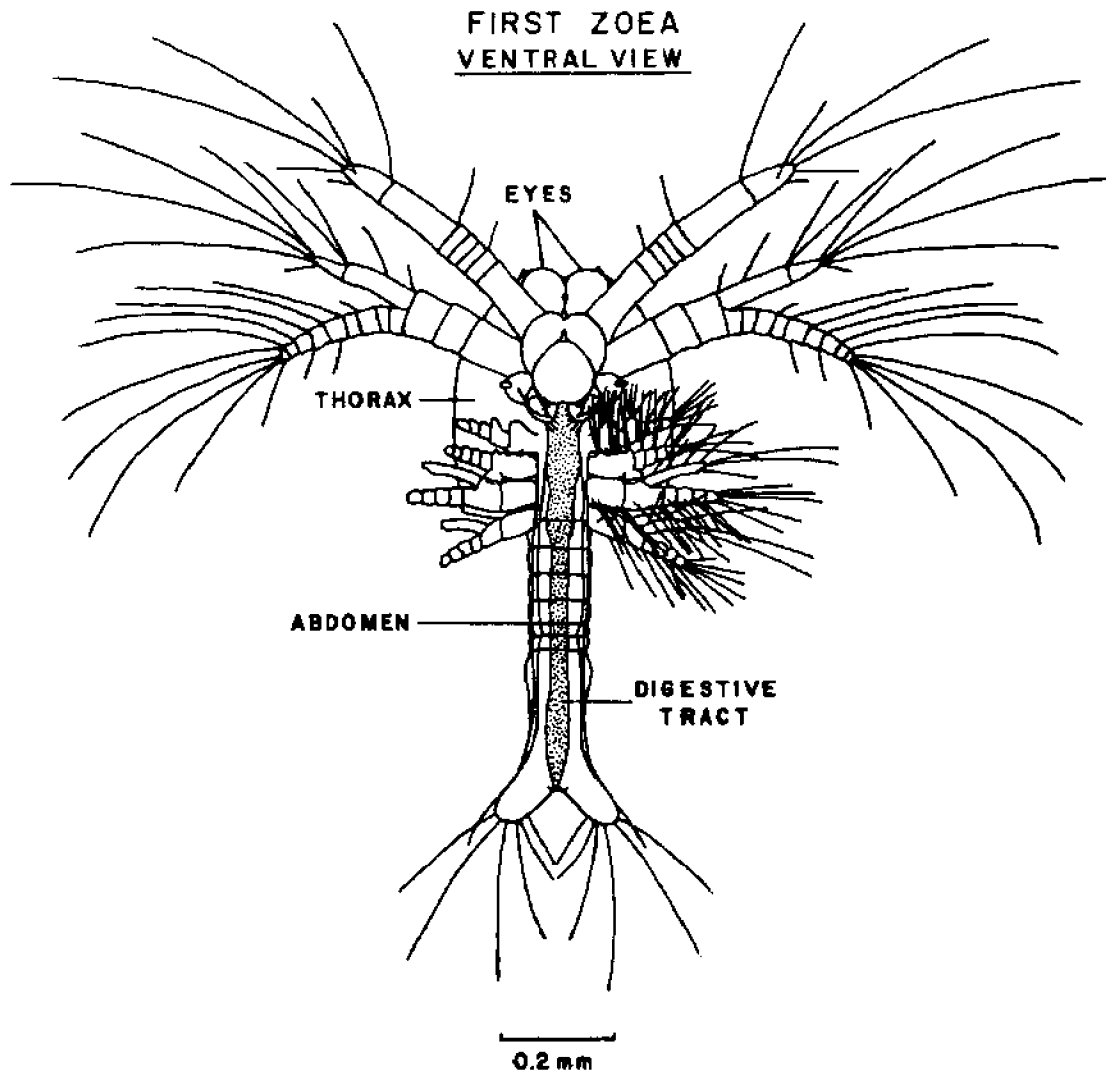


Figure 6.

Zoea I (= Z1)

- Body length, approximately 1.00 mm
- Body width, approximately 0.49 mm at widest part of carapace
- Eye present, sessile
- A radical change has occurred and the body is now clearly divided into 2 parts, the carapace or thorax (the "head") and abdomen (the "tail"); the difference from N5 is so obvious that the distinction can be made with the naked eye
- Digestive tract runs from mouth to anus at posterior end of body; food can be seen in it if larvae are feeding

SECOND ZOEAL
DORSAL VIEW

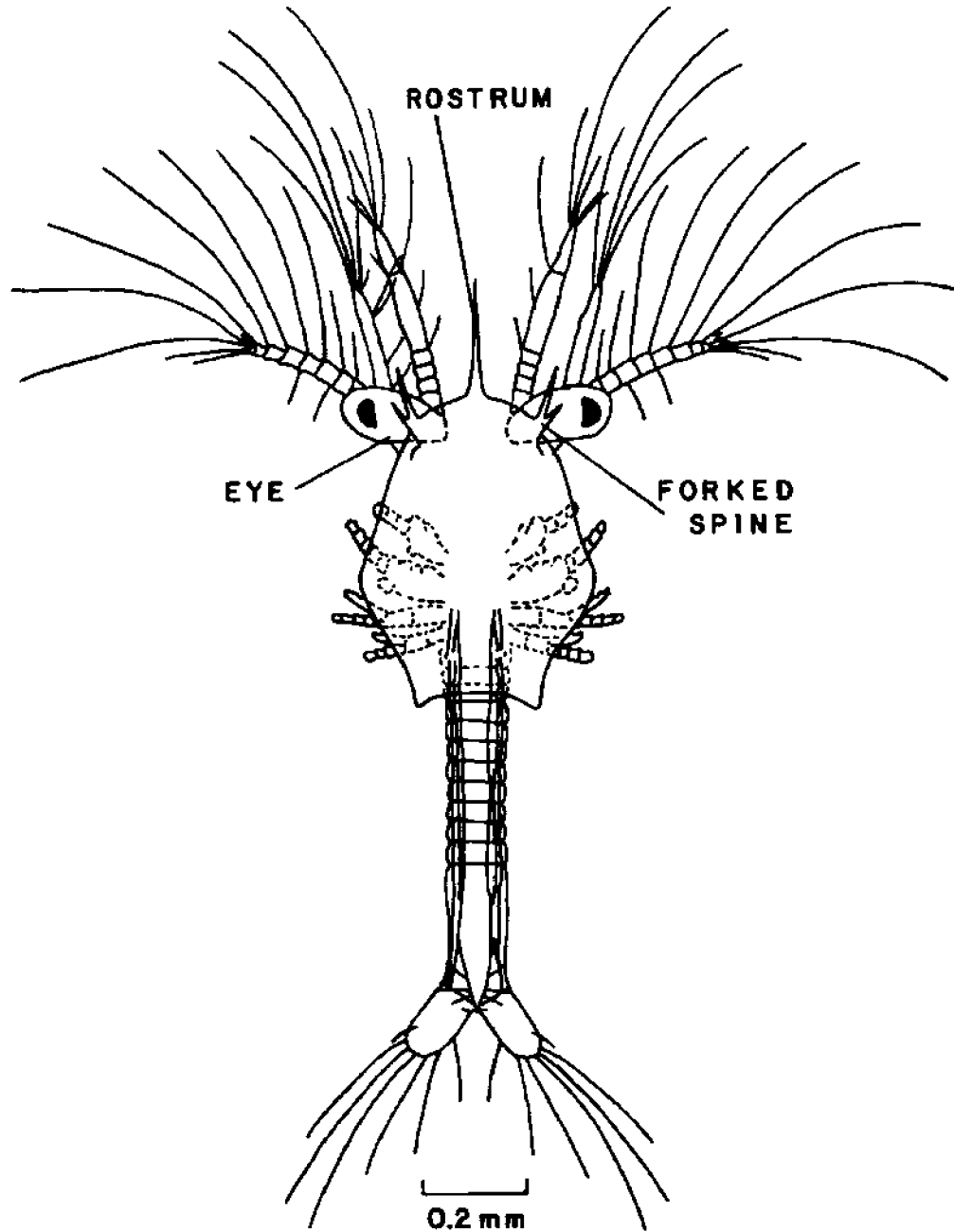
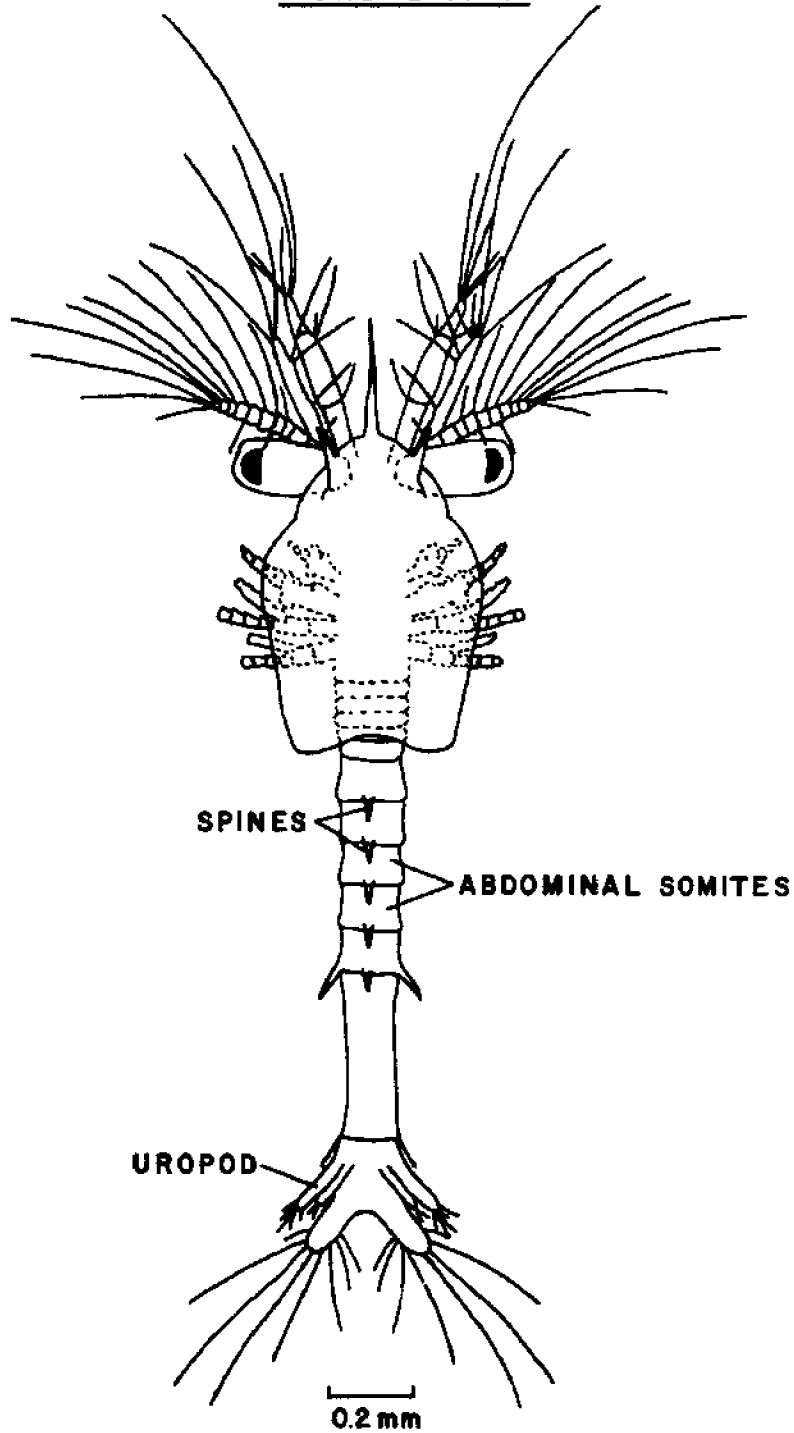


Figure 7.

Zoea 11 (= 22)

- Body length, approximately 1.9 mm
- Presence of stalked eyes, free from the carapace
- Rostrum present as well as forked spines above the eyes

THIRD ZOEAE
DORSAL VIEW



Zoea III (= Z3)

Figure 8.

- Body length, approximately 2.7 mm
- Appearance of a pair of biramous (doubly-branched) uropods
- Spines appear on abdominal somites (segments)

FIRST MY SIS **LATERAL VIEW**

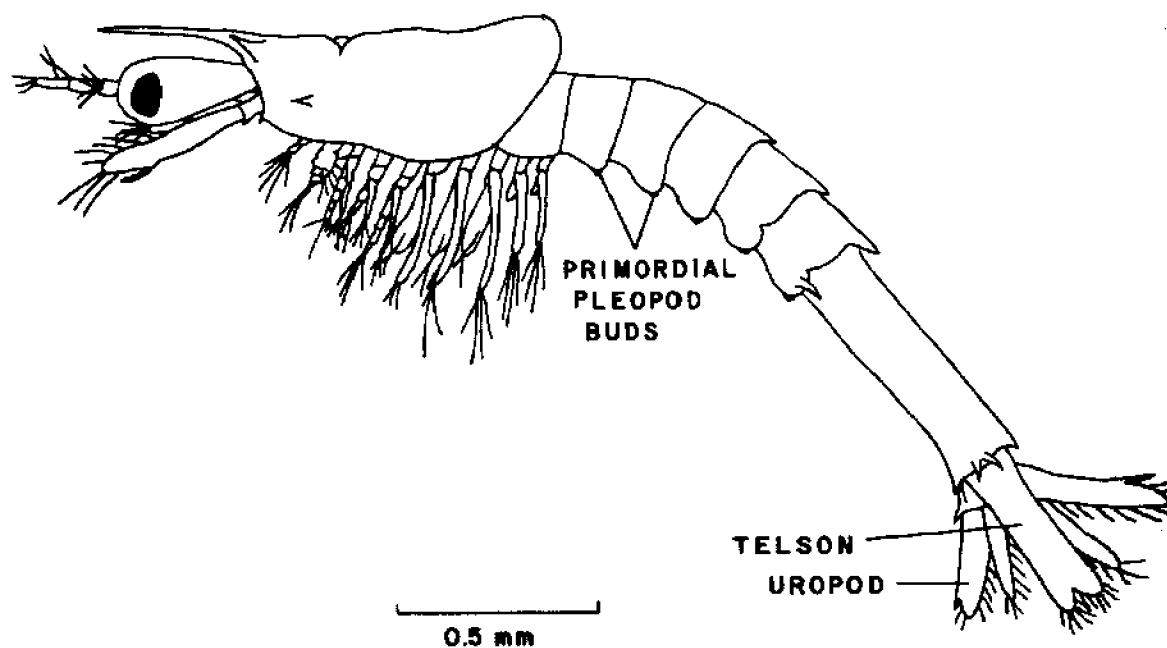
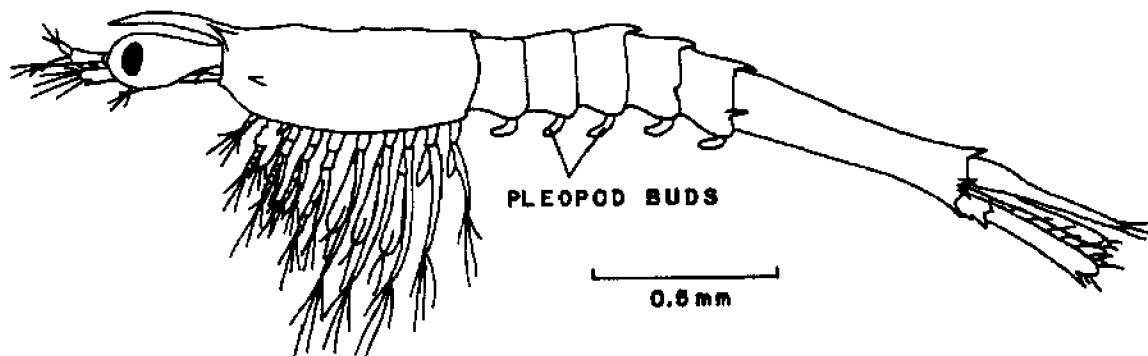


FIGURE 9

Mysis I (= M1)

- Body length, approximately 3.4 mm
- Now shrimp-like structure
- Development of a telson ("tail fan") and uropods, with great reduction in size of long spines which are present on the furcae in stage Z3
- Primordium of pleopod buds (swimming appendages) on the first 5 abdominal segments

SECOND MY SIS
LATERAL VIEW



DORSAL VIEW

FIGURE 10

Mysis II (= M2)

- Body length, approximately 4.0 mm
- Pleopod bud present, unsegmented
- Notch in end of telson is shallower than in M1 (when viewed dorsally)

THIRD MY SIS LATERAL VIEW

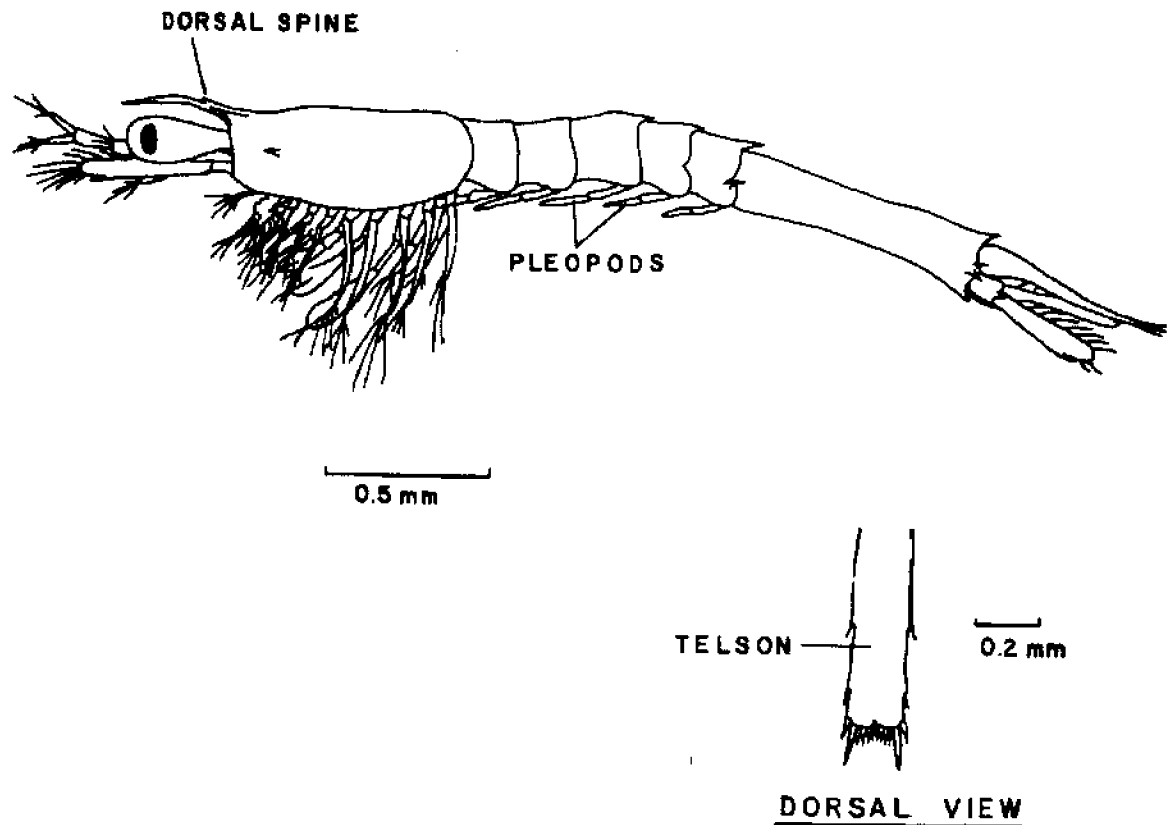


FIGURE 11

Mysis III (= M3)

- Body length, approximately 4.4 mm
- Longer, segmented pleopods
- Appearance of first dorsal spine on rostrum
- Notch in end of telson is even shallower than in M2 (when viewed dorsally)

FIRST POSTLARVA
LATERAL VIEW

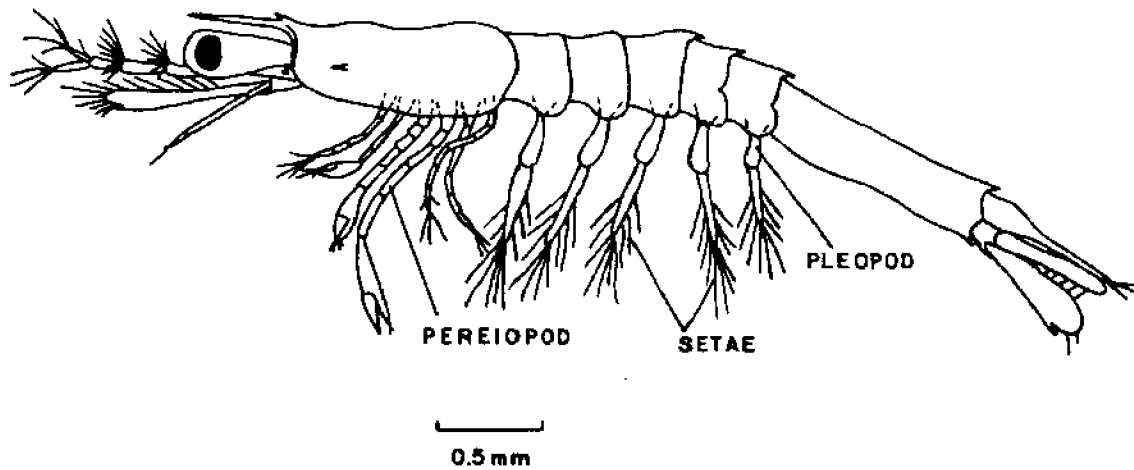


Figure 12.

Postlarva I (= PL I)

- Body length, approximately 4.8 mm
- Swimming setae present on pleopods
- Pereiopods have lost their exopods in most cases

SECOND POSTLARVA
LATERAL VIEW

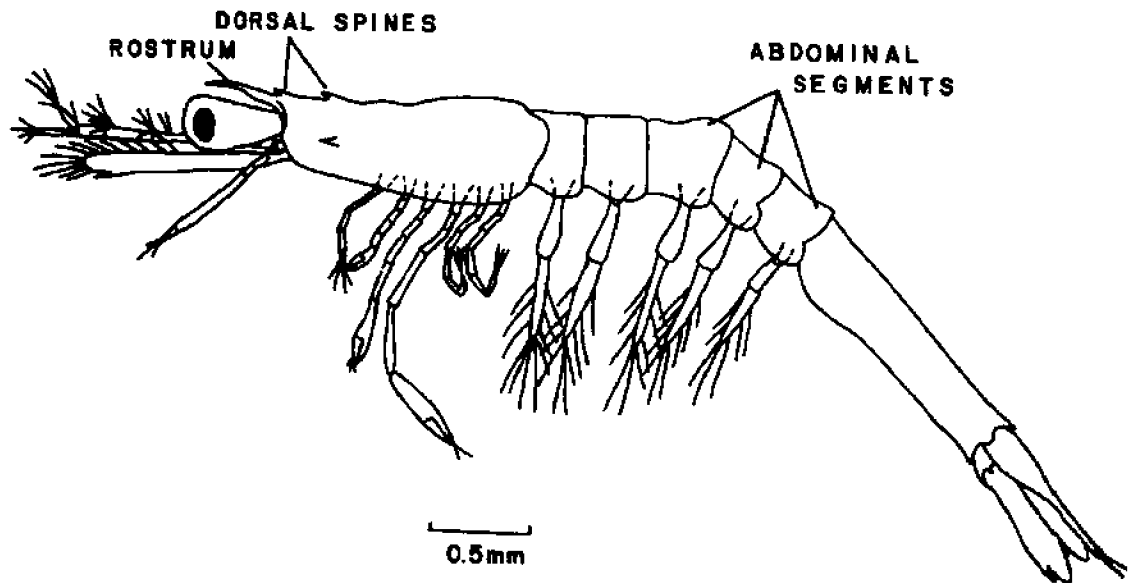


FIGURE 13

Postlarva II (= P1 2)

- Body length, approximately 6.6 mm
- Appearance of second dorsal spine on rostrum
- Rostrum no longer extends beyond the eye stalk
- There are no spines on the third through fifth abdominal segments



FIGURE 14
Two-ton indoor hatching tanks

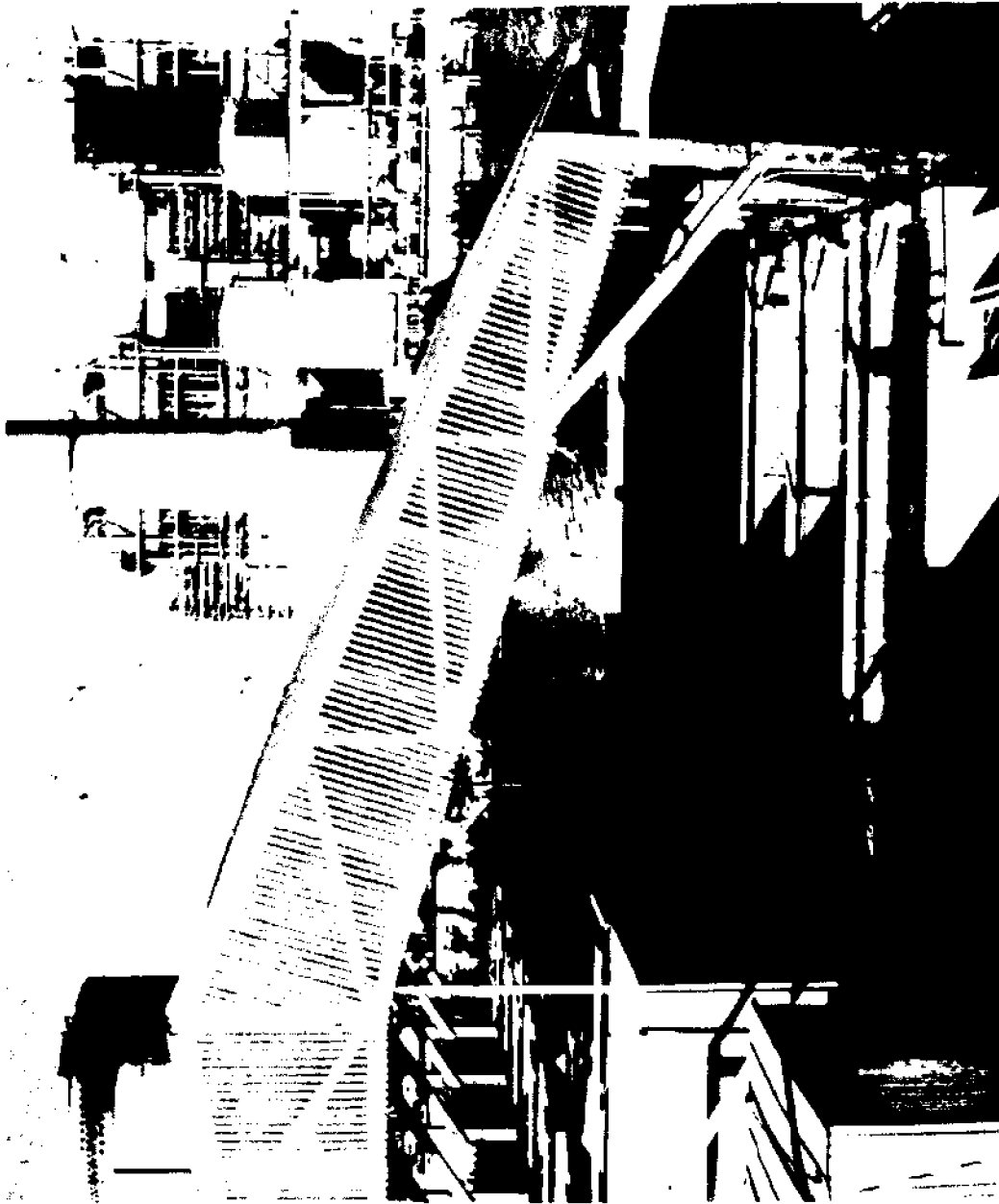


FIGURE 15

Twenty-ton outdoor hatchling tanks



FIGURE 16
Mass cultures of phytoplankton being grown under
lights for feeding larval shrimp

DIFFERENCES IN STRUCTURE AND LOCATION OF
PINK SHRIMP (Penaeus duorarum) GENITALIA

VENTRAL VIEW

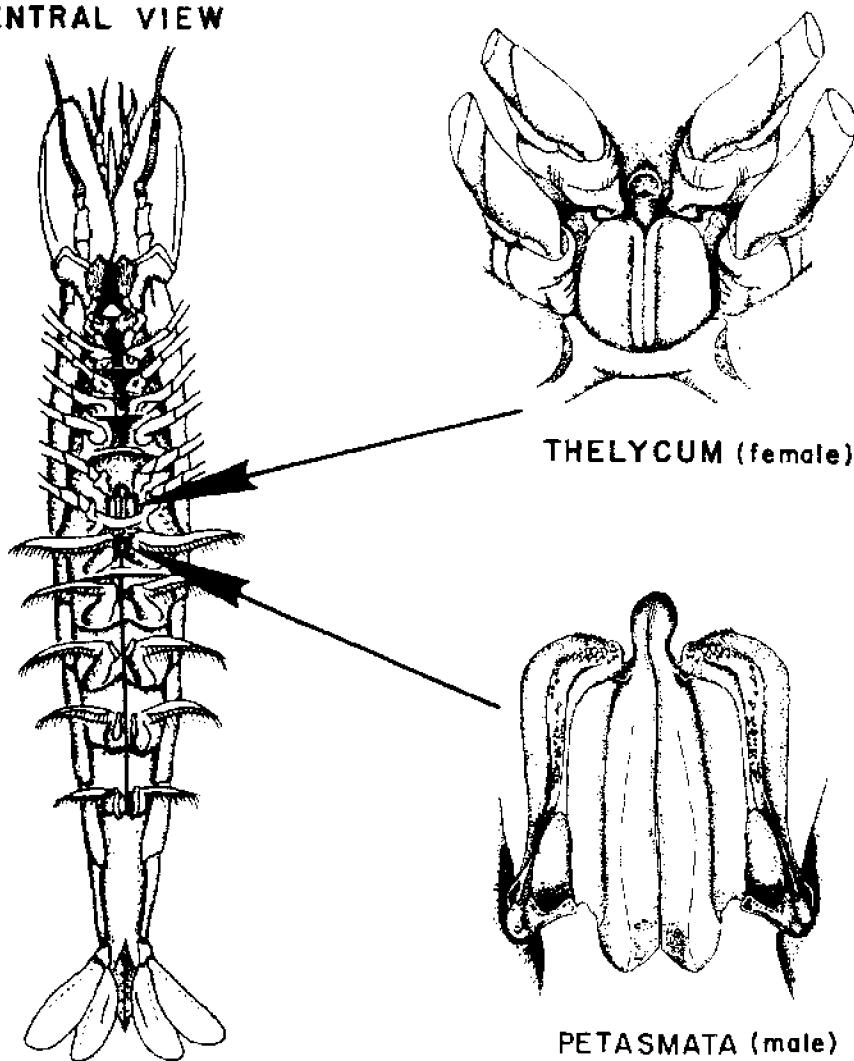


FIGURE 17

External genitalia of male and female pink shrimp

Note: Details of petasma and thelycum after Farfante (1971)

DORSAL VIEW OF GRAVID FEMALE PINK
SHRIMP Penaeus duorarum

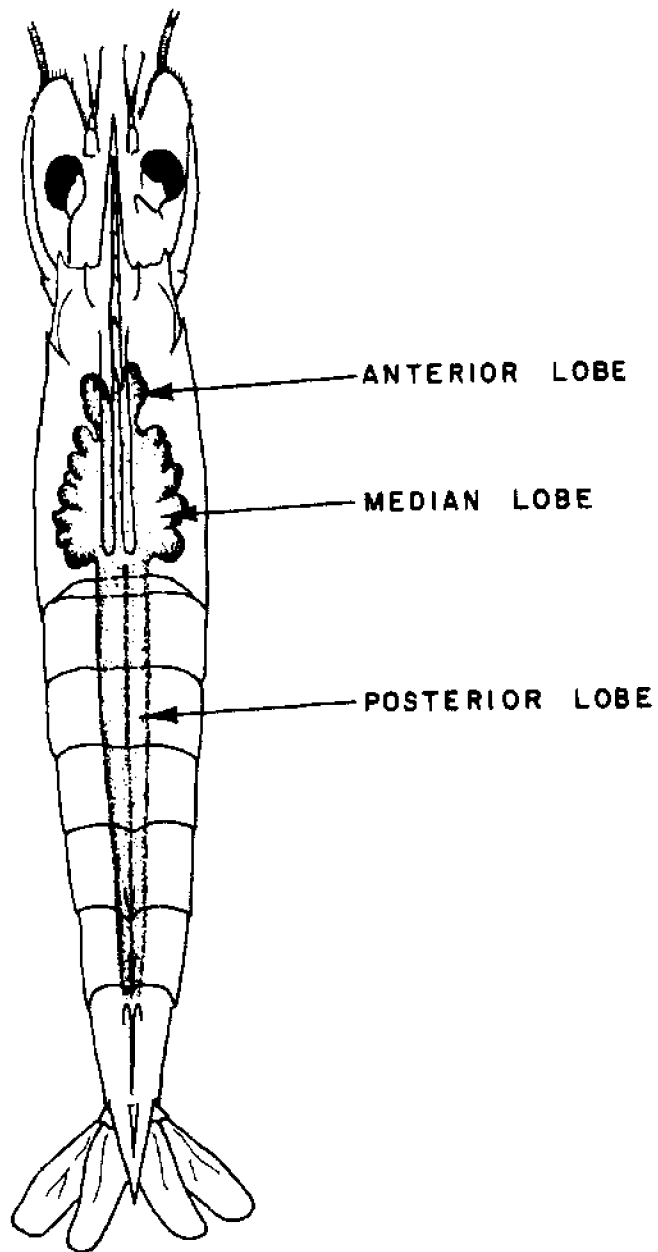


FIGURE 18

Dorsal view of gravid female pink shrimp

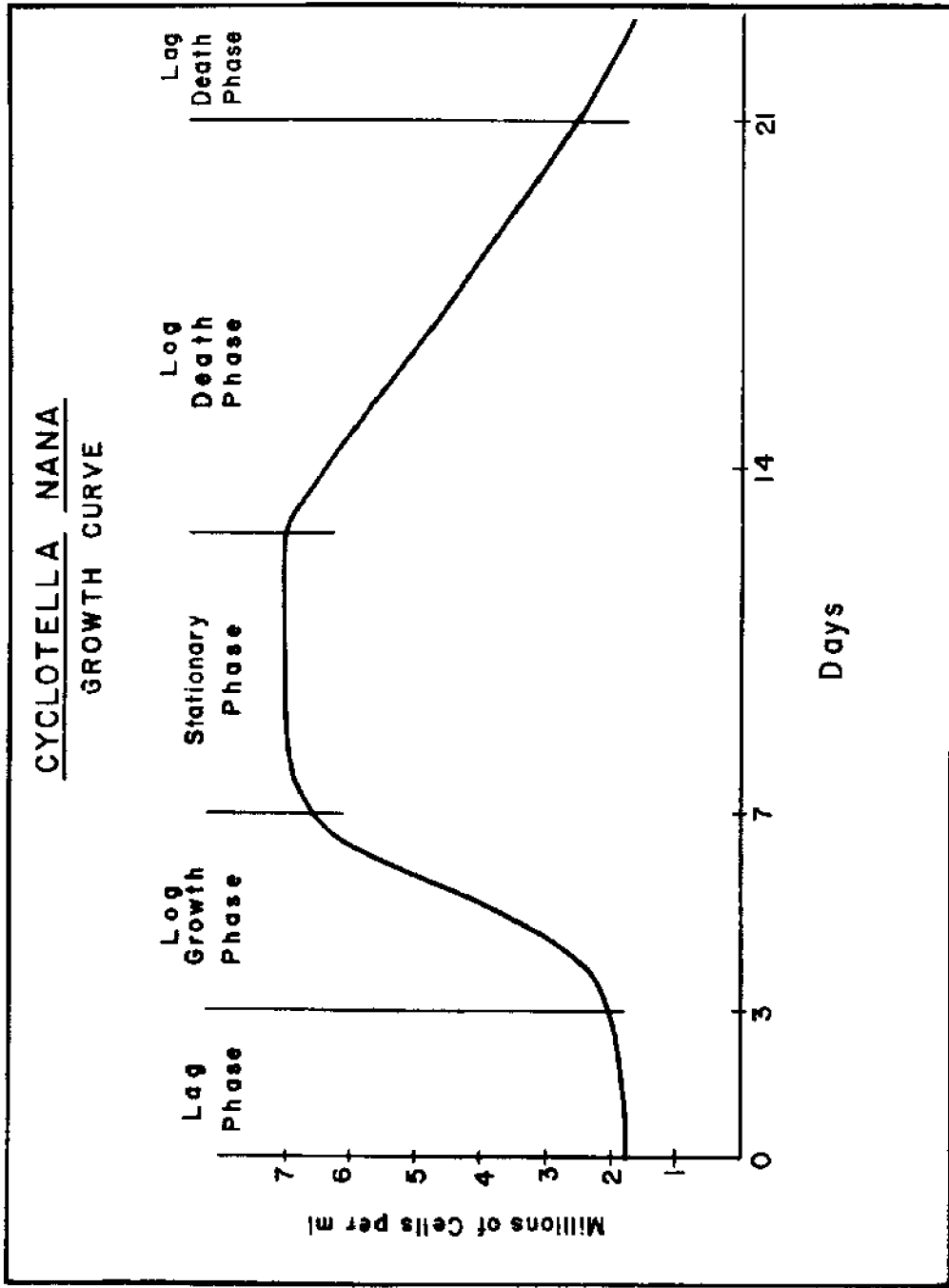


FIGURE 19

Growth curve of *Cyclorella nana*

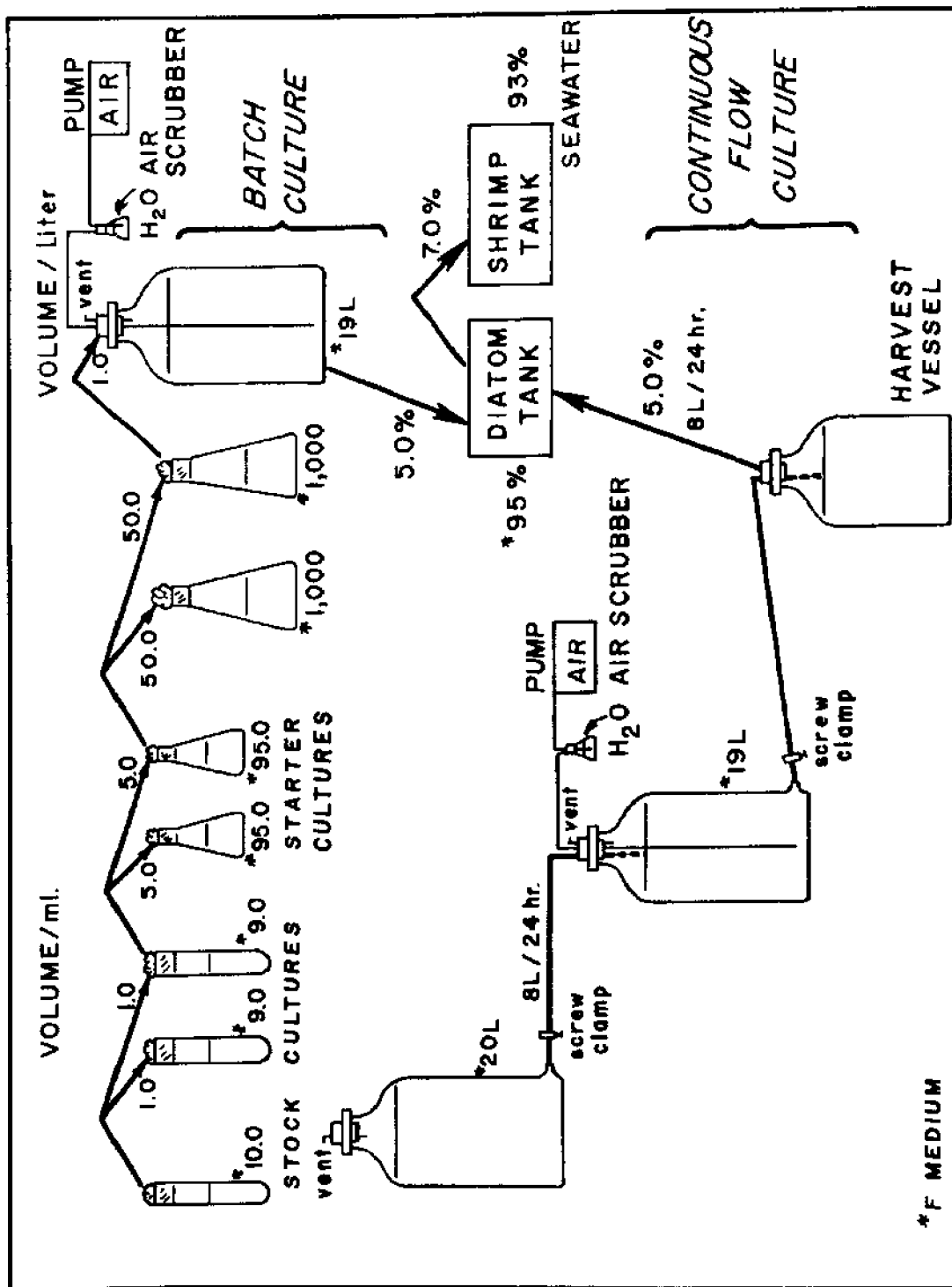


Figure 20.
Batch-culture and continuous flow culture for producing diatoms

A P P E N D I X B

TABLES

TABLE 1

Stage duration of Penaeus duorarum (hours elapsed from the appearance of a given stage until the appearance of the next stage).

Stages	Indoor Tanks 27-28°C		Outdoor Tanks ^{1/} 27-28°C		23-25°C	
	Average	Range	Average	Range	Average	Range
Egg	14 hours	13-15	13	13	19	18.5-19
Nauplius I	4	3-6	9	9	8	8
Nauplius II	7	6-7	7	7	8.5	8-9
Nauplius III	9	8-10	7	7	13.5	13-14
Nauplius IV	9	8-11	8	8	10	10
Nauplius V	15	12-16	10	10	24	24
Zoea I	45	35-56	35	35	30	30-47
Zoea II	46	36-58	25	25-26	43	40-51
Zoea III	38	29-55	34	33-36	45	42-51
Mysis I	33	26-39	30	29-40	29	28-30
Mysis II	26	14-38	24	24	29	28-30
Mysis III	32	18-41	24	24	29	28-30
TOTAL TIME	278	264-313	226	224-239	288	286-313

^{1/} Based on 2 tank experiments at 27.0°-28.0°C and 4 tank experiments at 23.0°-25.0°C

TABLE 2

Pre-spawning Schedule for Ten Indoor 2-Ton Larval Culture Tanks

	<u>Days Before Spawning</u>
A. Diatom Preparation	
(1) Inoculate 20 extra 250-ml Erhlenmeyer flasks, each containing 50 ml of F/2 medium (Table 3) with 5% of stock culture (see Stock Culture). ^{1/}	24
(2) Transfer the entire contents from each pair of 250 ml Erhlenmeyer flasks into ten 2,800 ml Fernbach flasks, each containing 1,000 ml of sterile F/2 medium and set the flasks on light room shelves under about 800 foot-candles of illumination. Agitate each culture by gentle swirling motion of the flasks at least once daily during the growing period of one week. Culture at 23° to 25°C	17
(3) Use all 1,100 ml of growing culture in each 2,800 ml Fernbach flask to inoculate each of ten 5-gallon carboys, each of which contains 19 l of sterilized F/2 medium. Place carboys on light table under 700-1000 foot-candles of continuous illumination. Turn air in each carboy to a vigorous "boil". Culture at 23° to 25°C.	10
(4) Use 5 to 10 l of growing culture from carboys having highest cell counts as inoculum for each of six 210 l mass culture, polyethylene tubs containing 150 l of sterilized F/2 medium. Diatom cell counts in the carboys used to inoculate the mass culture vessels should be at least 2 to 5×10^6 cells/ml for <u>Cyclotella nana</u> . The number achieved with other species will vary. If cell counts are lower than indicated above we use 1 or 2 extra liters of inoculum. Set overhead lights to produce 1,000 foot candles of illumination at medium surface in the open-top mass culture tubs. Two weighted airstones should be supplied for each tub and set to produce a rolling boil circulation. Culture at 23° to 25°C.	3

TABLE 2
(Continued)

	<u>Days After Spawning</u>
(5) Cell counts in mass culture vessels should be between 2 to 5 x 10 ⁶ cells/ml. This stock should be added to larval rearing tanks early in the first zoeal stage (Figure 6) when about 10% of the nauplii have transformed to 1st zoea. We try to achieve and maintain cell densities of at least 20 to 50 thousand cells per ml in the 2-ton indoor shrimp culture tank. Twice daily counts must be made to ensure this level of feeding.	2
	<u>Days Before Spawning</u>
B. Preparation of Two-ton Indoor Tanks for Hatching	
(1) Wash and disinfect inner surfaces of tanks with alcohol if slime present from previous hatches. Rinse with tap water.	4-5
(2) Fill with 1,400 l (i.e., 70 cm depth in a 1 m wide x 2 m long x 1.2 m deep tank) with seawater filtered through a 5µ filter. Salinity should be between 30 and 32 ppt. Set room air temperature or immersion heater thermostats at 26°C.	
(3) Drop 5 weighted air stones to the bottom of each tank, one in center and four others equally spaced about 1 ft out from bottom corners of tank but do not begin aeration. If, in the interest of economy, one is forced to re-use airstones and air tubing, these should be sterilized by boiling for 3-4 hours. Darken room or cover tanks with plywood covers to exclude airborne contaminants. (Note: Never use insecticides in the hatchery).	
(4) Begin aeration at a metered volume of 0.2 cfm or, lacking airflow meters, until mild circulation is visible at water surface. Water temperature should be stabilized at about 26°C. Keep tanks covered or room darkened until females are introduced.	1

^{1/}The starter cultures in 250 ml Erlenmeyer flasks will have been made up as part of the routine, year around sub-culturing required on a weekly basis to maintain diatoms in an active growth phase.

TABLE 3

Guillard F Medium (Modified) -- (1,000 X, Stock Solutions)

Part A. Minerals:	NaNO_3	150.0 g
	$\text{NaH}_2\text{PO}_4 \cdot \text{H}_2\text{O}$	10.0 g
	NaFe Sequestren	10.0 g
	$\text{Na}_2\text{SiO}_3 \cdot 9\text{H}_2\text{O}$	30.0 g

Part B. Trace Minerals:

$\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$	19.6 mg
$\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$	44.0 mg
$\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$	20.0 mg
$\text{MnCl}_2 \cdot 4\text{H}_2\text{O}$	360.0 mg
$\text{Na}_2\text{MoO}_4 \cdot 2\text{H}_2\text{O}$	12.6 mg

Part C. Vitamins:

Thiamine · HCl	0.2 g
Biotin	1.0 mg
B_{12} (Cobalamine)	1.0 mg

Part D. Preparation:

- (1) Add sterile distilled water to parts A, B, and C above as follows: Part A: 300 ml, Part B: 300 ml, Part C: 1,000 ml. (2) Combine parts A and B, add sterile distilled water to 1 liter and mix thoroughly (solution I). (3) Dispense solution in sterile screw cap bottles of desired size and autoclave at 121°C and 10 lbs pressure for 10 minutes. (4) Filter part C (solution II) through a membrane filter of 0.47 μ pore size and distribute in sterile screw cap bottles of desired size. (5) Solution I can be held refrigerated indefinitely. Solution II should be stored frozen if more than a 30-day supply is kept on hand.

TABLE 3
(Continued)

Part E. Use:

Add 1 ml each of solutions I and II per 1 liter of sterile
or pasteurized sea water.

Note: Preparation modified from Guillard (1967)

F/2 medium = 1/2 strength or 1: 2,000 dilution
of stock solutions.

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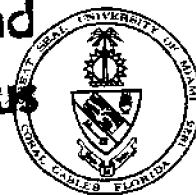
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