I. INTRODUCTION

This manual does not purport to be a complete text on shrimp culture, nor even on larval culture. It is, rather, designed as an aid to a short general introductory laboratory course in shrimp culture. To this end, several sources were used, in some cases almost in toto. We wish to extend our sincere thanks to all those who, wittingly or unwittingly, helped us. A bibliography is included herewith and acknowledgments are made at the end of the manual.

In worldwide commercial shrimp aquaculture, penaeids are primarily cultured. These species include monodon, japonicus, vannamei, stylirostris and others. Because P. vannamei is, at the moment, the most popularly cultured species in the Western Hemisphere and is the most available to us, we have chosen to concentrate on its culture in this laboratory manual. However, for the most part, the general techniques presented here can be used in the cultivation of any of the above species. Where applicable, the differences between the specific species will be mentioned.

To learn the conditions required for the survival of shrimp, zoologists have studied the natural environmental conditions of the various stages of metamorphosis and maturation. Hence, they have learned to raise shrimp through all phases of their development, including: maturation, spawning, hatching, larval rearing and grow-out. (See Figures 1 and 2, Page 2). It is these ideal natural environmental conditions which we attempt to emulate in the laboratory and commercial facility.
Figure 1
Major Aspects of Shrimp Mariculture

Figure 2
Penaeid Shrimp Life Cycle

OPEN OCEAN
II. INTRODUCTION TO THE LABORATORY

During this course, we will spend a considerable amount of time in the laboratory. Each participant will get hands-on larval rearing experience. You will be asked to work together in groups, each group being allocated a larval rearing tank.

It is intended that each group will stock their own larvae and raise them through their "most critical" stages of metamorphosis. This will require daily inspection and recording of their stage and condition. The water quality (including temperature, salinity, pH and contamination) and availability of food (first algae, then Artemia) will be monitored, recorded and controlled. We will all participate in raising algae and Artemia (brine shrimp) and preparing them for use. Each team will then ascertain the requirements of their larvae and feed them appropriately.

We will become acquainted with and make use of the instruments, equipment and procedures most often used in larval rearing facilities. Most of these will be covered in this laboratory manual.

Wet Laboratory

SYSTEMS:

Larval Rearing Tanks (LRT): The LRTs consist of a rack of 12- by 16-liter (5 gallon), conical-bottomed carboys. They are of clear plastic and are open topped to afford inspection and allow handling. The stopper in the bottom has two penetrations. One is for drainage and the other, for aeration (see Figure 3, Page 4).

Seawater: Seawater, with a salinity between 28 to 36 ppt. (parts per thousand), is held in settling tanks. Here, the coarser suspended particles are allowed to precipitate out before the water is filtered and pumped into a reservoir. In the laboratory we pass it through a 10- and a 1-micron cotton cartridge filter to remove fine particles and finally an ultraviolet filter to sterilize it. New water is allowed to recirculate through the filter system continuously until it is used. Chlorination of the seawater may be necessary, depending upon the location of the laboratory.

The drain in the tank stoppers should not be used, as larvae could themselves be lost. Rather, when exchanging water, a siphon with a Nitex® filter is used to drain the medium, while excluding the larvae. The Nitex® screen should be just fine enough to exclude the larvae (this will range from 100 μm, at stage N1, to 300 μm, at the mysis stage).

Electricity: Electricity is delivered by a socketed strip, mounted above the tanks.
A  THERMOSTATICALLY CONTROLLED HEATER
B  SIPHON, WITH MESH SIEVE
C  AERATION WITH AIRSTONE
D  DRAINAGE PIPE; DO NOT USE

FIGURE 3: LARVAL REARING TANK
WARNING: PLEASE BE SURE THAT HANDS, PLUGS, ETC., ARE DRY BEFORE WORKING WITH THE ELECTRICAL EQUIPMENT.

Lighting: Lighting is supplied continuously by two neon tubes, at an intensity of 950 to 1,200 LUX at the medium surface.

Temperature: The temperature of the medium is maintained at approximately 28°C, by thermostatically controlled immersible heaters. The "knob" at the top of the heater allows adjustment of the thermostat (clockwise rotation will increase temperature, while counter-clockwise rotation will decrease it). This should be done initially by immersing the heater in 28°C water. The thermostat is then adjusted until the light just flickers on and off. It should then be monitored constantly for 15 minutes after and intermittently for two hours thereafter. Should the temperature be more than 1°C above or below 28°C, a very slight adjustment should be made, followed by careful monitoring. The thermostats are very volatile and lack of patience can quickly result in "boiled" shrimp.

NOTE: WHEN NOT FULLY IMMERSED IN LIQUID, THE HEATERS WILL BURN OUT. THEY SHOULD BE UNPLUGGED BEFORE THEIR REMOVAL FROM THE TANKS.

Air: Air is supplied by a compressor, located above the LRT rack, via PVC piping and a PVC needle valve. Clear plastic (Tygon®) tubing is used between the valve and the airstone.

The aeration should be set at a slow, steady, stream of bubbles. The aeration should be strong enough to facilitate a gentle circulation in the tank, but not strong enough to batter the larvae or to prevent them from feeding. When inspecting the larvae, fold or pinch the plastic tubing, rather than shut off the valve.

INSTRUMENTS: The instruments which we will use in the lab are primarily water quality measuring devices. They include: the thermometer, the pH meter (for measuring acidity/alkalinity) and the refractometer (for measuring salinity). Further, we will be introduced to the chemical analysis kit (with this we run various titrations for ammonia, nitrates, etc.).
Dry Laboratory

SYSTEMS: The only additional supply which we will use here is distilled water.

INSTRUMENTS: In the dry lab, we will use both dissecting and compound microscopes, the hemacytometer (for counting algal cells) and various other general lab instruments. Basic information on the use of the major instruments can be found in the Technical Sheets beginning on Page 68.

RECORDS: The systematic maintenance of records can be a useful aid in larval care. They serve as a means of communication between the hatchery team members. When several tanks are to be tended, late at night, it is easy to forget the various measurements and even entire steps in the process. Well formulated tables for record keeping can minimize mistakes and can also aid in training the new team members.

Records can be used, not just for day-to-day planning, but also for "fine-tuning", ordering supplies, long-term planning and expansion. They can also be used to pin-point a problem or potential problem. One way to keep records, which has proved to be useful in a commercial operation, is by recording the collected daily data on a "Larval Rearing Tank Data Log" (see Figure 4, Page 7). Another important log is the "Maturation/Hatchery Egg-Nauplii Data Log" (see Figure 5, Page 8). The "Larval - State of Health" form (see Figure 6, Page 9) provides a format for the subjective grading of new batches of larvae. By grading each new batch for various criteria, it is used to build an overall numerical score for that batch. The form can be used to train new hatchery personnel in evaluation techniques. Afterwards, its use can be discontinued, but the principles are still followed when evaluating a batch.
<table>
<thead>
<tr>
<th>Date</th>
<th>Time</th>
<th>Stage</th>
<th>Health State</th>
<th>Temp</th>
<th>pH</th>
<th>Food Type</th>
<th>Comments</th>
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Larval Rearing Tank Data Log

Figure 4

Nauplii Score: ________________________________
LAR Volume: ________________________________
# Nauplii Stocked: ________________________________
# P.L.s. Harvested: ________________________________
% Survival N-P.L.: ________________________________
% Survival P-1-P.L.: ________________________________

Batch Number: ________________________________

After Counts/Data: ________________________________
# Art/ml: ________________________________
# Des/red: ________________________________

Cell Count: ________________________________
Before: ________________________________

N. Larval Rearing Tank Data Log

Figure 4

Nauplii Score: ________________________________
LAR Volume: ________________________________
# Nauplii Stocked: ________________________________
# P.L.s. Harvested: ________________________________
% Survival N-P.L.: ________________________________
% Survival P-1-P.L.: ________________________________

Batch Number: ________________________________

After Counts/Data: ________________________________
# Art/ml: ________________________________
# Des/red: ________________________________

Cell Count: ________________________________
Before: ________________________________

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### Figure 6
Larval-State of Health

<table>
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<th>Batch #</th>
<th>Activity</th>
<th>General Appearance</th>
<th>Absence of Deformities</th>
<th>External debris</th>
<th>Bacteria</th>
<th>Absence of Bent Setae</th>
<th>Photo-Positive Reaction</th>
<th># Dead</th>
<th>Date</th>
<th>Time</th>
<th>Name</th>
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</table>

**Score Ratings:**
- #3. Excellent, Highest, Most.
- #2. Fair, Mid-Range, Average.
- #1. Poor, Lowest, Least.
III. LARVAL CULTURE

Introduction

In their natural environment, adult shrimp make their way offshore to clean, stable, oceanic water (see Figure 2, Page 2). Here they mature, mate and the females spawn their eggs. The eggs will sink, but within about 14 hours the eggs hatch and the nauplii, being strongly phototropic, swim towards the surface.

These larvae will pass through three distinct stages, naupliar, protozoal (or zoal) and mysis, before metamorphosing into postlarval shrimp. Their diet ranges from the hereditary yolk sack, during the early naupliar stage, to phytoplankton (microscopic plant organisms) and then to zooplankton (microscopic animals). Finally, at mysis stage and beyond, the shrimp is able to eat a wide variety of organisms, including Artemia (brine shrimp).

During this period, the larvae drift with the currents. A small percent of them are swept into the bays and estuaries by the currents. Here, the postlarvae remain, through their juvenile months, until they too mature and seek the offshore spawning grounds (see Figure 2, Page 2). It has been estimated that only 1 percent of those spawned in nature actually reach the adult stage.

LARVAL STAGES AND THEIR IDENTIFICATION (Yang, 1975): It is important that the aquaculturist be able to ascertain not only the major stages of metamorphosis, but also the substages. This allows the requirements for food, tanks, etc. to be anticipated accurately and for the correct rate of feeding to be made.

Naupliar Stage: The Indo-Pacific species (monodon, indicus and japonicus) have 6 naupliar substages, whereas the New World species (vannamei and styliosiris) have only 5 substages. A general pattern of 3 substages for each of the zoal and mysis stages is universally reported, with only a few exceptions. The duration of each substage varies with the species and rearing temperature.

Data on the larval development of Penaeid shrimp is presented in Table I, Pages 13 and 14. For each substage, this includes the approximate duration of the substage, the size of the larvae and their required food and environmental conditions. Additional comments, of value to the culturist, are also included.

The nauplii hatch from the eggs in a folded-over position, but quickly straighten out. After several minutes they begin to swim, slowly at first, but within half an hour, more briskly. Swimming is accomplished by movement of the three pairs of appendages in paddle-like fashion, which produces a zig-zag roll of the body (Hudinaga, 1942). They
swim briefly and then rest. The nauplii are strongly phototropic and swim in the direction of the light source. A rapid response by the nauplii to a light source indicates that they are in good health. When at rest, the nauplii are suspended in a somewhat perpendicular position with the dorsal side of the body downward and the appendages slanted upward. During the last naupliar substage, the body becomes somewhat flattened.

Figure 7, Page 15, illustrates the substages of the shrimp larval stages, emphasizing those characteristics which can be used for identification. More detailed characteristics of the five naupliar substages of the species *P. duorarum* are shown in Figure 8, Page 16. Characteristics used in the identification of the individual substages are listed in the legend and indicated on the figure by arrows.

**Protozoal Stage:** In the zoeal stages, swimming is accomplished with the first and second antennae, as in the naupliar stage, but these are now aided by the well-developed first and second maxillipeds. The swimming stroke is slower than that of the nauplii, the movement appears less jerky. Characteristic of the zoeae is their continuous feeding. The culturist can judge how well the zoeae are feeding by the contractions of the digestive tract and the presence of long trail of feces. Active feeding and a continued prompt response to a light source are indications of healthy zoeae. Towards the end of the last zoeal substage, the body becomes slightly flexed.

Detailed characteristics of the 3 zoeal substages of *P. duorarum* are shown in Figure 9, Page 17 (from Dobkin, 1961). As in the illustration of the nauplii, characteristics used in identifications of the zoeal substages are indicated by arrows. The size ranges given in the diagnoses below are based on measurements of *P. japonicus*.

**Z1:** 1. Body length 0.86 - 1.32 mm.
   2. Body flattened; carapace distinct.
   3. Sessile eyes present.
   4. First and second maxillae and first and second maxillipeds functional.
   5. Furcal processes present.
   6. Digestive tract visible.

**Z2:** 1. Body length 1.33 - 2.13 mm.
   2. Stalked eyes present.
   3. Rostrum developed.
   4. Supraorbital spines developed.
   5. Abdominal segmentation apparent.

**Z3:** 1. Body length 2.14 - 2.70 mm.
   2. Abdominal segmentation distinct; dorsal and/or lateral spines present on most somites.
   3. Rudiments of uropods present.
Mysis Stage: In the mysis stage, the antennae are reduced and swimming becomes a function of the pereiopods, with some assistance from the three pairs of maxillipeds. In swimming the mysis body is flexed, with the head lowered; movement is in a backward direction. In this stage, there is less tendency for the myses to be attracted to light.

Detailed characteristics of the three myses substages are shown in Figure 10, Page 18 (from Dobkin, 1961), and are based on P. duorarum. As in the illustration of the naupliii, characteristics used in identifications of the mysis substages are indicated by arrows. The size ranges given in the diagnoses below are based on measurements of P. japonicus.

   2. Body shrimp-like in shape.
   3. Pereiopods well developed.
   4. First and second antennae reduced.
   5. Uropods well developed.
   6. Primordial pleopod buds present.

   2. Unsegmented pleopod buds present.

M3: 1. Body length 3.70 - 4.52 mm.
   2. Pleopods developed, segmented.

POSTLARVAE: During the first 4 or 5 days of postlarval life, the animals are planktonic. In subsequent stages, they can be seen to cling to the walls of the tank or will take up a completely demersal life. By Substage Pl7 (7-day-old postlarvae), the larvae of burrowing species often are able to burrow in the sand. Feeding by the postlarvae is accomplished by the chelate pereiopods, which are able to grasp and hold food. Pleopods are used in swimming.

Detailed characteristics of the first Pl substage are shown in Figure 10, Page 18. The size ranges given in the diagnoses, on this page, are based on measurements of P. japonicus and P. duorarum. As in the illustration of the naupliii, characteristics used in identifications of the mysis substages are indicated by arrows.

Pl1: 1. Body length 4.79 - 5.00 mm.
   2. First three pairs of pereiopods chelated.
   3. Pleopods with setae.
<table>
<thead>
<tr>
<th>Form or Stage and Various Names</th>
<th>Time in Stage</th>
<th>Size at End of Stage</th>
<th>Common Food</th>
<th>Parameters [opt.]</th>
<th>Additional Comments and Observations</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 Egg</td>
<td>Approx. 14 hrs @ 28°C</td>
<td>Approx. 220 Microns in dia. (0.22mm)</td>
<td>Water as clean as possible. Surface area of container important as little aeration as necessary to maintain high D.O. (5ppm*), but prevent abrasion of egg membrane. pH 8.0. Eggs sink and should not be overcrowded (Layered). Temp. 28°C (opt)</td>
<td></td>
<td>Eggs are flexible, elastic and can be forced through a 202 micron screen mesh if under any type of water pressure. To eliminate the possibility of eggs passing. A #155 nitex screen is used.</td>
</tr>
<tr>
<td>2 Nauplius (N) (plural Nauplii) NI, NII, NIII, NIV, NV</td>
<td>48 hrs @28°C (Range: 36-51 hrs)</td>
<td>NV Mean Total Length = 0.50 mm Range (0.43-0.58mm). Mean Width = 0.20mm. Range (0.18-0.22mm)</td>
<td>Water clean as possible pH 8.0. Gentle aeration to maintain high D.O. (5ppm*) or water current to keep them suspended, they will surface otherwise. Photopositive at this stage. Temp. 28°C</td>
<td>Yolk</td>
<td>Swim only occasionally but with more frequency as they develop. (Example: Stage NI may swim 3 strokes, lie dormant for 30 seconds, then swim 3 strokes, lie dormant 5 sec. then swim.) (#155 Nitex)</td>
</tr>
<tr>
<td>3 Protozoa I (P1) (P*1) also referred to as Zoea I or Zl.</td>
<td>40 hrs. Average (Range: 36-48 hrs.) @28°C</td>
<td>Mean Total Length 1.0 mm Mean Caudal (tail) Length 0.3 mm</td>
<td>(Minimum algal cell count which animals are exposed to is 1 x 10^6 algal cells/ml.) pH 8.0 (opt.) Temp. 28°C Ammonia - Less than 25 micro gram atoms/liter</td>
<td>Phytoplankton 3-5μ in size</td>
<td>Continuously swimming and consuming phytoplankton. Fecal threads visible and are often longer than animal. #202 Nitex used to retain animals</td>
</tr>
<tr>
<td>4 Protozoa II (Pll) (P*2) also called Zoea II or Zll</td>
<td>40 hrs. Average (Range 36-48 hrs.) @28°C</td>
<td>Mean Total Length 1.71 mm (Range 1.28-2.01 mm). Mean Caudal (tail) Length 0.80 mm (Range 0.72-0.87 mm)</td>
<td>&quot;&quot;</td>
<td>&quot;&quot;</td>
<td>Presence of stalked-compound eyes #202 Nitex used to retain animals</td>
</tr>
<tr>
<td>Form or Stage and Various Names</td>
<td>Time in Stage</td>
<td>Size at End of Stage *</td>
<td>Common Food</td>
<td>Parameters (opt.)</td>
<td>Additional Comments and Observations</td>
</tr>
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<tr>
<td>Protozoa III (PIII) (PI) (also called Zoea III or ZIII)</td>
<td>40 hrs. Average (28°C) Range 36-48 hrs.</td>
<td>Mean Total Length 2.59 mm (2.4-2.59 mm Range) Mean Caudal (tail) Length 1.06 mm (Range 0.93-1.40 mm)</td>
<td>Phytoplankton Mixed Species</td>
<td>pH 8.0 (opt.) same as PI &amp; PII Ammonia - Less than 25 µg at/l Temp. 28°C</td>
<td>Biramous uropods and spines on abdominal segments #202 Nitex used to retain animals.</td>
</tr>
<tr>
<td>Mysis I (MI) (M.1)</td>
<td>24 hrs. (28°C)</td>
<td>Mean Total Length 3.5 mm Mean Caudal (tail) Length 1.2 mm</td>
<td>Artemia and phytoplankton Fed the combination of phytoplankton and Artemia. pH 8.0 Same parameters as protozoa. Temp. 28°C Ammonia - Less than 25 µg at/l</td>
<td></td>
<td>Pleopods small just beginning to protrude from ventral side of abdominal segments. Animals able to flip backwards as well as swim forward. #202 Nitex effluent screen used to retain Artemia nauplii.</td>
</tr>
<tr>
<td>Mysis II (MII) (M.2)</td>
<td>24 hrs.</td>
<td>Mean Total Length 3.8 mm (Range 3.3-4.2 mm) Mean Caudal (tail) Length 1.3 mm (Range 1.2-1.4 mm)</td>
<td>Artemia and phytoplankton</td>
<td>pH 8.0 Temp. 28°C Ammonia - Less than 25 µg at/l.</td>
<td>Unsegmented pleopods, but more pronounced and curving to anterior #202 Nitex used to retain Artemia nauplii.</td>
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<tr>
<td>Mysis III (MIII) (M.3)</td>
<td>24 hrs.</td>
<td>Mean Total Length 4.3 mm (Range 3.9-4.7 mm) Mean Caudal (tail) Length 1.4 mm (Range 1.3-1.5 mm)</td>
<td>Artemia and phytoplankton</td>
<td>pH 8.0 Temp. 28°C Ammonia - Less than 25 µg at/l.</td>
<td>Pleopods composed of 2 segments and 2 - 3 terminal setae. #202 Nitex to retain Artemia</td>
</tr>
<tr>
<td>Post larvae</td>
<td>24 hrs.</td>
<td>Mean Total Length 4.6 mm (Range 4.2-5.0 mm) Mean Caudal (tail) Length 1.5 mm (Range 1.4-1.6 mm)</td>
<td>Artemia and phytoplankton</td>
<td>pH 8.0 Temp. 28°C Ammonia - Less than 25 µg at/l.</td>
<td>Generally - Post-larvae spend approx. 6 days suspended in water column but prefer to be bottom dwellers thereafter. #202 - to retain Artemia (food supply). ( #500 Nitex to retain animals).</td>
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</tbody>
</table>

*These sizes are based on measurements using P. acutus, P. sty. and P. vann are slightly larger (example: we have measured a number of P. sty., PI's at 6 mm total length).
Figure 7  Shrimp Larval Stages
(after Motoh, 1979)
Figure 8 Naupliar substages of *Penaeus duorarm*:

a1 – first antenna; a2 – second antenna; en – endopod; ex – exopod; fr – frontal organs; fu – furca; lm – labrum; md – mandible; mx1 – first maxilla; mx2 – second maxilla; mxp1 – first maxilliped; mxp2 – second maxilliped; o – ocellus; sc – scaphognathite (from Dobkin, 1961).

a) Nauplius I: Body pear-shaped.
b) Nauplius II: 1 long, 1 moderate and 1 short terminal setae on 1st antennae.
c) Nauplius III: 2 distinct furcal processes, each with 3 spines.
d) Nauplius IV: Each furcal process with 5 spines (1); segmentation of appendages apparent (2), 1st and 2nd maxillae and maxillipeds present (3).
e) Nauplius V: Body more or less depressed; swollen knoblike structures at bases of mandibles present (1); frontal organs present (2).
Figure 9  Zoeal substages of *Penaeus duorarum*: ab – abdomen; c – carapace; dt – digestive tract; e – eye; la – labium; md – mandible; mx1 – first maxilla; mx2 – second maxilla; mxp1 – first maxilliped; mxp2 – second maxilliped; r – rostrum; su – supraorbital spine; th – thorax; u – uropod (from Dobkin, 1961).

a) Zoea I: Eyes stalked (1); rostrum present (2); supraorbital forked spines present (3).

b) Zoea II: Pair of biramous (doubly branched) uropods developed (1); spines appear on abdominal somite (2).

c) Zoea III:
Figure 10  Mysis and Postlarval substages of *Penaeus duorarum*: p1 – pleopod (from Dobkin, 1961).

a) Mysis I:  Shrimp-like body structure.
b) Mysis II:  Pleopod buds apparent but unsegmented.
c) Mysis III: Pleopods elongated and segmented.
d) Postlarva I (PL₁): Swimming setae present on pleopods.
EXERCISE I. DAILY LRT INSPECTION

OBJECTIVES:

1. To make an initial visual inspection of the larvae, the larval rearing tank and the supporting systems. This will include a judgment of the larval stage and health, the water quality and temperature, the light, heat and air supply and the amount of food in the tank.

2. Inspect at least three of the larvae under the dissecting or compound microscope. Accurately judge and record the substage. Inspect for and record any deformities or contamination.

3. Read and record the water temperature, salinity and pH. Take action, should these be unacceptable.

4. Perform routine water exchange.

5. Count and record larval food remaining in the LRT (algal cell density and/or Artemia concentration).

6. Calculate, record and administer additional feed requirements.

MATERIALS AND EQUIPMENT:

- 16-liter larval rearing tank, including larvae (100 per liter) and all support systems.
- 500 ml beaker.
- Eye dropper or pipet.
- Glass slide and cover plate.
- Dissecting microscope.
- Compound microscope.
- Daily record sheets.
- Thermometer.
- Refractometer.
- pH meter.
- Siphon tube with filter (see Figure 3[b], Page 4).
- Two buckets (one for discarded and the other for fresh seawater).
- Hemacytometer.
METHODS:

(1) The initial inspection will not be easy for the neophyte aquaculturist. However, within a few days, a very quick inspection of all the tanks will allow him/her to recognize possible problem areas. Immediate closer inspection and action can then be taken.

Check the rate of aeration. This should be a steady flow of little bubbles, but should not "boil." Put your hand against the side of the tank to judge the temperature. It should feel a little cooler than your hand. Fold and squeeze the air supply tube, so as to cut off the air. Inspect the quality of the water. It should be clear with a slight yellow or green tinge, if algae is being fed. It should be neither milky nor dark yellow and there should be no strands of algae or bacteria developing on the sides of the tank.

Using the identification charts (Table I, Pages 13 and 14, and Figures 7 through 10, Pages 15 through 18) and with a little experience, the major stage of metamorphosis (nauplius, zoeal or mysis) can be distinguished with the naked eye. Notice the size, shape and swimming characteristics. The condition of the larvae can also be judged. Are they swimming as they should (if at all)!? Note the presence or absence of fecal threads, which will indicate whether or not they are eating well.

(2) To remove a sample from the tank, scoop some water with a clean 500-ml beaker. Use the eye dropper or pipet to draw up the larvae and place them, in single drops of water, on the glass slide. It might be interesting to look at the animals under the microscope before killing them, as they might become discolored, distorted or damaged when killed. A drop of Lugol's stain can be placed on each animal. They are extremely sensitive and die quickly.

The general condition of the animal should be inspected, with particular attention to the appendages. Setae should be straight and whole. Disfigurement might include curled, kinked setae or appendages. Bacteria (a sign of poor water quality and contamination) may also be present, and may appear in the form of debris or as described in the larval diseases section on Page 61. Side effects of bacteria on larvae may cause numerous symptoms, including localized necrosis or discoloration on any appendage, or a yellow-vermilion and red color permeating the entire nervous system. The muscles should be reasonably translucent and not milky or discolored, brown or have "little bubbles" in them (signs of disease). Is the hepatopancreas full and is the gut full and straight? At very high magnification, are there signs of bacterial colonization of the gills, setae, etc.?

Should abnormalities be found, further samples may be taken to gauge the extent of the problem. Once again, the beginner will soon learn what a healthy animal should look like and experience will lead to ease in identifying abnormalities.
The stage and substage of the larvae can now be determined (see Table I, Pages 13 and 14, and Figures 7 through 10, Pages 15 through 18). Record the above information on the "Larval Rearing Tank Data Log" for each tank (Figure 4, Page 7).

(3) Measure and record the temperature. Ideally, it should be 28°C. Should it be more than 1°C above or below this, the thermostat should be adjusted, as described in Chapter II, "Introduction to the Laboratory," on Page 5.

The salinity is read with a refractometer and recorded. This should be 32 (±4) ppt. Should it be outside the range of 28 to 36 ppt., water exchange or slow dilution with distilled water should be carried out.

A pH meter is used to tell if the medium sample is acidic, basic or neutral. The optimum pH is 8.0. If outside the 7.6 to 8.4 range, water exchange is used for correction.

NOTE: TO PREVENT DAMAGE, CARE SHOULD BE TAKEN NOT TO IMMERSE THE pH ELECTRODE DEEPER THAN INDICATED ON THE INSTRUMENT.

Should the pH or salinity levels be unacceptable, the water exchange required could exceed 50 percent. The tank should then be monitored closely for the next 24 hours.

(4) Routine water exchange is between 25 percent and 33 percent per day.

NOTE: TO PREVENT THE IMMERSION HEATER FROM BURNING OUT, IT SHOULD BE UNPLUGGED BEFORE THE TANK IS DRAINED.

Before the water is removed, the aeration can be increased so as to agitate the water and mix the heavier particles into suspension. The filter end of the siphon is then lowered into the tank and approximately 5 liters is siphoned into the "discard" bucket. Now the aeration is decreased to the normal level.

Before the water is replenished, the temperature and salinity of the fresh seawater is checked. Should it be unacceptably higher or lower than that of the tank, it must be adjusted or be added slowly so as to acclimate the larvae. The rate of change should not exceed 1°C or 1 ppt. every 15 minutes. Return the immersion heater to the tank and plug it in. Adjust the aeration.

(5) To ascertain the amount of food remaining in the LRT after the water change, refer to two other exercises. If algae is to be fed, see Exercise III, Page 45. If Artemia are to be fed, see Exercise IV, Methods 3, 4 and 5, Page 54.

(6) Refer to Table II, Page 22, for food requirements of each larval stage. To calculate the amount of food to be added to the LRT, refer to the same two exercises as in "5" above.

A thorough record of all larval and LRT conditions should be kept. This will not only improve the management of that particular batch of larvae, but also allow better preplanning and "fine tuning" of future work.
Table II
LARVAL REARING FEEDING REGIME
(from Treece, 1985)

| Days of Larval Culture | -1  | 0 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 | 20 | 21 | 22 | 23 | 24 | 25 | 26 | 27 | 28 | 29 |

Larval Stage

Eggs Hatch 14 hrs @ 28°C
N₁ to N₅
P₁
P₂
P₃
M₁
M₂
M₃
P₁₁
P₁₂
P₁₃
P₁₄
P₁₅
P₁₆
P₁₇
P₁₈

Phytoplankton (1 x 10⁶ cells/ml) Minimum
Highest Feed Levels Maintained Cell Count Minimum 1 x 10⁶ cells/ml

Artemia/ml

0.25
1
3
6
8
6
3
1
1

Ground Pellet or Flake

Flake or ground pellet diet fed until P1's are sold or stocked (any excess Artemia are fed to P1's)

Full Size Pellet

NOTE: Almost always 11 days from N₅ to P₁ (28°C), but the periodicity of each stage may vary considerably during this 11 day span.