MIAU-H-75-001 C2

A Manual for Large-Tank Culture of Penaeid Shrimp to the Postlarval Stages

Won Tack Yang

A MANUAL FOR LARGE-TANK CULTURE OF PENAEID
SHRIMP TO THE POSTLARVAL STAGES

WON TACK YANG[1]
Division of Biology and Living Resources
Rosenstiel School of Marine and Atmospheric Science

[1]Present Address: F.A.O.
United Nations Development Programme
Projekt UDANG
P.O. Box #1
Jepara, Indonesia

Price: ~~$5.00~~

Out of print

Library of Congress Catalog Card Number: 75-23538

NTIS# PB 247 923/AS

Under Florida law

This manual is dedicated to the late

Dr. Motosaku Fujinaga

who devoted his life to the study of

penaeid shrimp culture.

PREFACE

Previously, the author participated in the publication of a Sea Grant study titled _A Manual for Culture of Pink Shrimp, Penaeus duorarum, from Eggs to Postlarvae Suitable for Stocking_. That manual dealt primarily with small-tank cultures and many of the techniques described have since been improved. The present manual presents some of the highly successful yet simplified large-tank culture techniques which have been developed.

It is hoped that this manual will present shrimp rearing technology in a clear and practical manner so that many prospective culturists may find it useful as a source of information. Furthermore, information on new data and techniques summarized from the Japanese also are presented for the interest of all who are concerned with the rearing of penaeid shrimp. This text is presented in the spirit of Sea Grant; to promote excellence in education, research and information services in the University.

TABLE OF CONTENTS

ACKNOWLEDGEMENTS

INTRODUCTION

The mass production of high quality, viable postlarvae is the key
to successful aquaculture. When compared with agriculture, aquaculture
still must be considered an art rather than a science. In technology and
expertise, mariculture is less advanced than its freshwater counterpart.
With the exception of oysters, clams, the American lobster and *Penaeus*
shrimp, larvae of few other marine invertebrate animals have been pro-
duced successfully. Many of the present-day techniques used in shrimp
larval production are the result of the life-long studies of the late
Dr. Motosaku Fujinaga. Credit must also be given to Dr. Jiro Kittaka
for the development of much of the large-tank culture system that is
described in this manual.

The successful production of postlarval shrimp has been an important
contribution to the development of mariculture, and has enabled the trad-
itional shrimp fisheries to advance from catch fisheries to farming
fisheries. In Japan, the great demand for shrimp (*Penaeus japonicus*)
has provided the impetus to this evolution in mariculture. Currently,
many of the Japanese catch fisheries operate, in part, to provide
gravid female shrimp for the culturists. It is anticipated that
similar cooperative fisheries and large-scale shrimp culture facilities
can be developed in other parts of the world.

Two distinctly different culture methods have been developed for
postlarval shrimp production, i.e., the small-tank culture and the
large-tank culture. The small-tank culture results in higher productivity
per unit volume; however, the techniques involved are quite complex.

The large-tank culture, although resulting in lower production per unit volume, provides far greater overall production, and requires only simple techniques and equipment. The Japanese Seedling Centers of the Seto Inland Sea Culture Fisheries Association alone produced a total of 120 million penaeid shrimp postlarvae in 1970 (Oshima, 1972) and more recently Imamura (1974) reported that production had been increased to 150 million postlarvae per year. With the successful production of one million postlarvae, Fujinaga (1967) proposed the term mega to represent a unit of one million postlarvae.

Although shrimp farming has proved profitable in Japan, the mass production of postlarvae does not, in itself, guarantee that commercial shrimp culture necessarily will be a profitable venture. The farming process from the postlarvae to marketable size currently is a major problem, as far too often operational expenses exceed the market value. However, for government sponsored mariculture programs, large-tank culture is of immediate practical value. The emphasis in this manual is placed on large-tank culture methods.

The major portion of this manual has been taken from the various reports on Penaeus japonicus, the only species for which the most detailed information is available, and from the author's own work at the, now closed, University of Miami's Turkey Point mariculture facilities. The methods described, however, are applicable to all penaeid species. Refinements in large-tank culture techniques are continually being sought, particularly in the area of early larval food sources. Although some of the newly developed experimental techniques may be valuable, others are of questionable merit. The basic procedures outlined in this manual should enable the prospective mariculturist to successfully rear penaeid postlarvae.

TERMINOLOGY

The terminology applied to the larval stages of penaeids differs
among authors. Williamson (1969) and Kurata (1973) have attempted
to standardize this terminology for all decapod larvae. However, in
shrimp mariculture, most authors have tended to follow the terminology
used by Fujinaga (who published first under the name of Hudinaga).
To avoid confusion, Fujinaga's (Hudinaga, 1942) terms have been used in
the present manual: egg (E), nauplius (N), zoea (Z), mysis (M) and
postlarva (PL). The substages for the naupliar, zoeal and mysis stages
are written in Roman numerals, (e.g., Z-III indicates the third zoeal sub-
stage). The Arabic number after PL is the age (in days) of the
postlarva.

Two terms, "planting" and "seedling", as translated from the Japanese,
appear to have been borrowed from the botanists. Because these terms
have very particular connotations in Japanese that are not easily trans-
lated into English, these terms have been used in this manual, although
it is recognized that the English reader will think of them most readily
as agricultural terms. The term "planting" refers to postlarvae that are
returned to the sea for further development under natural conditions. The
term "seedling" refers to postlarvae suitable for stocking in a shrimp pond
(for further development under controlled conditions) or for planting in
the sea. The age of such seedlings varies with culturists.

The word "ton", as conventionally used in tank capacity, refers to
the water volume (one metric ton); a one-ton tank has a capacity of one
cubic meter of freshwater. All references to volume are actually less
(e.g., 180 m^3 of seawater is maximum in a 200-ton tank).

Small-tank culture refers to the method that utilizes a small water volume (less than five tons) and the separate culture of phytoplankton for larval shrimp food.

Large-tank culture refers to culture techniques involving volumes greater than 15 tons of culture water, with the phytoplankton cultured simultaneously in the same water with the shrimp larvae. Except when otherwise specified, large-tank culture, as described in this manual, refers to cultures greater than 50-ton.

CONSIDERATIONS FOR SELECTION OF HATCHERY SITES

The selection of the site for hatchery facilities should be based on the following considerations:

1. The site should be in an area where a shrimp fishing industry exists so that ovigerous females from the natural grounds can be readily, easily and cheaply obtained for spawning purposes.

2. Transportation and communication facilities, which are essential to the acquisition of gravid adults and larval food materials, as well as for the transfer of seedlings to farming ponds, should be conveniently located near the site.

3. For year-round hatchery operations, variations in the abundance of local stocks must be considered. The site should be so chosen as to insure that gravid females brought from other areas will be able to reach the hatchery facilities within a few hours after capture.

4. Electrical power is essential to hatchery operations, therefore the site should be provided with a reliable power source.

5. The seawater in the area should be clean and relatively free from silt. To avoid lowered salinities, the site should not be situated near river mouths. The hatchery should be set up away from sources of industrial pollution as well as from agricultural areas where the heavy use of pesticides may contaminate the water.

6. For outdoor and large-tank cultures, the site should be in an area well protected from adverse weather conditions. Subtropical and temperate regions where warm winters and breezy summers prevail are generally best suited for these culture purposes. Areas in which the local weather patterns include an abundance of clear bright days are much more desirable than areas where overcast skies are common.

FACILTIES AND EQUIPMENT

Culture Tanks

The size and number of the culture tanks will vary depending on the scope of the hatchery operation. In the methods discussed herein, it is the author's belief that the minimum workable tank size should be 15-ton (5 X 2 X 1.5 m depth); even larger tanks are preferable. The 200-ton (10 X 10 X 2 m) tank (Fig. 1) has become the Japanese standard after successful mass-culturing of Penaeus japonicus by Hudinaga and Kittaka (1967). In their work, they made use of modified 200 m^3 brine solution tanks formerly used for salt manufacture. However, Shigueno (1972a) considered a square or rectangular concrete tank with a depth of 2 m and a holding capacity of 100 tons to be the most desirable and efficient structure. P. japonicus seedlings also have been produced in a 1800-ton (50 X 20 X 1.8 m) municipal swimming pool (Hirata and Wada, 1969) and in a 2800-ton capacity tank (Kureha and Nakanishi, 1972), both at the Shibushi Seedling Center, Seto Inland Sea Culture Fisheries Association.

The ideal shape of the larval culture tank is cylindrical, as better water circulation can be achieved by eliminating dead corners. Small-capacity cylindrical tanks have been utilized for indoor culture by Cook and Murphy (1969) and Mock and Murphy (1971). A small circular tank equipped with a rotator and blade insures better food suspension so that early larvae can feed on detrital materials (Imamura and Sugita, 1972) and the feeding of pelletized artificical diets to the mysis stage and beyond can be facilitated.

The main shortcoming of the large-capacity cylindrical tank is the expense of construction. Precast, reinforced 20-ton (2 X 5 X 2 m) cement tanks, such as those designed by the author and used at the University of

intake water valve

screen plankton net

filter screen for water exchanger

water exchange siphon

inside catch basin
plastic screen
drain

3/100 slope

sump

large aeration - stone

drain canal

adjustable dam board

sump

adjustable dam board

drain canal
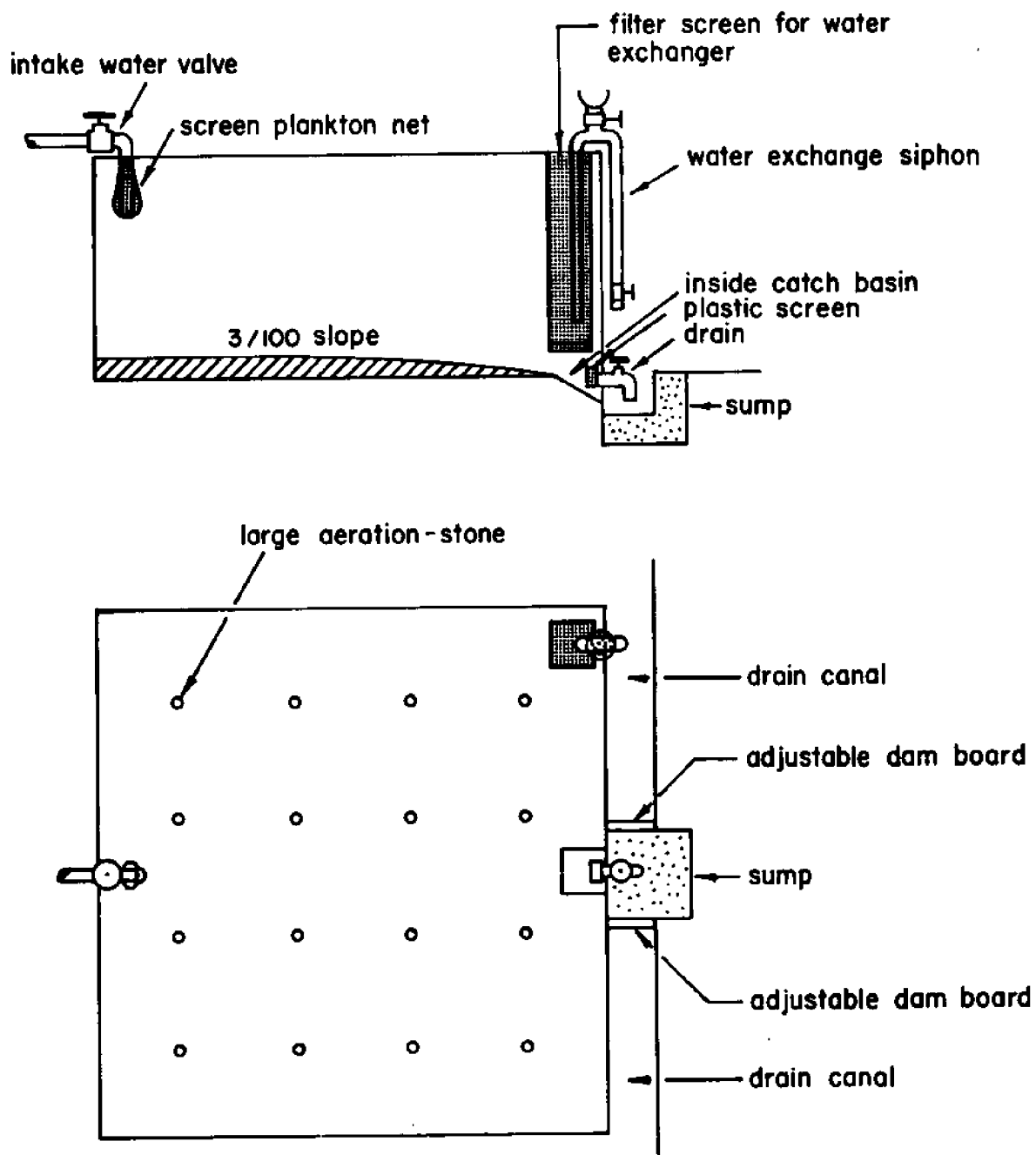
Figure 1. Schematic views of larval culture tank.

Miami's Turkey Point shrimp culture experimental station (Tabb, et. al., 1972) (Fig. 2), large molded fiberglass tanks, or commercially available vinyl-lined, above-ground swimming pools that meet the specifications presented here may also be used. Before plastic tanks can be utilized, the chemical constituents of the plastic must be specifically known and possible toxicity, if any, determined. These smaller tanks, i.e. 15 to 20 tons, also may be made of cement blocks with partial reinforcement. Larger culture tanks should be constructed of reinforced concrete. Depending on the size, a depth of 1.5 to 2 m is desirable, with 1.2 m as the minimum allowable depth for convenient operation. A reinforced concrete wall (thickness of about 20 cm) is sufficient to withstand the water pressure within a 200-ton tank and provides a convenient walkway around the tank. The tank bottom should be gradually sloped at a rate of 3/100 (Shigueno, 1972a) toward one side to facilitate drainage. It is advisable to have the inside walls and bottom smoothed with mortar for ease in cleaning, especially if the tank is partially constructed of cement blocks since these have granular holes in which organisms can grow.

Through the use of collapsible plastic swimming tanks and mobile laboratories, it is also possible to develop portable, large-tank culture facilities. Such operations can be relocated frequently to take advantage of changing seasons, sources of gravid female shrimp, and water quality.

For water exchange and mixing, and for the population estimates necessary for proper feeding, a depth marker on the inside wall of the tank is essential. A glass window on one side of the tank is desirable to facilitate inspection of the population, especially after the post-larvae have settled.

The diameter of the drainage pipe will depend on the size of the tank. A polyvinyl-chloride drain pipe and valve of at least 15 cm diameter is
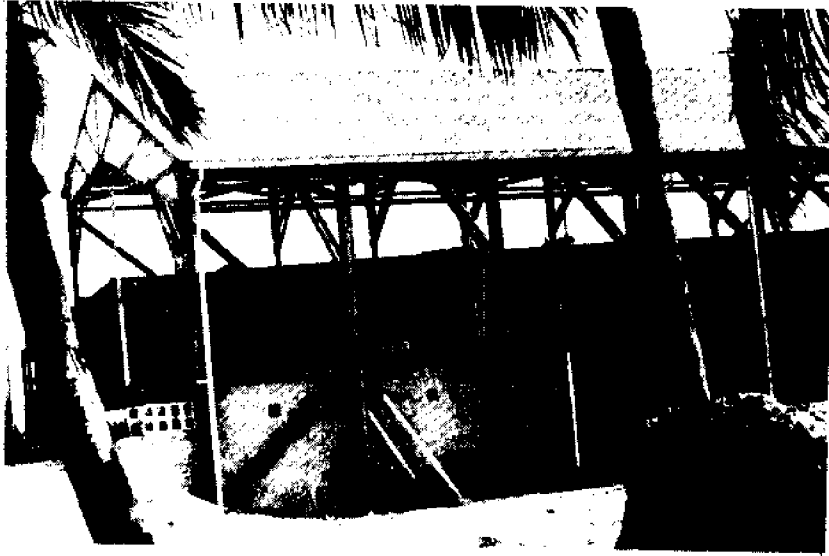
Figure 2. Two tiers of 20-ton tanks (5 X 2 X 2 m) at the Turkey Point
facilities. Note the corrugated plastic roofing over the
tanks. The bulky wooden frame reduces illumination.
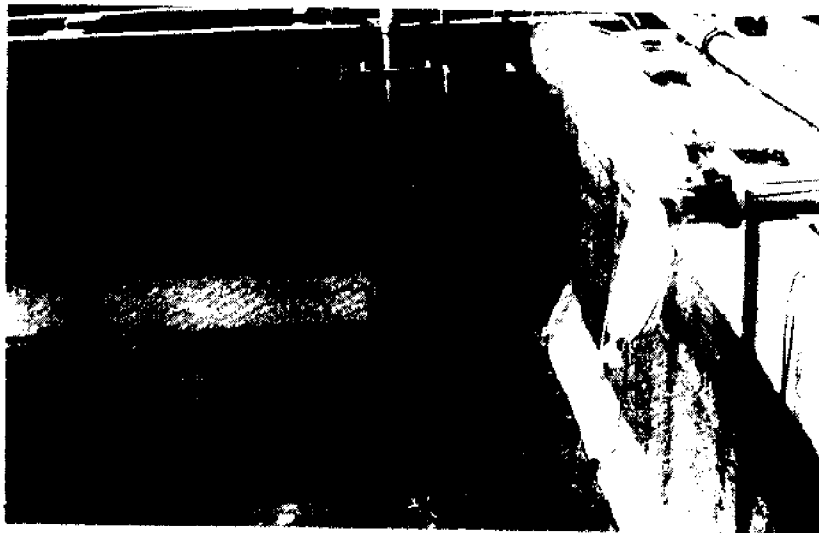
Figure 3. 200-ton tank just harvested. Two water exchange filters are
situated at the corner of the tank; one with a water exchange
siphon. The plankton net filter bag is on the water intake
valve. Note the heavy barnacle growth on the tank wall. The
clean bottom indicates the culture was well managed.

recommended. The drain hole inside the tank should be provided with a plastic screen cap to prevent the female shrimp from hiding in it during spawning. A small catch basin at the intake of the drainage pipe is desirable for convenient harvesting.

At the discharge point of the drain pipe, that part of the drain canal can be expanded into a sump (Fig. 1) that would be wider and deeper than the canal. At both ends of the sump along the canal, there should be grooves for the dam-boards so the water level in the sump can be controlled during draining and during the harvesting of the postlarvae.

To avoid undue shading, tanks should not be constructed adjacent to large buildings. When arranging small, deep rectangular tanks, these should be placed in an east-west direction, parallel to each other. Such positioning will allow the tanks full benefit of sunlight with no parts of the tanks shaded.

New concrete tanks should be built at least two months prior to use in the hatchery operation. After the tanks have hardened, they must be filled with water and leached out thoroughly for up to 50 days before use. When there is insufficient time for adequate leaching, acetic acid may be added to the water in the tank to hasten the buffering and stabilization of alkalinity caused by the Portland cement.

Before each use, the culture tank must be cleaned of all attached organisms and detrital material. All surfaces should be scraped clean and rinsed with freshwater. At least two days should be provided for the tank to dry thoroughly. All these precautions will prevent an unwanted bacterial bloom when the seawater is fertilized. Any major bacterial bloom will curb diatom growth.

10

The tanks should be covered with translucent roofs made of colorless, corrugated plastic or fiberglass materials to prevent dilution of the culture water by rain and, for better phytoplankton culture, to block severe direct sunlight. The use of a roof also will reduce the ambient temperature of the culture water slightly. Using tanks in a given locality, experiments have shown that seedling production is much better in covered tanks. Tank roofs should be of corrugated plastic roofing material, covered externally with Mylar to prevent rapid deterioration of the roofing material from the sun's ultraviolet rays. Even with the Mylar covering, the roofs will deteriorate in time, so it is advisable, initially, to use roofing material with 80 to 90% light transmission capability. Steel or aluminum is preferable to bulky wood for the roof frame as the latter tends to interfere with illumination (Fig. 2).

In temperate regions, temperature control devices are required during the colder months of the year. One means of temperature control is the provision of removable walls, made of the above-mentioned roofing material, to encase the culture tanks. Proper temperatures also can be maintained through the use of large-size electrical immersion heaters. Shigeno (1969) used one 40 kw submersible heater for his 57-ton capacity tank and found the culture results were average. However, as such heaters can give severe electrical shocks, extreme caution in their use is required. Another method, more expensive in installation, but with a low operating cost, is a pipeline through which steam or warm water is circulated. If such a pipeline is used, it should be placed about 20 cm above the tank bottom. Temperature control devices are usually not necessary in tropical regions, but some provisions for cooling may be needed during the summer months.

Penaeid shrimp have been reared at a variety of salinity levels. Mock (1974) pointed out that salinities of 27 to 35 parts per thousand are most satisfactory for larval culture. In outdoor, large-tank cultures, it is preferable to use seawater with salinities near the lower limit of acceptable culture salinities (approximately 27 ppt) for better phytoplankton growth in the tanks. In areas with low salinities or where seasonal rains greatly dilute the seawater, the salinity of the culture water may be raised by the addition of artificial salts such as Instant Ocean (Cook and Murphy, 1969), "rock" or "solar" salts or brine. Also, in areas with a prolonged rainy season, there may be problems resulting from reduced solar illumination.

Water Exchange Filter

To prevent the loss of larvae during water exchange, a filter box is used (Figs. 1, 3). The structure should have dimensions of 1 X 1 X 1.8 m high or 50 cm X 50 cm X 1.8 m in a 200-ton capacity tank. Preferably, the frame should be constructed of hardwood, treated with a polyester resin coating. Two layers of screening material should be attached to the frame. The inside screen should have approximately 3.0 to 4.0 mm mesh openings to reinforce and support the outside screen. For the late mysis and postlarval stages, the outside screen of plankton net material should have about 350 micron openings. For harvesting large postlarvae (i.e. PL_{20}), the mesh opening of the outside screen should be about 1 mm diameter, which permits fast siphon drainage without much clogging.

Water Exchange Siphon

The water exchange siphon is of an inverted U shape and, depending on tank capacity, made from 5 to 10 cm diameter PVC pipe (Figs. 1, 3). The length of each arm is also dependent on the size of the tank and the inside water filter box; a length of less than 1.8 m is suitable for a 200-ton tank. A water inlet funnel with a very tight stopcock is affixed at the top of the siphon and a valve is set near the end of the outside arm. To operate the siphon, shut off the outside valve, open the top valve stopcock and pour in water until the tube is full, then close the stopcock. When exchanging water in the tank, simply open the outside valve.

A long, flexible, large-diameter hose can be attached to the end of the outlet valve. Adjustment of the height of the terminal end of the hose permits control of the water level in the tank. By lowering the end of the hose, the tank can be drained; in the elevated position water can be added into the tank.

Water System

As mentioned previously, the hatchery site should have available clean seawater that is free from silt. If the site is near a tidal flat, the intake pipe of the seawater pump should be extended well beyond the low tide level. Seawater should be taken in only during the high tide period. Asbestos or PVC pipes and valves should be used for the water pipe system. Galvanized iron or other metallic pipes should be avoided; partial use of metal-based connecting joints and valves is permissible (Fig. 3).

Preferably a rubber or plastic-lined water pump should be used to pump in seawater for the culture operations. The capacity of the pump depends on the size and number of tanks utilized as well as the maximum volume of water needed for culture operations.

In the culture techniques explained herein, no sand filter for the water system is needed. The seawater, when first pumped in at the start of the culture operation, should be filtered only through a filter bag made of plankton net material (165-micron mesh opening). During the mysis stages of the larvae a larger filter mesh size may be used; a 345-micron mesh is usually suitable for preventing the introduction of unwanted large organisms into the tanks (Figs. 1, 3).

A reliable source of freshwater, either the municipal water system of the area or a water well, is also necessary for hatchery operations. In case of high salinity seawater, the salinity may be lowered by addition of freshwater, preferably well-water.

Depending on the size of the culture facility, an adequate quantity of seawater should be stored for subsequent use and for flushing the culture tanks during harvesting. This reservoir should always be kept

full; any excess pumped in can be allowed to overflow and drain away.
The minimum useful reservoir size is about five tons.

Aeration System

Aeration is important not only in providing oxygen in the culture
water, but also in circulating the water in the tanks, in expelling
excessive ammonia, and in keeping food materials in suspension.

Ordinarily, air compressors are used in many culture institutions
because they are readily available, and provide high pressure air. How-
ever, compared with a blower having the same power consumption, a compressor's
air volume capacity is less. Frequent checks of the compressor's air tank
(which accumulates vapor condensation) and regular drainage are necessary.
Both Kittaka (1971) and Shigueno (1972a) recommended the "roots blower"
as a source of air for the aeration system because it provides oil-free air.
For culture tanks where the maximum water depth is 2 m, a low pressure,
high volume aerator may be used; a line pressure of about 0.2 - 0.3 kg/cm^2
has been suggested by Kittaka (1971). Since the blower purchased may have
more capacity than initially required, care must be taken to bleed out
excessive air to reduce back pressure and stress on the blower.

Another method for increasing water circulation and movement of
suspended food materials in the water column is through the use of air-
lift tubes. The best consist of polyvinyl chloride (PVC) pipes (>3 cm)
held vertically in the tank, extending from near the bottom to the water
surface. With an airstone positioned inside each piece of pipe at the
bottom, water may be lifted to the surface as the air is released by the
airstone. Many useful modifications of this general principle may be
made; a review of designs and operating efficiencies is given by
Spotte (1970).

The size of the aeration system depends on the extent of the
hatchery operations. As a guide, Kittaka (1971) has estimated that for

a 1 m deep tank, 0.036 m^3/min of air is required for every square meter of area. The range of aeration rates is 250 to 400 liters per minute in each 200-ton tank (Akazawa, 1973). A convenient way to measure air volume for each outlet of the aeration system is to immerse a pail, with volume markings, into the tank water, turn it upside down and introduce air into it for a known period of time. The volume may then be read from the markings in the submerged pail.

Regardless of the type of aeration provided, there should be two aerator systems, one electric-powered, and the other an auxiliary, diesel or gasoline-powered system, housed in one room. Caution must be taken to insure that the exhaust fumes are directed away from the blower or compressor room.

Stoppage of aeration is fatal to the larvae, and sudden power failures can be disastrous. Therefore, a battery-powered warning system must be installed to notify the culturist of any stoppage of the aeration system. For the compressor type, a warning system that is pressure-oriented to the air tank is required. For the blower type, a relay-type of warning system is needed.

Galvanized iron pipes, in part, may be used for the air pipe system; however, PVC pipe is more highly recommended. A 5.1 cm or 7.5 cm diameter pipe (Schedule 40) can be used for the main air supply system from the blower. This can then be branched into a multi-valved outlet system.

Airstones to be used in larval culture should be made of non-water-soluble materials. Inexpensive freshwater aquarium airstones usually are not adequate as they may dissolve in seawater. About 16 airstones (3 cm diameter and 9 cm long) made of carborundum grinding stone can be used in a 200-ton tank. These stones are hollowed inside, with an

inside-wall thickness of 0.5 cm. In this type of airstone, air is introduced into the hollowed inside through a plastic line; the orifice is stoppered with a rubber stopper. A carborundum airstone should be used with 9 mm (inside diameter) plastic tubing and a heavy sinker tied at the neck of the airstone (Fig. 4). Kittaka (1971) mentioned that this type of airstone may be used at a rate of one airstone per 5 - 10 m^2 of area.

If the smaller aquarium-type airstones are used, small air-line plastic tubing is needed since these require smaller outlets. About 40 - 60 small airstones with air-line tubing (4 mm inside diameter) will be required for a 200-ton capacity tank. Shigeno (1972) advocated setting one airstone for every 3 m^2 of tank bottom area. Akazawa (1973) used 25 airstones (5 in a row) in 200-ton tanks as an experimental control. The outside 16 airstones were about 80 cm from the wall and the rest of the stones were 2 m apart. Preferably, both the tubing and airstones should be securely anchored at the bottom of the tank (Fig. 5).

Recent research at the Tamano Seedling Center of the Seto Inland Sea Culture Fisheries Association (Akazawa, 1973) has shown that a rotor blade combined with a modified aeration system significantly improves larval culture techniques with respect to oxygen and nitrite levels, accumulation of tank detritus and survival rates in PL_1 to PL_{20}. In a square 200-ton tank, the rotor (8 m long) was suspended 5 - 10 cm above the bottom and driven at one RPM by an overhead motor. Air is released at the center of the tank (under the drive shaft) and all around a circle under the perimeter of the blade. Perforated air tubing was used for this purpose. This system has been particularly applicable when suspended foods, especially "bacterial floc", are used (Imamura and Sugita, 1972).
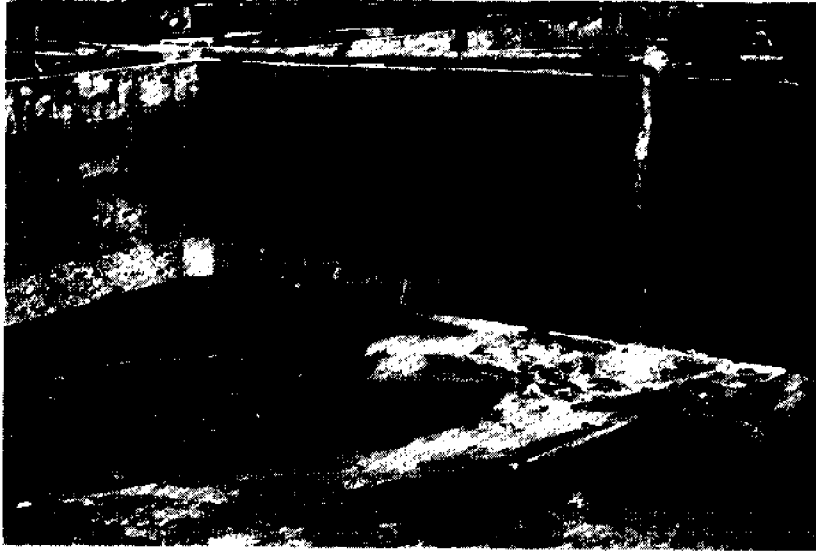
Figure 4. 200-ton tank with new culture water being added. Note the large airstones with anchors.

Figure 5. 200-ton tank with many small airstones and airline tubing securely attached to the tank bottom.

REPRODUCTION

Induced Gonad Maturation

One of the major problems in shrimp culture at present is how to
complete the organism's life cycle under culture conditions. Culturists
still rely on the wild stock as a source of the gravid females needed
for spawning. Recently, however, attempts have been made to induce
maturation in the shrimp in captivity. Gonad maturation and subsequent
spawning have been achieved in Penaeus japonicus with several generations
reared, but the number of eggs spawned has been much less (Maeda, personal
communication). Hara, et al (1969) and Maekawa (1970) reported that
second generation seedlings of P. orientalis were produced at the
Yamaguchi Prefectural Marine Seedling Center. Of the seedlings produced,
4,800 were used in planting studies in the Inland Sea area. P. latisulcatus
was reared in indoor tanks, induced to spawn and a successful larval
culture was achieved by Shokita (1970). Griessinger (1975), with the
C. O. P. Aquaculture Team in French Polynesia, reared postlarvae
and juveniles of five species of shrimp from different areas to maturity
in the laboratory. They reported about one thousand spawnings and two
generations in captivity for P. merguiensis in one year. Spawning of
P. japonicus and Metapenaeus ensis were also achieved by the team;
twenty spawnings of P. aztecus occurred only after removal of one eyestalk.
Liao (1973) successfully reared P. penicillatus and P. monodon to sexual
maturity. The latter species spawned but the eggs did not cleave.

21

Collecting and Transporting Gravid Females

Live ovigerous female shrimp may be obtained either by commercial trawling or by the use of gill-nets. The females should be collected from the best fishing grounds.

Where ovigerous females can be caught in shallow bay areas during the spawning season, as in the case of P. japonicus, gill-nets can be used effectively. These nets measure 5 m X 90 cm each, and about 50 are used by each boat. Twenty nets are joined to make a single line and the other 30 connected together in a second line, to form a nearly T-shaped structure. The nets are set at sunset, and the catch is checked at midnight and at sunrise. Gravid females are then placed in live-baskets made of netting.

Commercial trawling is usually used in the collection of gravid females both in Japan and in the United States. Long trawling times should be avoided. Cook and Murphy (1969) suggested 10 to 30 minutes towing time, depending on the amount of fish and trash taken along with the shrimp; Mock and Murphy (1971) considered towing times of 10 to 20 minutes to be sufficient.

As soon as the catch is taken aboard the boat, the wriggling, live shrimp may be put immediately in large tanks containing clean seawater, and the selection of gravid females made later, or, if enough skillful men are aboard, large ovigerous females may be picked out immediately and placed in aerated holding tanks.

When required, water in the holding tank may be chilled with ice in one-gallon plastic canisters with tight-fitting caps. Tabb, et al (1972) reported successful transport of up to 55 females per 75-liter plastic drum during an 18 hour period using this method. During warm weather, water temperature should not exceed 24°C; cooling of the water

22

can be achieved by placing plastic bags of ice in the container (Mock and Murphy, 1971). In Japan, P. japonicus are usually collected during the night and early morning, put into live-cages, and then brought to the market that morning where they are placed in cement tanks containing water chilled to 16 to 18°C; shrimp culturists then make their selection.

Aeration should be provided in all holding containers. It is advisable to have a portable automobile-battery-powered aerator on board. The boat's generator may also be used as a source of power for the aerator. To avoid stressing the shrimp, frequent water exchanges should also be made, especially during long distance transport.

In Japan, for short distance transport, about 20 to 30 ovigerous females are placed in a collapsible, two-ring-frame net-basket (cf. Figs. 6, 7), approximately 30 cm diameter X 40 cm high, made of fishing net material (about 1 cm mesh opening). The baskets are put inside holding tanks containing chilled seawater. For long distance transportation the shrimp are packed in dry, chilled sawdust. In midsummer, if the water or sawdust temperature is brought down to 14-15°C and overcrowding is avoided, the gravid shrimp can withstand 10 to 12 hours in transit. There is not much difference in survival rates between the two methods, but the method using tanks with chilled seawater is recommended (Maeda, 1968).

When gravid females were in short supply, spawners were imported from Taiwan to the different Seto Inland Sea Seedling Centers. Over a three-year period, a total of 1,572 females was imported. These were packed in dried and chilled sawdust surrounded by bags of ice. Shipment by air required approximately 28 hours, not including the time for collection, packing or release. At 10 to 15°C, 1,275 animals survived;
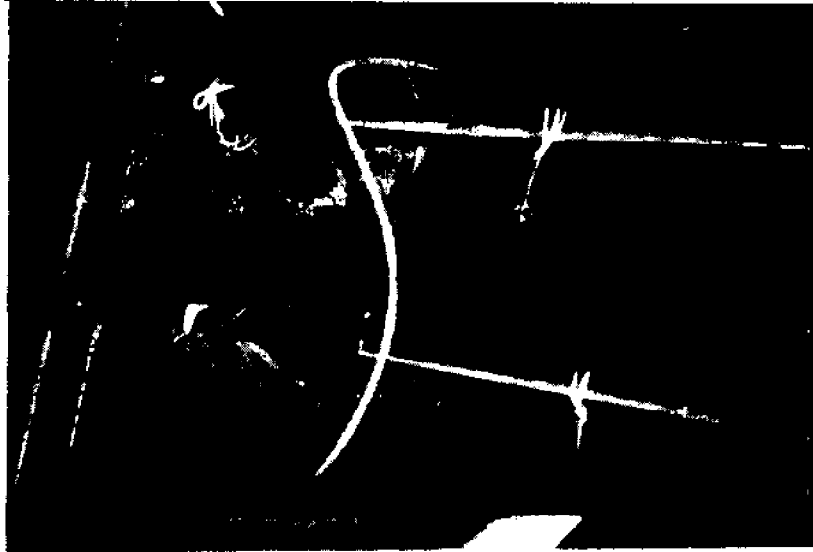
Figure 6. Gravid females (Penaeus japonicus) are placed in the net-basket
for transport. The net-baskets are hung in a chilled seawater
tank. Note the protein bubbles on the surface, which indicates
that some of the females have spawned.
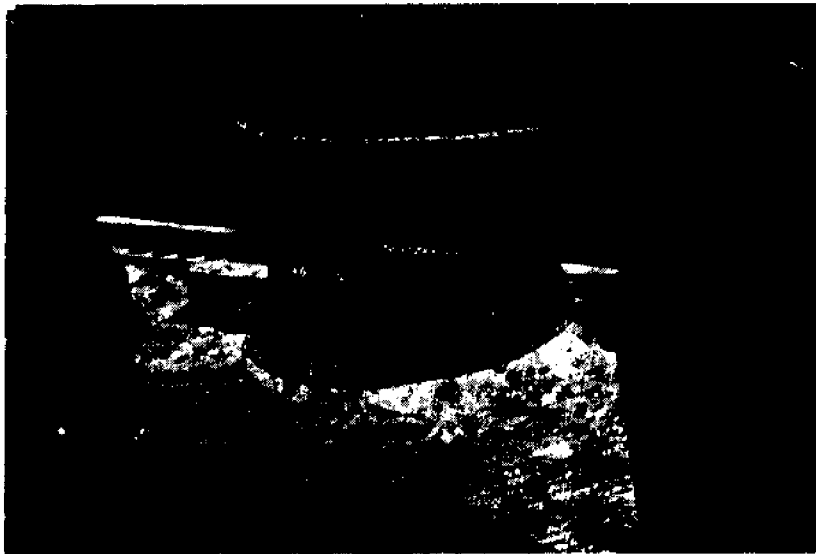
Figure 7. Gravid females purchased for spawning. Note the size of the
females. These shrimp were transported in chilled seawater in
the wooden pail shown at the left.

however, only 411 or 32% spawned (Imamura, 1974).

During transportation, sudden temperature change and unnecessary shock or agitation should be avoided, as these can cause the abnormal spawning of unfertilized or coagulated eggs. If possible, after arrival at the hatchery, the females should be kept in running seawater tanks in a dark place and allowed to rest for several hours before being put into culture tanks (Maeda, 1968). For a better spawning rate, the collected females should be placed in the culture tanks by the evening following collection (Oshima, 1969).

During the transportation of the gravid females, or while in holding tanks of chilled seawater, some individuals may spawn. Spawning can be readily recognized by the presence of large, protein bubbles (Fig. 6). Since nothing can be done about this unscheduled spawning, it is best to leave the females undisturbed and to change the water periodically.

If the shrimp are brought into the hatchery in an unrestricted container, they should be collected by dip-net and placed into the culture tank. If the net-basket is used, the shrimp may be taken out of the basket individually or all the females may be released into the culture tank simultaneously.

Determining Ovarian Maturation

The coloration of the ovaries is very important in determining the degree of maturity of the female penaeid shrimp. The color of the ripe ovaries of thirteen penaeid species, as reported by various workers, is shown in Table 1. The differences in the color in the same species may represent the subjective interpretation of the individual authors. Generally, dark green or dark greyish-green is the typical color of the ripe ovary. Brown and Patlan (1974) have provided good color photographs showing the distinct coloration of the matured ovary in Penaeus aztecus.

Sometimes, the coloration is not distinct. The degree of maturity also can be determined as follows: with the left hand, gently hold the shrimp at the anterior part of the carapace; to calm the animal down, the shrimp's eyes should be hidden within the palm. With the right hand, hold the abdomen of the shrimp, then slowly and gently bend the shrimp between the carapace and the abdomen. Check the median lobe of the ovary through the thin membrane. A well-developed gonad appears as a dark and broad lobe and the ova have a granular texture. This method of detecting gonad maturation is also mentioned by Liao and Huang (1972).

Ovarian maturity also may be checked by holding the shrimp against a well-lighted background and looking through the dorsal part of the abdomen. Observe the shape of the posterior lobe of the ovary. Usually, a developing ovary has a narrow, tapering, light-colored lobe. A ripe or well-matured ovary has a wide and dark lobe with distinct margins. Certain species or individuals within a species, in addition to having dark and wide lobes, may have noticeable, nearly oblong bulges in

26

TABLE 1. Color of ripe ovaries in penaeid females.

Species	Color	Reference
Penaeus		
aztecus	olive-green green to dark green golden brown	Cook and Murphy (1969) Brown and Patlan (1974) Perez-Farfante (1969)
duorarum	olive-green dark greyish green	Cook and Murphy (1969) Perez-Farfante (1969)
japonicus	yellow-green or blackish grey dark green or black-grey dark yellow or dark green	Maekawa (1961) Maeda (1968) Liao and Huang (1972)
merguiensis	olive-green	Tuma (1967)
monodon	deep brownish green greyish green	Villaluz et al (1969) Liao and Huang (1972)
orientalis	greenish	Oka (1970)
schmitti	drab olive or brownish yellow-green	Perez-Farfante (1969) Pinto and Ewald (1974)
semisulcatus	greyish green	Liao and Huang (1972)
setiferus	olive-brown drab olive-brown	Cook and Murphy (1969) Perez-Farfante (1969)
teraoi	light or dark brown	Liao and Huang (1972)
Metapenaeus		
monoceros	grass green	Liao and Huang (1972)
joyneri	grass green dark green	Liao and Huang (1972) Lee (1968)

the ovarian lobe, especially at the first abdominal somite. The
spent ovary is broad but appears empty and is translucent. A schematic
drawing of the various stages of the ovarian maturity as noted for
this method is shown in Figure 8.

Sometimes, even after careful scrutiny for ovarian maturation,
where the selected ovigerous females have met the above requirements,
some individuals will not spawn after being placed in the culture tank.
This is probably related to some kind of physiological stress. Matsunaga
(1973) discussed the collection of 10,495 gravid females from different
localities by four of the Seedling Centers. The females were carefully
selected for ovarian color, shape and form, and overall vitality. Even
with such selection, only 50% spawned, and of those spawning, 15%
were partial spawnings only. He concluded that the criteria for the
selection of gravid females is poor at present.
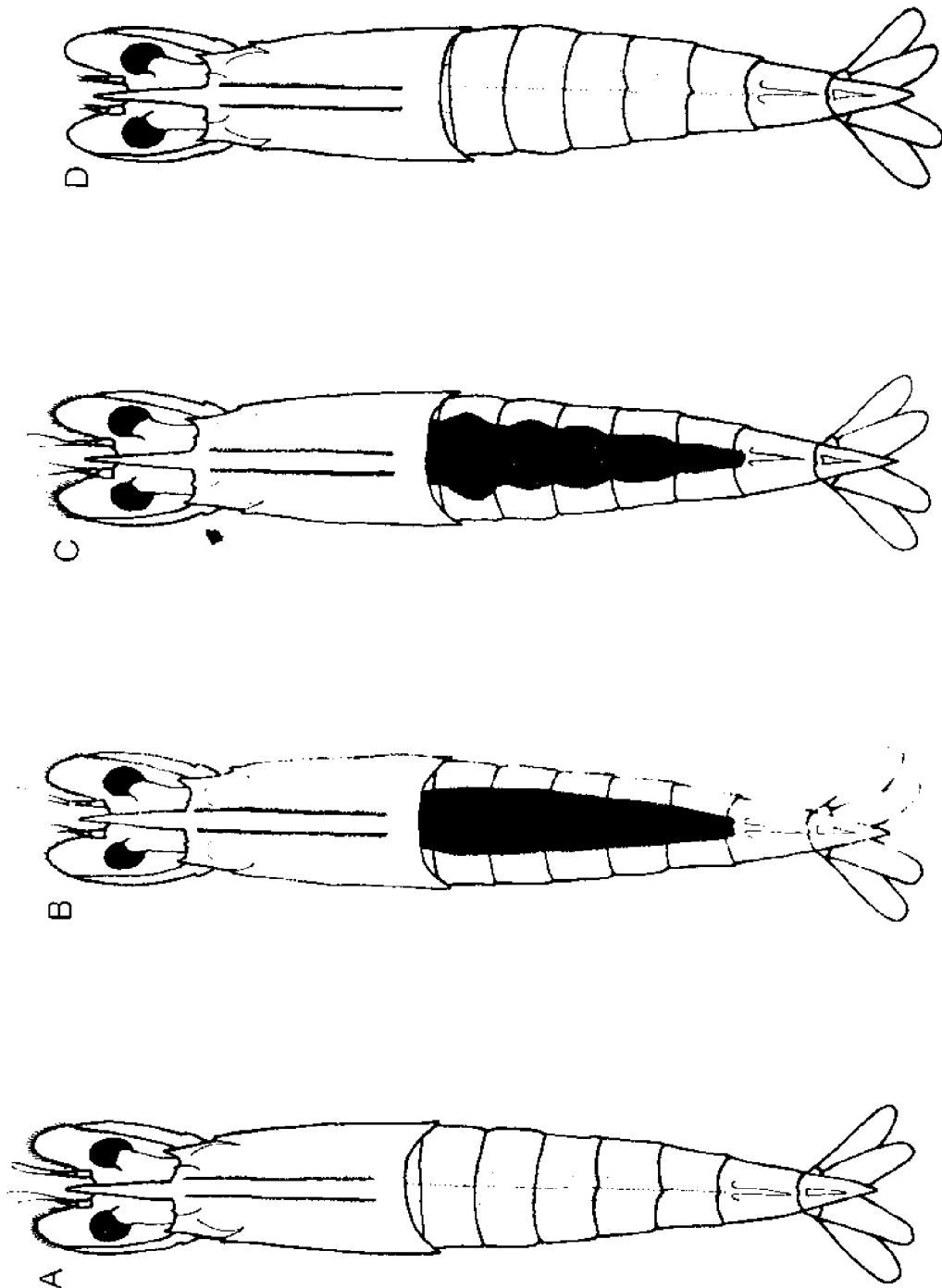
Figure 8. Schematic view of the developmental stages of ovarian maturity
(indicated by stippled area) in female penaeid shrimp (dorsal
view): A) developing ovary, ovarian lobe is narrow; B) ripe
ovary, lobe is broad and dark; C) ripe ovary, broad lobe bulges,
especially at first abdominal somite; D) spent ovary, lobe is
broad but translucent.

Conditions Governing the Number of Spawners to be Placed in a Tank

Several factors must be considered in determining the number of ovigerous females to be placed in the tank. The number of larvae that will hatch out will vary with the size of the spawning females, the period within the spawning season and the physiological well-being of the spawners.

Cook (1969) reported that only about 33% of ovigerous shrimp produce viable eggs. Shigeno (1969) pointed out that for each batch of gravid females brought in for spawning purposes, spawning can be expected from 30 to 68% of the shrimp from May to September. An average spawning of 52% can be expected from a size-group of females having an average body weight of 89 g. Maekawa (1961) noted that the size range of spawning females of P. japonicus was from 4.0 to 8.0 cm in carapace length. The major spawning group had a range of 4.5 to 6.5 cm, although females with the highest percentage of spawning in the group were in the 6.0 to 6.5 cm range.

Oshima (1972) stated that the average spawning rate of P. japonicus, e.g., 3,818 good females purchased by Tamano Seedling Center in 1970 and 1971, ranged from 30 to 40 percent. The gravid females collected at a nearby locality had a better spawning rate than females transported from remote distances. Differences in spawning rates were also noted during the spawning season; a higher rate was observed in the later part of the season.

Kureha and Nakanishi (1972) observed two spawning groups of P. japonicus in the Seto Inland Sea which they catagorized as the "large group", with females having an average body weight of 100 g, and the "small group", with the body weight of females averaging only 70 g. Forty-six percent of the large individuals usually spawned, whereas 57% of the smaller females generally spawned. Although the percentage of larger females spawning was lower, the number of nauplii produced was greater. Generally the larger females

30

produced up to 400,000 nauplii, whereas the smaller females produced only 200,000. In the author's experience, smaller specimens of P. duorarum have higher percentages of spawning than do the larger females. Oshima (1972) gave a relationship between the size (by body weight) of gravid females of P. japonicus and the number of nauplii hatched (Figure 9). The number of nauplii produced varied from 200,000 to 700,000. Although the more easily obtainable small ovigerous females had a higher spawning rate, Oshima noted that the nauplii produced by small females was only one-third to one-half the number produced by the large females.

Tables 2, 3 and 6 give examples of the number of ovigerous females used by Japanese culturists in their spawning tanks. In the first successful experiments in large-tank culture systems, Hudinaga and Kittaka (1967) found that an average of 69 females was needed to produce 800,000 or more postlarvae in a 200-ton tank. Maeda (1968) recommended 80 to 100 females for each 200-ton tank during the midseason of spawning (July-August); Shigeno (1969) suggested 30 to 50 females for a 57-ton tank and 50 to 100 shrimp for a 200-ton tank. For a 60-ton tank, Shigeno (1972b) recommended 30 ovigerous females. Kureha and Nakanishi (1972) used 381 to 814 spawners in their extra large 2800-ton tank culture.

The number of ovigerous females to be used to obtain the desired nauplii population should be determined by the size, maturity and general health of the females and by the period within the spawning season in which they are taken. When this information is known for the particular species under culture, it is possible to estimate the stocking density of the spawners more accurately.
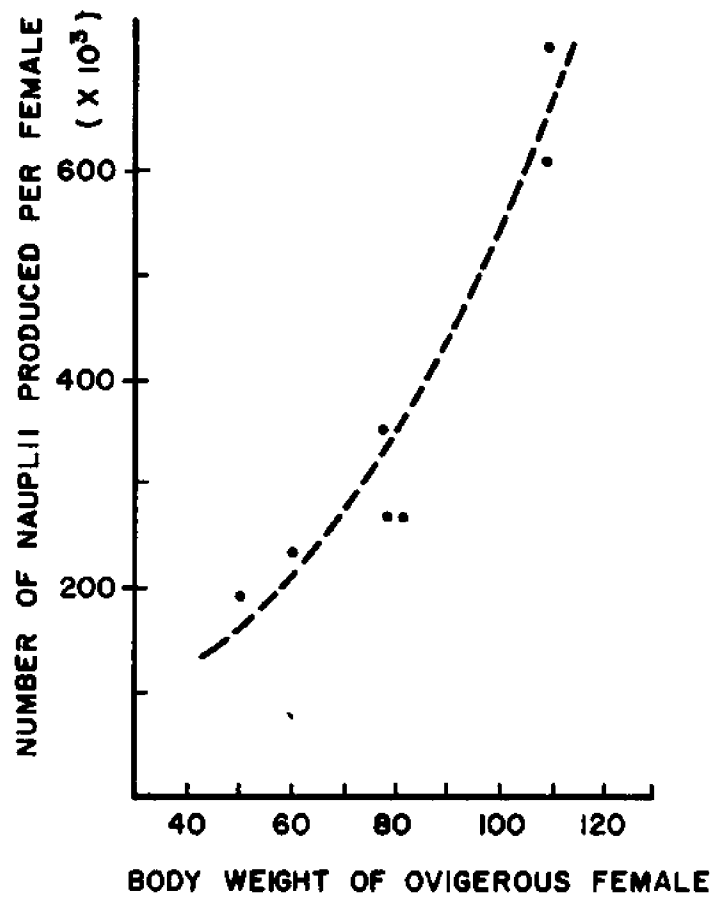
Figure 9. Relationship between body weight of ovigerous females and number of nauplii hatched from the eggs produced by the females (after Oshima, 1972).

TABLE 2. Fecundity of some cultured penaeid shrimp as reported by various workers.

Species	Number eggs spawned per female	Female length/weight	Reference
Penaeus			
japonicus	700,000	20 cm	Hudinaga (1942)
	78,760-555,910	17 - 19 cm	Maekawa (1961)
	1,000,000	>100 g	Miyamura (1967)
	230,000-559,000	--	Kittaka (1971)
	300,000	--	Liao and Huang (1972)
monodon	500,000-1,000,000	--	Villaluz and Villaluz (1971)
	300,000	--	Liao and Huang (1972)
orientalis	100,000	--	Oka (1967a)
semisulcatus	300,000	--	Liao and Huang (1972)
teraoi	300,000	--	Liao and Huang (1972)
Metapenaeus			
joyneri	100,000	--	Liao and Huang (1972)
monoceros	100,000	--	Liao and Huang (1972)

TABLE 1. The number of ovigerous females for optimum production of P. japonicus postlarvae (PL$_{20}$) in different size tanks. The percentages of survival for each stage and for cumulative survival.

Author	Maeda (1968)			Maeda (1968)			Maeda (1968)			Shigeno[+] (1969)			Hudinaga & Kittaka (1967)			Maeda (1968)			Oshima[+] (1972)			Oshima[++] (1972)		
Tank size (M³)	0.5			1-2			4-5			57			200			200			120-200			2800		
Recommended no. of females	1-2 2-3* 2-3**			2-5 4-10* 4-10**			8-10 20-25* 15-20*			30-50			69			80-100			60-101			381-814		
Stage	Pop./M³ x 10³	Survival Stage %	Cum. %	Pop./M³ x 10³	Survival Stage %	Cum. %	Pop./M³ x 10³	Survival Stage %	Cum. %	Pop./M³ x 10³	Survival Stage %	Cum. %	Pop./M³ x 10³	Survival Stage %	Cum. %	Pop./M³ x 10³	Survival Stage %	Cum. %	Pop./M³ x 10³	Survival Stage %	Cum. %	Pop./M³ x 10³	Survival Stage %	Cum. %
Nauplius	40.0	-	100	50.0	-	100	60.0	-	100	41.3	-	100	20.5	-	100	40.0	-	100	54.9	-	100	10.3	-	100
Zoea	30.0	75	75	35.0	70	70	35.0	60	60	-	-	-	-	?	-	20.0	50	50	-	-	-	-	-	-
Mysis	24.0	80	60	28.0	80	56	26.0	70	72	-	-	-	-	?	-	14.0	70	35	-	-	-	-	-	-
Early postlarva	22.0	90	54	23.0	80	45	20.0	80	34	16.4	-	39	-	-	-	11.2	80	28	-	-	56	-	-	42
Late postlarva	20.0	90	49	20.0	85	38	16.0	85	28	7.6	42	23	5.0	-	24	9.0	80	22	10.5	-	19	4.2	-	32

*Early season (March - May).

**Late season (September - October).

[+]Total of 15 rearing records; included two 200-ton tank cultures. Harvested age ranges from PL$_8$ to PL$_{20}$.

[‡]Included two 120-ton, two 170-ton, and three 200-ton tanks.

[++]Review of results from three 2800-ton tank cultures.

Spawning

Of the various species of shrimp, the spawning behavior of P. japonicus has been studied in the most detail. Early in the season, the majority of P. japonicus spawn in May, within two to three days after being placed in the culture tanks; by June spawning occurs in the first three days; in July to September, the majority of females spawn on the day they are placed in the tanks (Hudinaga and Miyamura, 1962).

Spawning usually occurs at night; however, the spawning hour also shifts as the season progresses. The majority of P. japonicus females spawn between eight and ten o'clock in the evening during the early part of the season; by September most shrimp spawn between two and four o'clock in the morning (Hudinaga and Miyamura, 1962). Occasionally females spawn in the daytime when they are placed in the tanks. This has been observed for P. japonicus in large-tank culture and for P. duorarum.

Observations of small-tank cultures have shown that during spawning, the female starts to release the eggs while swimming sideways along the walls of the tank; sometimes the female will spawn while moving up and down in the water column along the wall. In a small tank, the whitish eggs can be easily seen against a black background.

The eggs are planktonic for approximately 30 minutes after spawning; after the formation of the fertilization membrane, they become demersal. Since the eggs are quite delicate, FEEBLE AERATION of the culture water is required until the eggs hatch. Aeration will prevent unnecessary damage to the eggs and allow them to settle on the bottom quickly.

In large-tank cultures, tank size makes it difficult to observe the actual spawning, and abnormal spawning by some individuals may be overlooked.

The appearance of foam-like mucoid bubbles on the water surface is an indication that spawning has taken place (Fig. 6).

Maeda (1968) noted that during the peak season, delayed spawning may occur in some individuals. Eggs from such individuals usually have a lower hatching rate. Therefore, unless there is a problem in obtaining ovigerous females, late spawners should be discarded.

On the morning following confirmation of spawning, the females should be removed with long-handled dip-nets. A small cage may be floated in the tank to hold the shrimp as they are recollected. This floating cage is particularly useful in large culture tanks. The recollected females should be carefully recounted so that no females remain in the culture tank. It is especially important to carefully check the drain area and around the aeration stones. For ease in recollecting, a large cage-type net may be placed in the culture tank; the ovigerous females are released into this cage and recollected after spawning by pulling the entire net out of the culture tank.

Eggs

Even in the same species, the number of eggs spawned varies with the size of the females, the season, the locality, transportation methods used and the individual physiological conditions of the spawners. Maekawa (1961) noted that the hatching rate of eggs of P. japonicus differed with each month of the spawning season; the average hatching rate was 30.6%. The hatching rate was 38.5% in June (temperature: 19.0 - 24.8°C); 36.0% in July (23.1 - 27.8°C); 21.2% in August (23.7 - 28.2°C); 26.4% in September (23.1 - 23.9°C); and 53.8% in October (20.6 - 21.3°C). His basic experiments also indicated that differences in hatching rates were dependent upon the kind of culture water used. He obtained 37% hatching in raw seawater, 99% in sand-filtered seawater and 100% hatching when a chelating agent (EDTA) was added to the seawater and the culture medium then filtered. Cook (1969) also mentioned that the use of EDTA (1 g/ 100 l) in culture water resulted in no failure of hatching or unusual mortality in the larvae. Shigueno (1972b) mentioned that the average hatching rate was approximately 50%.

Differences in spawning time result in some differences in the embryonic stages. Observe the embryonic development while the nauplius is still in the egg; movement of the nauplius indicates that hatching is soon to follow. The embryonic duration of some cultured penaeid shrimp is tabulated in Table 4. Depending on temperature, most penaeid shrimp eggs have a range of 12 - 19 hours before hatching. Penaeus orientalis requires 37 hours to hatch; this is a generally temperate species that requires a lower temperature for development.

The uneven distribution of the spawned eggs on the bottoms of the tanks makes egg population estimates difficult, particularly in large tanks.

TABLE 4. Duration of larval stages of some penaeid shrimp as reported by various workers.

| Species | Temperature Range (°C) | Stage duration in hours (average/range) | | | | TOTAL Larval Hours | Reference |
		Egg	Nauplius	Zoea	Mysis		
Penaeus							
aztecus	28	12-14	--	--	--	--	Neal (personal communication)
	26 - 28	--	90 / 83-91	178 / 143-234	157 / 137-226	425 / 363-551	Krantz and Norris (MS)
	23 - 25	--	55.5 / 51-57	183 / 146-217	132 / 116-185	370.5 / 313-459	Krantz and Norris (MS)
		36	55	110	72	237	Mock and Murphy (1971)
duorarum	23 - 25 (outdoor tanks)	19	64 / 63-65	118 / 112-149	87 / 84-89	269 / 259-304	Tabb et al (1972)
	27 - 28 (outdoor tanks)	13	41	94 / 93-97	78 / 77-88	213 / 211-226	Tabb et al (1972)
	27 - 28 (indoor tanks)	14 / 13-15	44 / 37-50	129 / 100-169	91 / 58-118	264 / 195-337	Tabb et al (1972)
japonicus	27 - 29	13-14	36-37	120	120	276-277	Hudinaga (1942)
latisulcatus	28.8-29.7	12	37	71	72	180	Shokita (1970)
monodon	27 - 29	12-16	48-53	120-144	120-144	288-341	Villaluz et al (1971)
orientalis	18 - 21	37	111	202	161	474	Oka (1967b, 1970)
californiensis	27 - 28		44 / 37-49	135 / 120-180	83 / 72-96	262 / 229-325	Krantz and Norris (MS)
setiferus	27 - 28			95 / 71-147	117 / 96-125		Krantz and Norris (MS)

As mechanical disturbance of the eggs can be harmful, only a single egg sample for population estimates is recommended. As an aliquot of the eggs spawned will not necessarily reflect the population accurately, aliquot samples of the newly hatched nauplii are usually used as a standard estimate of the population density.

In the indoor small-tank culture system used at the Gulf Coast Fisheries Center, Galveston, Texas, Cook and Murphy (1969) maintained a nauplii population at a concentration of 266 nauplii per liter. Mock and Murphy (1971) reared the equivalent of 256 nauplii per liter and harvested PL_1 with a survival rate of 76%.

In large-tank culture, as may be seen in Table 3, both Maeda (1968) and Shigeno (1969) had initial nauplii populations of 40,000 per m^3. Similarly, Kureha and Nakanishi (1972) considered 40,000 nauplii per m^3 the standard initial population of P. japonicus for a 200-ton tank. Oshima (1972), in his review of the Japanese Inland Sea Seedling Center results, calculated that the average initial nauplii density was 50,000 per m^3. However, populations of nauplii in excess of 50,000 per m^3 did not necessarily result in increased yields per m^3. It is apparent that 40,000 to 50,000 nauplii per m^3 are optimal with the present culture techniques.

TABLE 5. Number of larval substages of penaeids reared
experimentally or commercially from known parentage.

Species	Nauplius	Zoea	Mysis	Reference
Penaeus				
aztecus	5	3	3	Cook and Murphy (1971)
brasiliensis	5	3	3	Lares (1974)
californiensis	5	3	3	UM Turkey Point data
duorarum	5	3	3	Dobkin (1961)
	5	3	2-5*	Ewald (1965)
japonicus	6	3	3	Hudinaga (1942)
kerathurus	5	3	3	San Feliu et al (1973
latisulcatus	6	3	3	Shokita (1970)
monodon	6	3	3	Villaluz et al (1969)
occidentalis	5	3	3	UM Turkey Point data
schimitti	5	3	3	Pinto and Ewald (1974)
semisulcatus	6	3	3	Liao and Huang (1972)
setiferus	5	3	2	Johnson and Fielding (1
	5	3	3	UM Turkey Point data
teraoi	6	3	3	Liao and Huang (1972)
Metapenaeus				
joyneri	6	3	3	Lee and Lee (1968)
monoceros	6	3	3	Liao and Huang (1972)
dobsoni	3	3	3	Menon (1952)**
affinis	6	3	3	Hudinaga (1941)***

*Due to effect of temperature on development.
**Cited by George (1970a)
***Cited by George (1970b)

-40-

LARVAL STAGES AND THEIR RECOGNITION

All penaeid species pass through three distinct larval stages, the naupliar, the zoeal, and the mysis, before metamorphosing into post-larvae. The naupliar stage subsists on its own yolk, and consequently does not feed. The zoea is a filter feeder, feeding on phytoplankton and occasionally on small zooplankters such as rotifers, or on newly hatched Artemia nauplii. The mysis stage usually feeds both on zooplankton and phytoplankton, or can develop adequately on an exclusively Artemia diet.

Data on the larval development of penaeids, which have been reared experimentally or commercially from known parentage, have been published for 12 species of Penaeus and 4 species of Metapenaeus (Table 5). It is interesting to note that the Indo-Pacific species have 6 naupliar sub-stages, whereas the New World species have only 5 substages. A general pattern of three substages each of the zoeal and mysis stages is universally reported. The duration of each larval stage or substage varies with the species and rearing temperature. Table 4 presents a summary of the durations recorded for these species.

Naupliar Stage (Figs. 10, 11)

The characteristics of naupliar stages representing 5 naupliar sub-stages (P. duorarum) and 6 naupliar substages (P. japonicus) are given below. Characteristics used in the identification of the individual sub-stages are listed in the legends of the figures (Figs. 10, 11) and indicated on the figures by arrows.

Figure 10. Naupliar substages of Penaeus duorarum: a_1--first antenna; a_2--second antenna; en--endopod; ex--exopod; fr--frontal organs; fu--turca; lm--labrum; md--mandible; mxl--first maxilla; mx2--second maxilla; mxpl--first maxilliped; mxp2--second maxilliped; o--oscellus; 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 antenna.

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 11. Naupliar substages of _Penaeus japonicus_ (from Hudinaga, 1942).

a) Nauplius I: Body pear-shaped.

b) Nauplius II: 1 long and 2 short terminal setae on 1st antenna.

c) Nauplius III: 2 distinct furcal processes, each with 3 spines (1);
                  buds of 1st and 2nd maxillae and maxillipeds
                  appear (2).

d) Nauplius IV: Each furcal process with 4 spines (1); exopod of
                  2nd antenna segmented (2).

e) Nauplius V: Swollen knoblike structures at bases of maxilli-
                  appear (1); frontal organs present (2).

f) Nauplius VI: Mastigatory processes well developed.

Characteristics of Naupliar Stages of P. japonicus[1] and P. duorarum[2].

	Setae of Antenna 1		Setae of Exopod Antenna 2		Furcal Spine Formula		Size Range	
	P. japonicus	P. duorarum	P. japonicus	P. duorarum	P. japonicus	P. duorarum	P. japonicus	P. duorarum
N-I	7	5	5	5	1+1	1+1	0.30-0.34	0.35-0.40
N-II	7	6	6	6	1+1	1+1	0.33-0.35	0.40-0.45
N-III	7	5	7	7	3+3	3+3	0.35-0.38	0.45-0.49
N-IV	7	5	9	8	4+4	5+5	0.38-0.42	0.48-0.55
N-V	7	8	10	9	6+6	7+7	0.42-0.45	0.53-0.61
N-VI	8	-	12	-	7+7	-	0.48-0.51	-

[1]Hudinaga (1942)

[2]Dobkin (1961)

The nauplii hatch from the egg 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). The nauplii are strongly phototropic and swim in the direction of a light source; however, they do avoid direct sunlight. 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.

Zoeal Stage (Fig. 12)

The size ranges given in the diagnoses are based on measurements of P. japonicus and P. duorarum. As in the illustrations of the nauplii, characters used in identifications of the zoeal substages are indicated by arrows.

Z-I.   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.

Z-II.   1. Body length 1.33-2.13 mm.

        2. Stalked eyes present.

        3. Rostrum developed.

        4. Supraorbital spines developed.

        5. Abdominal segmentation apparent.

Z-III. 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.

Swimming in the zoeal stages is accomplished with the first and
second antennae, as in the nauplier stage, but these are now aided by the
well-developed first and second maxillipeds. The swimming stroke is slower
than that of the nauplii, and movement appears less jerky. Characteristic
of the zoeae is their continuous feeding. The culturist can ascertain
feeding in the zoeae by the quick contractions of the digestive tract and
the long trail of feces. Active feeding and a continued prompt response
to a light source are indications of healthy zoeae. Toward the end of the
last zoeal substage, the body becomes slightly flexed.
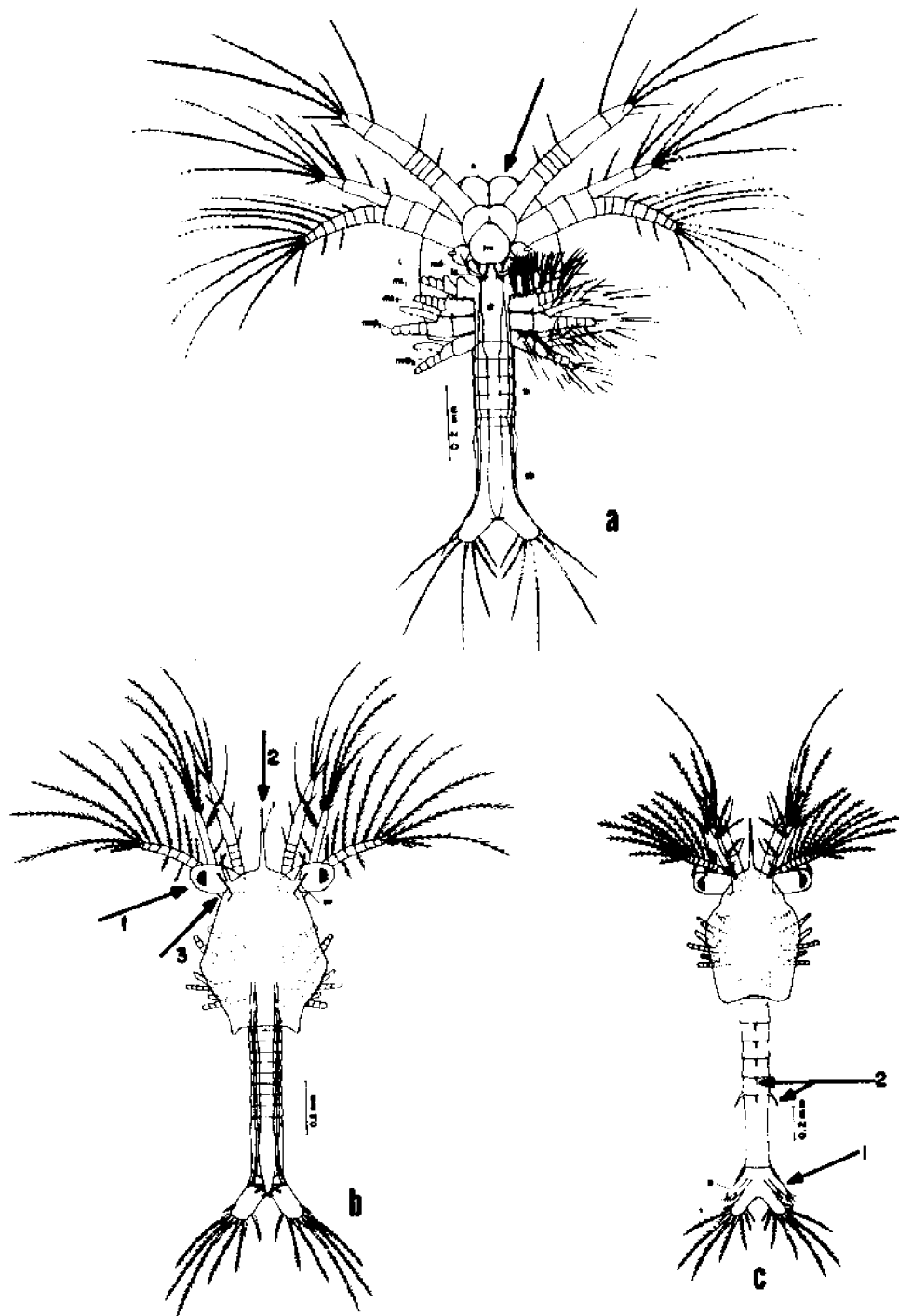
Figure 12. Zoeal substages of _Penaeus duorarum_: ab--abdomen; c--carapace; dt--digestive tract; e--eye; la--labium; lm--labrum; md--mandible; mxl--first maxilla; mx2--second maxilla; mxpl--first maxilliped; mxp2--second maxilliped; r--rostrum; su--supraorbital spine; th--thorax; u--uropod (from Dobkin, 1961).

a) Zoea I:

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

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

Mysis Stage (Figs. 13 a-c)

M-I.   1. Body length 2.67-3.40 mm.

        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.

M-II.   1. Body length 2.99-3.90 mm.

        2. Unsegmented pleopod buds present.

M-III. 1. Body length 3.70-4.52 mm.

        2. Pleopods developed, segmented.

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.

Postlarvae (Fig. 13d)

PL$_1$.   1. Body length 4.79-5.00 mm.

        2. First three pairs of pereiopods chelate.

        3. Pleopods with setae.

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 PL$_7$
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.

47
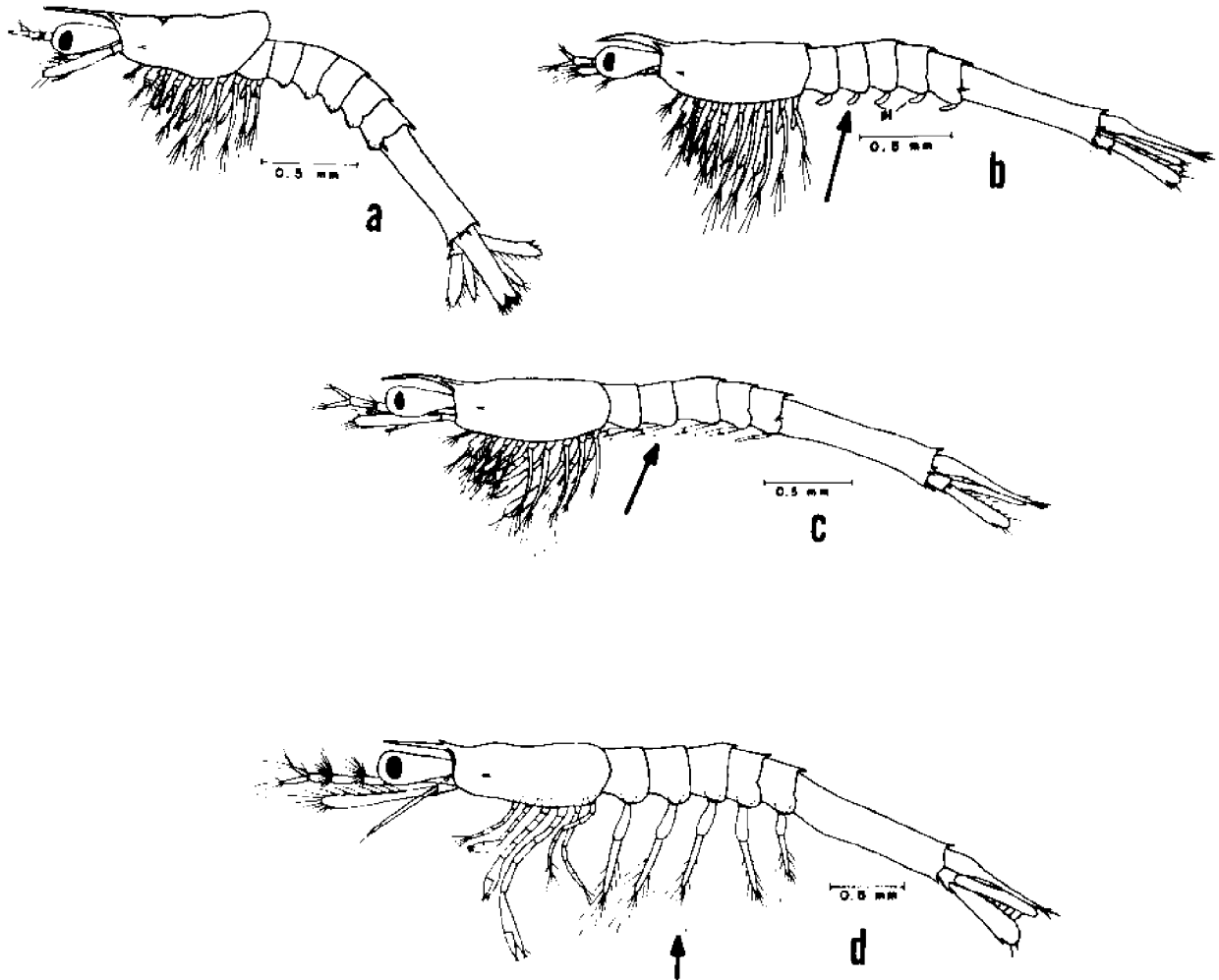
Figure 13. Myses and Postlarval substages of _Penaeus_ _duorarum_: pl--pleopod
(from Dobkin, 1961).

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 (PL_1): Swimming setae present on pleopods.

Seedlings

The harvest time of postlarvae varies with individual culturists. As a result of the initial work of Hudinaga and Kittaka (1967), most Japanese culturists harvest about PL_{20}. Villaluz et al (1969) have reported harvesting at PL_{29}, and Oshima (1972) has recommended PL_{30-35}. In contrast, some culturists prefer to harvest early postlarvae (Cook and Murphy, 1969; Mock and Murphy, 1971; Mock, 1974). An early harvest requires that the postlarvae be cultured in low density nursery ponds until the desired planting size has been reached. Kurata (1973) and Setoguchi et al (1974) have recommended that seedlings have a body length of 9-12 mm and an average body weight of 10 mg. Oshima (1972), on the contrary, has stated that seedlings with a body weight of 8-10 mg are somewhat small to be planted in the sea, and has suggested that seedling body weights should be 12-14 mg before planting.

ESTIMATION OF POPULATION

Population estimation is one of the most important factors in routine culture. Since the feeding schedule must be correlated to the population density, estimations of such density are a key point in successful penaeid shrimp culture. There have been attempts to make electronic counters for population estimates (such as _Artemia_ counters), but their accuracy is still questionable and their cost unreasonable. There are too many variables in light levels and particle sizes in shrimp culture to make the present-day counters useful. An additional drawback is that the electronic counter cannot judge the viability of the organisms, which is also an important factor in determining optimum population densities. At present, population estimates by sample aliquots are still the best.

An aliquot sampler can be easily made by attaching a plastic, one-liter beaker to the end of a long stick; the beaker is dipped into the middle of the water column and the sample removed. The sample then is transferred to a glass beaker for observation. Avoid excessive loss of shrimp due to any sampling technique.

The following techniques are helpful:

(1). The population should be checked early in the morning when the light distribution throughout the tank is even.

(2). Aliquot sampling points should be placed diagonally across the tank. Where permissible, a cat walk across larger tanks is very convenient for sampling. Such a structure should not cast excessive shade on the tank.

(3). In the naupliar and zoeal stages, counts conveniently can be made in a large eye dropper with a long glass tube. The count is

usually made at tank-side and the aliquot sample returned to the tank.

(4). Myses and early postlarvae can be easily counted in a small container painted white inside (an ordinary teacup is satisfactory). This estimate for the M-III and PL_1 is used as the standard estimate of population density until harvest.

(5). As many difficulties and inaccuracies are encountered in the population estimates of settled postlarvae after PL_4 as are encountered in the previously mentioned egg counts. In certain tanks equipped with windows, it may be possible to determine the population density and behavior of the settled postlarvae. A flat-sided dip-net may be used to sample the settled population and obtain a general estimation of density. If the population estimate discloses an over population, one should be prepared to divide the population into separate tanks.

In addition to an estimate of population density, the aliquot provides a convenient means for making the following important observations:

(1). The healthiness of the animals as indicated by their swimming activity and by phototaxis in the naupliar and zoeal stages.

(2). The degree to which the animal has fed, i.e., is the digestive tract filled?

(3). An approximate estimation of the density of viable food organisms and particles.

If dead animals are present in the aliquot, the cause of the mortality must be determined. If heavy mortalities occur in very early stages, the entire culture should be discarded and started anew. If postlarval mortalities are great, especially if the head region is damaged, cannibalism may be indicated.

DENSITY REDUCTION IN OVERPOPULATED TANKS

For large-tank cultures, the population densities reported by
Maeda (1968) appear to be optimal densities for the different larval stages
(Table 3). Whenever an overpopulated condition exists, a fraction of the
culture population should be transferred to another tank. One must always
keep in mind the future feeding program, based on the volume of water in
use and the shrimp density. The density of the food organisms per unit
of water volume is more important than the gross amount of food in a tank,
i.e. the food level should be such that the later stage larvae can find
food easily. If an overpopulated condition is allowed to persist, reduction
in larval size throughout the culture will result.

In a small tank one can collect nauplii and zoeae at night utilizing
their phototactic response. A pail then can be used to remove the nauplii
to another tank. Zoeal stages actually are too delicate for safe transfer;
mysis or older stages, since they are stronger, are the best for
population division. Also, calculated feedings start in the mysis stage.
In any case, always make use of another tank adjacent to the original
culture tank.

The following techniques can be applied when dividing a population:

(1). One or two days before the transfer, fill the new tank with
fresh seawater and aerate as previously described. Only partially fill
the tank (50 cm depth) at first, to permit the gravity flow of water by
siphon from the original tank.

(2). As the tank is filled, it should be fertilized as herein
described in the following fertilization section. The diatom bloom is
permitted to grow.

(3). If the transfer is to be carried out on the mysis stage larvae, *Artemia* nauplii should be present in the tank.

(4). A large diameter hose (>5 cm) should be used as a siphon between the tanks. If the tank volume is great, more than one siphon may be used for rapid transfer. The siphon intake should be positioned in the middle of the water column.

(5). During the transfer, aliquot samples should be taken from the new tank to estimate the population and adjust it to the desired level.

FEEDING OF ZOEAL STAGE

Fertilization Method

After years of research, a culture technique involving large culture tanks and the fertilization of seawater has been developed. As sufficient phytoplankton inoculum usually is present in the coarsely filtered seawater, when nutrients are added to the culture water in these large tanks a phytoplankton bloom develops in the course of a few days. If daily additions of nutrients are continued, the bloom can be maintained. The initial character of the bloom will gradually change, as reflected in the color of the culture water. A great diversity of primary consumers will appear in this highly productive water, again as a result of the coarse filtration of the culture water. As the phytoplankton itself provides foods for the early zoeae, copepods and other minute consumers appearing later provide supplemental foods suitable for late zoeae and subsequent larval stages. Therefore, three factors determine the effectiveness of the fertilization method: the phytoplankton inoculum present in the seawater, nutrients supplied and solar energy.

The use of fertilized seawater (7-9-0 inorganic fertilizer) was used first by Johnson and Fielding (1956) for the culture of the white shrimp, P. setiferus. Hudinaga and Kittaka (1966) briefly described their use of fertilizers to develop phytoplankton blooms in 200-ton shrimp culture tanks. Subsequently, they (Hudinaga and Kittaka, 1967) published growth and survival results using this method that confirmed the value of fertilized phytoplankton blooms for penaeid shrimp culture. Shigeno (1965) and Fujinaga (1969) summarized the development of shrimp culture as evolved by Dr. Fujinaga. One of the problems throughout his 33 years of research was the production and use of phytoplankton, Skeletonema,

as a zoeal and mysis food. Even though almost 30 years ago, Fujinaga (Hudinaga, 1942) reared penaeid shrimp zoeae on a diet of Skeletonema costatum, this diet has continued to have a major drawback. At the temperature suitable for the shrimp larvae (>27°C), the Skeletonema blooms diminished, as the latter cannot be sustained at temperatures above 27°C. Consequently Skeletonema usually has been cultured separately.

Much of the success of the fertilization method is based on the fact that a phytoplankton bloom is developed in the same tank in which the larvae are reared. In typical circumstances, the culture water should be filtered to exclude particles greater than 165 microns. Such filtration permits sufficient inocula of both phytoplankton and the eggs or larvae of the primary consumers to enter when the tanks are filled; a diverse community of food organisms develops as the shrimp larvae metamorphose from herbivorous early zoeae to omnivorous late zoeae and myses. Although the laboratory culture of such food organisms is often difficult, under the more natural conditions afforded by large tanks, many copepods and the larvae of sea urchins, barnacles and annelids thrive. From the later zoeal stages onward, this multiple diet increases the larval shrimp survival and growth significantly. The diversity of particle size of the phytoplankters and prey species provides readily available food sources for every larval stage.

As the non-feeding nauplii metamorphose into early zoeae, they initally are capable of ingesting food particles of approximately three to five microns in size. Yeasts and Chlorella spp. are of this size range. The diatoms Chaetoceros calcitrans forma pumilis (Takano, 1968) and Cyclotella nana range from three to eight microns and provide some of the best larval foods for various species. By the third zoeal substage, food particles larger than 200 microns can be consumed.

When the cultures are to be fertilized, fresh seawater must be used. If the seawater is allowed to remain in the tank for more than two days before fertilization, the phytoplankton bloom may not develop sufficiently for the shrimp larvae. The natural population of phytoplankton in seawater differs with location and tidal conditions. If the phytoplankton does not bloom rapidly at first, seawater (10 cm or more in a 200-ton tank) should be added to guarantee a sufficient natural phytoplankton inoculum. Shigeno (1969) suggested that a small amount (unspecified) of ferric chloride should be added when the diatom bloom is not thriving.

Many nutrient formulae are used for phytoplankton culture. For the stock culture of a wide range of phytoplankters, the author uses "F" media (Guillard and Ryther, 1962). The formulation and preparation of this media is comparatively simple. However, many of the nutrients required are expensive (notably the vitamins) and it is not practical for large-tank culture even though large volumes of phytoplankton blooms may be maintained well on one-thirtieth the "F" media (F/30). The quality of any nutrient used should be at least analytical reagent grade. The cost of these nutrients, in view of their effectiveness, is minor when compared with the overall cost of shrimp culture.

The nutrients used by various authors are tabulated in Table 6. In addition, Oshima (1969) used a fertilizer formula based on a ratio of 100:10:1 of KNO_3 : K_2HPO_4 : $FeCl_2$, in a large-tank culture system. Liao and Huang (1972) mentioned the use of a fertilizer formula of KNO_3 : Na_2HPO_4 : K_2SiO_3 at a ratio of 100:10:5 for 200-ton tanks. Shigeno [Shigueno] (1969, 1972b) used potassium nitrate at 2 parts per million. He continued this fertilization through the fifteenth day of the postlarvae (PL_{15}) which exceeds the time when the phytoplankton bloom requires nutrients in addition to those provided by metabolic and food wastes

TABLE 6. Tank size, fertilizers, food and number of females used in some large-tank larval cultures in Japan.

Author(s)	Tank Capacity (m^3)	Nutrients and Food	Number of Females	Final Production of PL_{20}/m^3 (X 10^3)
Hudinaga and Kittaka (1967)	200 (10 x 10 x 2 m)	KNO_3 180 mg/m^3/day K_2HPO_4 . 20 mg/m^3/day Artemia nauplii Clam (Tapes)	69	5.0
Maeda (1968)	200 (10 x 10 x 2 m)	$(NH_4)_2SO_4$ K_2HPO_4 Urea Artemia, Tapes, fish	80 – 100	9.0
Shigeno (1969)	57 (4.2 x 7.6 x 1.8 m)	KNO_3 2.0 ppm K_2HPO_4 0.2 ppm $FeCl_3$. $6H_2O$ occasionally Artemia nauplii clam (Tapes)	30 – 50	7.6
	200 (10 x 10 x 2 m)		50 – 100	7.6
Shigeno (1972b)	62 – 200	KNO_3 2.0 ppm K_2HPO_4 0.2 ppm Artemia nauplii shell-meat	30 per 60 m^3 tank	12.0
Kureha and Nakanishi (1972)	2,800	Powdered soy sauce cake Rotifers, Artemia nauplii clam (Tapes) artificial diet (SF_1 and Tetramin)	381-814	[4.2] *

*From Oshima (1972)

When the culturist observes that the nauplii are visible inside the egg or that they have hatched, the appropriate amount of nutrients should be added. Depending upon the duration of the naupliar stages of the species in culture, adjustments in the amount and timing of fertilization must be made. The objective is to provide a sufficient phytoplankton bloom as the majority of nauplii metamorphose into the first zoeal stage. It is important to know the duration of the last naupliar stage since premature fertilization may cause the phytoplankton to foul the body surfaces of the nauplii. After some hours under such conditions, the entire culture will die and will have to be discarded. This fouling phenomenon, although not uncommon in small-tank cultures, is rare in large culture programs. Shigeno (1969) mentioned that an application of a one-half dosage of fertilizer during the egg stage produced no effects, either good or bad.

A working table (Table 8) derived from the present author's culture notes on a 200-ton tank culture of penaeid larvae, provides an example of a timetable for larval stages, nutrient addition, phytoplankton bloom changes, and feeding in such large-tank systems. In this table, 100 grams of potassium nitrate, 10 grams of dibasic sodium phosphate and 5 grams of sodium or potassium silicate (or 5 milliliters of the saturated potassium silicate solution) make up one unit of nutrients.

As the first nauplii hatch out, one unit of nutrients is dispersed in the tank by dissolving each nutrient in about 15 liters of seawater. The silicate salt must be dissolved in FRESHWATER prior to its addition to the tank to avoid precipitation of a silicate complex. When productive bay waters are used, the silica levels are usually such that no initial addition of silica is required. Furthermore, oceanic water requires a greater initial dosage of all nutrients than does bay water.

As long as the bloom is to be maintained, a regular daily dosage of nutrients must be added. If a sufficient bloom does not develop from the initial dosage, another unit, in addition to the daily dosage, should be given the following morning. Should the bloom become too dense, the regular daily dosage should be reduced or omitted entirely for one day. A daily appraisal of the bloom density and, therefore, the nutrient dosage must be made. As the changes in phytoplankton blooms are noted, it is important to inspect the diatoms microscopically. When the blooms become more dense and the water color changes, the diatoms should appear as long chains.

The changes in water color after fertilization indicate the rapidly changing character of the phytoplankton bloom. In most cases, during the day following the first application of nutrients, a slight greenish color will develop by late afternoon. Each day, a definite water color change from morning to afternoon will be apparent. As the initial water depth in 200-ton culture tanks is only 70-80 cm, the bottom of the tank gradually becomes no longer visible as the culture develops. A light brown shade will develop after two or three days, darkening into a dark brown coffee color at the peak of the bloom. When this density is reached, it is best to thin the phytoplankton population by adding fresh seawater (about 20-30 cm depth) along with freshwater so that the nutrient and population levels are reduced. In the mid-afternoon hours, strong photosynthesis will result in a pH in excess of 9.0. Zoeae do well even under these conditions, feeding continuously and producing long trailing feces.

Although the feeding level and phytoplankton population density are easily observed and controlled in a small-tank culture, the only phytoplankton population density determinations that can be made for large-tank systems

are by visual inspection of water coloration and by aliquot samples. Hudinaga and Miyamura (1962) mentioned that the optimal density of Skeletonema costatum in the culture tank should be between 5 and 10 X 10^3 cells per milliliter (small-tank culture).

The brown color of the phytoplankton bloom should remain in the culture through the early postlarval stages. Even though the inorganic nutrient dosages are reduced before or during the mysis stage, the organic nutrients derived from unused foods and metabolic wastes are sufficient to maintain the phytoplankton bloom. However, there will be a shift in the color of the culture water toward green during the postlarval stages. Even though in small-tank cultures a bloom may cease to grow and fall out of solution, in large systems this will be an extremely rare occurrence. If this does occur, foam will be seen on the water surface, and under microscopic examination the diatoms will be seen to clump.

In case of a phytoplankton failure during the critical zoeal stages, culture water from an adjoining tank of advanced or similar stage larvae can be pumped into the tank. Such emergency transfusions should be filtered through the water exchange filter (350 micron opening) to prevent the introduction of alien larvae.

In consideration of the phytoplankton culture problem, the outdoor tank system at the University of Miami's Turkey Point facility was designed to have two tiers, eight tanks in each row. Each tank had a capacity of 20 tons (2 X 5 X 2 m depth). The intention was that the constant culture of phytoplankton by fertilization in the top row of tanks would feed the larval culture tanks in the bottom row. The use of separate tanks for phytoplankton culture is now unnecessary but these can be used for the rotifer cultures mentioned below.

Other Zoeal Foods

In recent years, zoeal foods have been discussed at length. Hudinaga and Kittaka (1966) studied the possibility of using different zoeal foods including planktonic and benthic diatoms, oyster eggs and larvae, frozen oyster eggs and rotifers. All of these alternative foods were found to be as good as _Skeletonema_ with respect to survival. Powdered, fat-free rice bran also appeared to be a satisfactory zoeal food; however, although this food material was able to carry the larvae to the postlarval stage, more frequent water exchanges were required and a drop in pH became a problem (Ishida, 1967; Maeda, personal communication).

Bae (1969) and Nakanishi and Kureha (1971) used powdered soy bean cake, with some success, to feed zoeae in small tanks. Soy bean cake is the dried, plate-like material, which is a by-product of soy sauce fermentation, collected after the sauce is leached out. Its nutritive value for shrimp larvae appears to be significantly different from other soy bean food products. In extra large tanks (1,800 to 2,800 tons) Hirata and Wada (1969) and Kureha and Nakanishi (1972) used soy bean cake food with sufficient success to warrant its continued use.

Furukawa (1972) had some successes in small-tank culture feeding a marine yeast diet from late nauplii through postlarvae, with other foods provided only after the late mysis stage. Recent studies at the National Marine Fisheries Service laboratories at Galveston, Texas, indicate that _Tetraselmis_ sp. is also a useful food for zoeal stages (Mock and Neal, 1974). Brown (1972) demonstrated the suitability of frozen and freeze-dried concentrates of _Skeletonema costatum_ and _Thalassiosira_ sp.

Imamura and Sugita (1972) attempted to develop other alternate foods for the zoeal stages. Using artificially produced activated sludge, they

obtained very inconsistent results. When they used "bacterial floc" cultured in glucose and inorganic nutrients, over 80% zoeal survival was routinely obtained. The material was kept in suspension by a slow moving rotor in the culture tank. Analyses showed that bacteria having high percentages of crude protein provided increased survival of the larvae. In these studies, 330 grams per day (dry weight) of this organic material was required to feed ten million larvae. The cost to produce this daily ration was less than two dollars.

FEEDING OF MYSES AND POSTLARVAE

As the zoeae metamorphose into myses, their feeding patterns tend to change from phytoplankton feeding to omnivorous feeding. The concurrent changes in mouth-part structure are apparent. Even in the second zoeal stages, newly hatched Artemia nauplii (approximately 330 microns) may be ingested, as indicated by Artemia nauplii yolk coloration visible in the gut of the zoea. Conversely, mysis stage larvae may continue to consume planktonic diatoms. Hudinaga and Kittaka (1966) noted that 48.5% mysis survival is possible on an exclusive diet of planktonic diatoms. As previously mentioned, the fertilization of culture water will produce a diversity of food organisms. Occasionally, a mass culture of copepods will flourish.

However, at present, despite the high cost of cysts ("eggs"), Artemia is still the most commonly used food for myses and postlarvae (Hudinaga and Miyamura, 1962; Maekawa, 1961). The locality where the cysts are produced is important, as is the rate at which the eggs will hatch (the hatching rate will govern the number of Artemia nauplii present as food). To determine the hatching rate, 100 or 200 cysts should be placed in approximately 100 millimeters of seawater. After twenty-four hours, the hatched nauplii should be counted and the percentage hatched calculated. As a rule of thumb, 19 hours are required for hatching at 30°C. If stored under conditions of high humidity (e.g. an opened can, no longer under vacuum), the hatching rate will drop. For this reason, it is best not to hold cysts for the next season's use. If they must be stored for later use, the hatching rate must be redetermined. Bowen (1962) has stated that the cysts resist desiccation for up to fifteen years; therefore, it is best to store the cysts in a dry vacuum.

Kurata (1967) discussed his extensive studies of _Artemia_ and gave valuabl[

data for mariculture operations. He presented figures on egg size, egg weight

and hatching rates in different salinities of California, Canada and Utah

Artemia strains. Table 7 has been constructed from Kurata's data. The

California strain has a consistently better hatching rate and the number of

eggs per gram is generally double that of Utah cysts. Both Utah and Canadian

strains hatch inconsistently; however, it appears that these latter nauplii

are larger than those of the California strain. In their culture system,

Hudinaga and Kittaka (1967) estimated the hatching rate of California cysts t(

be approximately 80%, while the Canadian eggs hatched at about 40%. Shigeno

(1969) regarded a 50% hatching rate for Utah cysts as average.

Bookhout and Costlow (1970) found DDT levels in _Artemia_ nauplii which

caused death and deformities in crab megalopae. Utah _Artemia_ cysts contained

approximately three times higher DDT levels than California cysts. However,

in large-tank culture systems where the water is fertilized and many

natural populations including diatoms flourish, the feeding of the Utah

strain nauplii does not have any apparent adverse effect on the shrimp larvae.

Maekawa (1961) in early basic studies, found little difference in the cor

sumption rates of _Artemia_ by myses when _Artemia_ was present at concentrations

0.5 or 1.0 nauplii per milliliter. However, he suggested that an _Artemia_ con-

centration of 0.25 mauplii per milliliter was insufficient for the myses. Hud

and Kittaka (1967) showed that at concentrations of 0.25, 0.50 and 1.00 Artemi

nauplii per milliliter (with 10 shrimp postlarvae per liter) all nauplii were

consumed, yet no differences in growth or survival could be detected. All of

these observations were made in the laboratory on a small scale using filtered

seawater. Although the temperature range of 25.9 to 27.9°C may have prolonged

the mysis stage, the data presented by Maekawa (1961) (Fig. 14) suggest that t

TABLE 7. Number of cysts per unit weight and hatching
rates of <u>Artemia</u> (from Kurata, 1967).

| | Number of Eggs | | Hatching Rate Range* |
	per mg	per g	%
Canada 1965	281–285	280,000	33–82**
Utah 1965	282–297	280,000	10–68[+]
California 1966	566–568	570,000	75–83

*Hatching rate salinity range: 27.7 – 35.6 ^{o}/oo.

**The hatching rates vary by production year.

[+]Utah cysts from two samples.
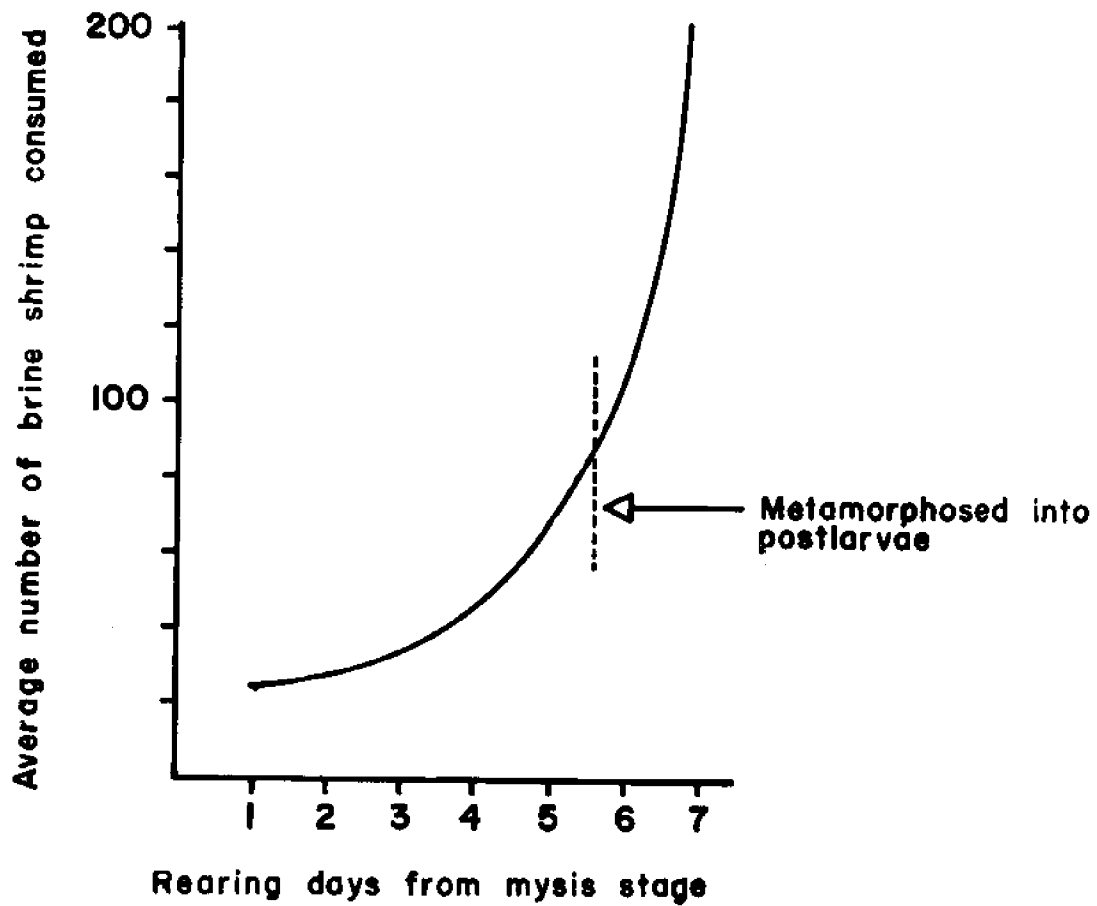
Figure 14. Average number of Artemia nauplii consumed by myses and post larvae (from Maekawa, 1961).

trend is for myses to increase their consumption of _Artemia_ nauplii quite significantly at metamorphosis. As early myses, approximately 25 nauplii are consumed daily, progressing to about 70 nauplii per day just prior to metamorphosis. As early postlarvae, feeding rates of over 140 nauplii per day are typical, the rates increasing as the postlarvae grow.

In a large-tank system (57-ton tank), Shigeno (1969) showed that a mixed population of third substage myses to second stage post-larvae consumed an average of 46.2 nauplii per shrimp larva per day. Twenty-four hours later, the same population consumed 84.6 nauplii per larva per day. The laboratory feeding studies by Maekawa (1961) are not comparable to those of Shigeno (1969) since the latter study was carried out in a large-tank system with diverse food sources available at metamorphosis. The latter resulted in reduced grazing on the _Artemia_ present in the tanks.

It is important to insure that the larvae are fed sufficiently during this significant change in feeding rates. It is better to diversify the diet by also feeding minced clams (as discussed below), as it is very difficult to culture sufficient quantities of _Artemia_ for the entire postlarval diet. If the larvae are on a diet of _Artemia_ nauplii, the water level should be kept low so that the nauplii population (food density) is high and readily available. In any case, the _Artemia_ diet can be continued only through the fourth day of the postlarval period as the larvae then commence demersal life.

In their mass culture experiments to produce one million postlarvae (PL_{20}) in 200-ton tanks, Hudinaga and Kittaka (1967) used from 1.3 to 17.7 kilograms of _Artemia_ cysts (total dry weight over

67

seven days, Canadian strain at 40% hatching rate). Extrapolating from

the conventional small-tank culture system (2-ton tanks), 8.75 kilograms

of the California strain (80% hatching rate) or 17.5 kilograms of the

Canadian strain (40% hatching rate) would be required to feed one

million postlarvae. In large-tank culture systems, the quantities of Artemia

cysts required per unit volume are much lower than in small-tank cultures.

Kittaka (1971) stated that the total amount of Artemia cysts needed

for the production of one million postlarvae (PL_{20}) would be about 5.0

kilograms (Canadian cysts at 40% hatching rate, or about 2.5 kilograms of

the California cysts with 80% hatching rate). Shigeno (1969) provided

separately hatched Artemia nauplii at the rate of approximately 1.0 kilograms

per day (measured dry weight of cysts, Utah strain at 50% hatching rate,

for 200-ton tanks) in the last day of the mysis stage. This diet was

continued for the first four days of postlarval life. However, his table

(Shigeno, 1969,1972b) indicated that the Artemia were provided from the first

mysis substage. In 57-ton tanks, feeding rates ranged from 1.1 to 2.9 kilo-

grams of cysts (total weight) over the seven-day period.

The ideal method for providing Artemia nauplii would be to hatch

the cysts in separate containers and add only the nauplii (no cysts or spent

capsules) to the culture tanks. However, when feeding shrimp larvae in large-

tank cultures, this method is laborious and the increased complexities involved

suggest that such a method should be avoided. The appropriate amount of

Artemia cysts may be added directly to the culture tank, taking into con-

sideration the hatching rate as previously determined and the time required

for hatching to commence at the prevailing water temperature. As Artemia

nauplii must be present as the zoeae metamorphose into the myses stage, cysts

68

should be added as the majority of the shrimp reach the third zoeal substage; at 28 - 30°C, the hatching will be completed in 19 - 24 hours, in time for the metamorphosis. Up to one-half of the appropriate amount of Artemia cysts may be added as early as the second zoeal substages in order to assure that a sufficient amount of nauplii will be present when needed. As previously mentioned, even these later zoeal substages are able to consume Artemia nauplii. As the aliquot samples described earlier are taken, observations on the success of the Artemia hatch and the density of nauplii present should be made. Subsequent daily additions of cysts are determined by the numbers of unconsumed Artemia remaining in the culture tank. Even if some nauplii are allowed to develop into adults, the later postlarvae will consume them. If Artemia populations are allowed to reach "excessive" levels, the phytoplankton in the culture tanks will be reduced to the point where the chemical balance of the culture may be affected.

As substitutes for Artemia, other foods have been investigated. Ito (1960) predicted the successful use of the rotifer Brachionus plicatilis for the culture of larval fish, penaeid shrimp, lobsters and crab larvae, etc. He noted several advantages of these rotifers over Artemia, e.g., appropriate size range, slower movement, etc. The maximum size (with eggs) of the Japanese strain is approximately 350 microns, while the California strain is approximately 245 microns. Hudinaga and Kittaka (1966) demonstrated that exclusive use of such a rotifer diet was sufficient for growth from zoea to postlarvae. Maeda (1968) recommended the Brachionus diet for the second zoeal substage onward. Hirata and Wada (1969) mentioned their use of rotifers

69

for shrimp culture in a 1800-ton capacity swimming pool. Kureha and Nakanishi (1972) also used rotifers in their extra-large-tank 2800-ton cultures, as did Furukawa (1972) for the later zoeal sub-stages onward.

Bae (1969) noted decreased survival and the production of more slender myses on the rotifer diet as compared with control diets in the mysis stage. However, the rotifer-fed myses metamorphosed into postlarvae which grew faster on a minced clam (Tapes sp.) diet than did the control group.

The major disadvantage to the use of the rotifer diet has been the difficulty of mass culturing these animals with sufficiently high yields to satisfy the demands of the shrimp larvae. Yang and Alon (a, b in press) recently have shown that the rotifer B. plicatilis (California strain) can be cultured to densities in excess of 400 per milliliter in two weeks with the addition of Na_2EDTA combined with a powdered Torula yeast diet.

When the postlarvae are cultured through PL_{20} an adequate supply of appropriate foods must be provided. The population estimates made during the first four postlarval stages are the last relatively accurate estimates that can be made. Once the larvae have taken up a benthic habitat, the flat-sided dip-net must be used to quickly scoop up a sample of shrimp from the tank bottom. Based on the area covered by this sampling, a very approximate population estimate can be obtained. To extrapolate an estimated total biomass, one must know the average weight of postlarvae of the cultured species for every day of the postlarval life. The first few times a species is cultured, daily samples of 500 - 1,000 postlarvae should be collected, drained,

weighed and counted. All measured samples should then be discarded.

Once the typical daily growth rate has been estimated for post-larval stages, only occasional samples of subsequent cultures need be taken about every five days (days PL_1, PL_5, PL_{10}, PL_{15} and PL_{20}). For P. japonicus grown in exceptionally well managed cultures, average postlarval weights are as follows: PL_1, 0.85 mg; PL_{10}, 5.0 mg; PL_{20}, 20.0 mg. Postlarvae can increase their weight over twenty fold in twenty days in such a culture.

Commencing with the first day of postlarval life, 100% of the total biomass weight must be provided in food (wet weight). This ration should be divided into four or five portions, which are fed to the shrimp four or five times a day (e.g., 8 AM, 11 AM, 2 PM, 5 PM, 10 PM). Shigeno (1969) also recommended four or five feedings a day but did not mention feeding rates. Theoretically, the shrimp cannot consume 100% of their body weight in food daily because, when young, they molt frequently and the mouth parts remain soft for a short time when they might otherwise be feeding. However, 20 - 25% body weight should be given in each of the daily feedings to assure that food will always be available. About two hours after each feeding, the tank should be inspected for any residual food left unconsumed. If any is present, the next food portion should be of smaller volume; however, no attempt should be made to remove any excess food from the tank. If cannibalism is noted, the rations must be increased. Liao and Huang (1972) have mentioned that 70 kilograms of minced clam meat is needed to produce one million 20-day-old postlarvae. Kittaka (1971) has stated that 80 kilograms is required for the equivalent production.

After receiving food, the shrimp will swim while holding food particles in their mouths. The food given during the early postlarval stages must be kept in suspension either by a constant slow moving rotor blade or a periodic (two or three times daily) stirring of the bottom with a long handled scraper. Debris from the diet may accumulate in areas where the aeration cannot create a strong current.

Starting with PL_4 or PL_5, metabolic and food wastes will accumulate in the culture water, soon reaching undesirable levels. At this time, water exchange should be commenced and approximately one-fifth of the tank volume should be replaced with fresh, filtered seawater each day. Shigeno (1969) has recommended an exchange of 20 - 35% of the water daily. Such daily exchanges will be required for the remainder of the culture period.

Hudinaga and Kittaka (1966) and Kittaka (1971) stated that cultured Skeletonema, benthic diatoms, oyster larvae and rotifers were as good as an Artemia nauplii diet. Planktonic diatoms and frozen oyster eggs were shown to be inferior but acceptable foods. For the first four to six days of postlarval life, rotifers, copepods, crushed barnacles and clams (Tapes spp.) are as good as Artemia. It has been shown that in the later postlarval stages, benthic diatoms, copepods, frozen amphipods (Gammarus), crushed barnacles, annelid worms, trash shrimp meat and certain synthetic diets are as good as the commonly used clam (Tapes).

In the mysis and early postlarval stages, it also is possible to satisfy the larvae's nutritional requirements with finely ground pelletized fish chow, kept in suspension by a slow rotor in the tank (Fujinaga, personal communication). Imamura and Sugita (1972) produced a bacterial floc on an artificially produced organic detritus in a glucose medium, which was relatively successful in feeding first stage myses to day-one postlarvae.

72

However, this diet resulted in reduced growth and survival as compared with Artemia, and results were less consistent. Imamura and Sugita have suggested that, with further refinements, this bacterial diet may be substituted for Artemia feedings.

As indicated, there are several types of commonly used food materials which are available for feeding shrimp postlarvae. Several bivalves are used, after appropriate preparation (see instructions below), such as the Japanese clams (Tapes spp.) and the mussel (Mytilus sp.). Crustaceans such as euphausiid "shrimp" and barnacles may also be used, but they provide less meat per unit of weight. Lean and non-oily fish may be processed and fed, as well as squid, the preparation of which will be described below. Since most Japanese researchers have relied on Tapes clams as a postlarval food, its preparation will be discussed first. The techniques involved are equally applicable to other bivalves.

The processing steps are as follows:

(1) Crush the entire clam, or shuck the animal out of the shell.

(2) Separate the shell from the meat by suspending the crushed material in seawater and stirring vigorously. The shell pieces will settle out. In order to maintain osmotic balance in the tissues, only seawater is recommended for this processing.

(3) The remaining meat and viscera are pressed and shaken over a 600 micron screen so that the juices and viscera will pass through. The meat then is washed thoroughly in seawater over this screen.

(4) All the water is squeezed or pressed out of the meat. Most of the natural juices are eliminated through this processing to prevent excessive fouling of the culture water.

(5) The meat is ground in a meat grinder, plate opening of 1.6 mm,

73

for the later postlarval stages (Shigeno, 1969). It is ground a second time to a finer size for earlier stages. Meat ground more than once may range in particle size from 0.4 to 3.0 mm, the average size being 1.5 to 2.0 mm.

(6) This fibrous ground meat is pressed and then refrigerated until used.

(7) To feed the shrimp postlarvae, the chopped meat is suspended in a pail of seawater and dispersed over the entire tank.

Any unneeded processed food may be frozen. Extra food may also be frozen to avoid any possible shortage in the food supply; however, once this type of processed meat is frozen solid, it will tend to float when placed into the culture tank. Floating foods may be left unconsumed, and these must be removed along with the protein bubbles or foam that they produce. It is best to rely on frozen foods for emergencies only.

In the case of clams, the processed ground meat represents only 5% of the total live weight of the clams. Therefore, an ample supply of the raw bivalves must be available to satisfy the ever increasing demands of the postlarvae as they grow.

Maeda (1968) used a simple technique to prepare postlarval foods. The flesh of small fish or bivalves was placed in a blender with seawater. The suspension produced by blending was strained through screens at 1500 microns, 600 microns and 350 microns and washed in seawater. The food particles from the 350 to 600 micron range were suitable for PL_2 to PL_3 while PL_4 to PL_7 could be given the 600 to 1500 micron particles. Food particles larger than 1500 microns were given larvae older than seven days and might consist only of fish flesh. When feedings consist only of fish, a constant slow water exchange rather than a daily 20% exchange is necessary to avoid rapid deterioration of water quality.

For $\underline{P}$. $\underline{\text{duorarum}}$, squid has been used by the author as a postlarval food. The viscera (including the ink sac) must be removed and the mantle and arms cleaned. The meat then is cut into five to ten centimeter pieces, frozen solid and subsequently ground with a meat grinder to the appropriate size. If the squid is not frozen solid before every grinding operation, an unsuitable fibrous mass will result.

Hirata and Wada (1969) noticed that underfed postlarvae (PL_{10} and older) tend to swim on the surface of the tank water when insufficient food has been provided. In their 1800-ton system when this happened to PL_{17} to PL_{20} larvae, they fed an emergency ration of one kilogram of TETRAMIN[R] each hour for three hours. This emergency procedure provided sufficient nourishment until more fresh foods could be provided.

A GENERAL EXAMPLE OF A WORKING CULTURE

A general example of a working table is provided for the reader's convenience (Table 8). A culture of P. japonicus was started in late August and completed in late September. The 200-ton system was covered by a roof as described earlier. During the course of this work, the water level was gradually increased to a depth of 180 cm (Figs. 15, 16). The water used in this culture was not highly productive bay water and, therefore, it required a greater than usual nutrient dosage. Because of the reduced temperatures of the season, the postlarval growth was somewhat less than typical, the final weights averaging 8.5 mg per postlarva at PL_{20}. As may be noted, the population was divided at the PL_3 stage, and 2 million post-larvae were transferred to an adjacent tank. Ultimately 1.6 million with an average body weight of 7 mg (PL_{25}) were harvested from this second tank.

The condition of the shrimp as they pass through each of the major stages must be noted as these are critical events. As may be seen in the table, any deaths should be noted and recorded, and an explanation for any mortalities sought. Though the majority of the larvae were healthy and active, the mortality at PL_3 to PL_4 in this example remained unexplained.

TABLE 8. Working table: a general example of a 200-ton tank culture to PL_{20} (P. japonicus).

Day (Weather)	Water Temp. (°C)	Salinity (°/oo)	Water level (cm)	Stage	Population estimated (x10³)	Nutrients or Food given	Remarks
0 (Bright)		28.0	FW 8+ SW 72 = 80 cm				Tank cleaned 2 days previously. Well water (FW) added to 8 cm depth. Then filtered seawater (SW) (160 micron opening plankton net bag) 72 cm added in PM hours.
1 (Bright)	26.0 AM 28.2 PM		80	Adults			50 females of average quality are placed in tank at 0730. One died; 49 females remain. Aeration started in AM hours; slow aeration, causing water surface movement. Two females in poor condition but regained normal position; these may not spawn.
2 (Bright)	27.0 AM 28.7 PM		80	Egg N–I	1,700 (0900 hrs) 2,400 (1600 hrs) 5,100 (1800 hrs)	Nutrients 1 dosage = KNO_3 100 g K_2HPO_4 10 g K_2SiO_4 5 g	Spawned during the night. Eggs sampled by stirring small area of bottom. A rough calculation of egg population made. Eggs developing well. One dosage of nutrients supplied at 1100 Bottom clearly visible. pH 8.4 at 1500 Nauplii hatched. Aeration increased – "boiling" on water surface. Water color greenish shade. Bottom clearly visable.
3 (Cloudy-Bright)	28.0 AM 29.1 PM		80	N–V (1200 hrs)	6,000	Nutrients 2 dosages (1000 hrs)	Water still greenish shade. Bottom visible; therefore double dosage of nutrients added. At 1330 water slightly brownish shade. Water color brownish at 1800
4 (Cloudy-Bright)	28.1 AM 28.6 PM		80	Z–I	5,500	Nutrients 1 dosage	Nauplii metamorphosed to zoeae; there are a few dead but the majority are healthy. Water color brown. Diatom bloom checked; cells appear healthy Zoeae swimming actively; long feces trailing. pH 8.8 at 1330
5 (Bright)	27.5 AM 28.7 PM	29.6	Added SW 40+ FW 5 (in AM) 125	Z–II	5,400	Nutrients ½ dosage Artemia cysts 300 g Nutrients ½ dosage	Water color still brown even after addition of seawater. Aeration increased because water level increased. Zoeae actively swimming. Artemia cysts added for the mysis stage. Diatoms examined – do not look good. Some diatoms clumped and floating on surface; dispersed by stirring surface. Added nutrients.
6 (Bright)	27.8 AM 29.5 PM		125	Z–II – Z–III Z–III	5,000 (1000 hrs) 5,300 (1700 hrs)	Nutrients ½ dosage (0900 hrs) Artemia cysts 300 g	Water color dark brown Diatoms appear healthy. Artemia nauplii hatch not good. Zoeae swimming actively; appears to be well fed.
7 (Partly Cloudy)	28.6 AM 27.9 PM		125	Z–III – M–I (1100 hrs)	4,250	Nutrients 1 dosage Artemia cysts 500 g	Water color dark brown. Myses appear at 1100; swimming actively. Compared to the next tank, more myses present; a better culture. Bottom stirred up with long handled stirrer. Artemia hatch not good; added more cysts than scheduled.
8 (Bright)	28.7 AM 29.6 PM	30.7	Added SW 25 150	M–II	4,000	Artemia cysts 1.0 kg (0930 hrs) Artemia cysts 300 g (1430 hrs)	Water color dark brown and diatoms in good condition. Population of myses large, therefore 1 kg of Artemia cysts added Water added in AM hours; water level to 150 Sampled myses; good health Myses population is too great; added 300 g more Artemia cysts in PM hours Aeration checked.

TABLE 8. (Continued)

Day (Weather)	Water Temp. ($^\circ$C)	Salin- ity ($^\circ$/oo)	Water level (cm)	Stage	Population estimated ($\times 10^3$)	Nutrients or Food given	Remarks
9 (Bright)	28.7 AM 29.8 PM		150	M-III	3,750	Artemia cysts 1.0 kg	Water color somewhat less dark than yesterday. Population still large, therefore large amount of Artemia was given. Seawater filter bag changed (350 micron opening size).
10	28.8 AM 29.8 PM		150	PL$_1$		Artemia cysts 1.0 kg chopped clams 3.0 kg	Postlarve appear; very active. Large copepod population appeared in AM hours; the population decreased in PM hours. Individual weight of shrimp is 0.85 mg (normal size). 3 kg of chopped clams were given 5 times a day, 600 g at each time.
11	28.6 AM 29.5 PM		Added SW 30 180	PL$_2$		chopped clams 4.0 kg	Postlarvae active. Seawater added 30 cm; tank now full. Copepod population decreased. Larval population is too great; prepared No. 2 for division of population (see text). Postlarvae feeding satisfactorily; many individuals with full stomachs.
12	27.8 AM 27.2 PM		180	PL$_3$	3,750 -2,000 1,750	chopped clams 5.0 kg	Aeration fully open. Postlarvae active; growth good. Two million postlarvae transferred to No. 2 tank by two siphons; finished transfer at 1500. The water removed was replaced. Protein bubbles on the water surface; clams may not be fresh.
13	24.5 AM 24.6 PM		180	PL$_4$		chopped clams 4.0 kg	Some left over food at bottom. Considerable number of PL found dead at bottom; because dipnet used yesterday? Live individuals very active. No dead PL found in No. 2 tank to which they were transferred. Water temperature dropped.
14			180	PL$_5$	1,500	chopped clams 4.0 kg	Some left-over food at bottom; still over feeding? Water exchange started today; 1/3 of water siphoned out through the screen and replaced by fresh seawater; greater volume than usual because many dead PL found. Water exchange started; delayed one day because of population division; large volume exchanged. Average body weight 2 mg.
15	23.3 AM 24.0 PM		180	PL$_6$		chopped clams 4.0 kg	One-fifth of water exchanged. No left over food Water temperature low.
16	23.4 AM 24.0 PM		180	PL$_7$		chopped clams 5.5 kg	One-fifth of water exchanged every day. Checked bottom for left over food.
17	23.0 AM 24.2 PM		180	PL$_8$		chopped clams 6.5 kg	Water exchanged. Checked bottom for left over food.
18	23.5 AM 23.3 PM		180	PL$_9$		chopped clams 7.5 kg	Water exchanged. Checked bottom for left over food.
19	22.6 AM 23.5 PM		180	PL$_{10}$		chopped clams 8.0 kg	Average body weight 3.5 mg. Growth is slow because of low temperature. Water exchanged. Checked bottom for left over food.

TABLE 8. (Continued)

| Day (Weather)	Water Temp. ($^{\circ}$C)	Salin- ity ($^{\circ}$/oo)	Water level (cm)	Stage	Population estimated (x10^3)	Nutrients or Food given	Remarks
20	22.7 AM 23.5 PM		180	PL$_{11}$		chopped clams 8.0 kg	Water exchanged. Checked bottom for leftover food.
21	22.0 AM 23.5 PM		180	PL$_{12}$		chopped clams 8.0 kg	Water exchanged. Checked bottom for leftover food.
22	22.4 AM 23.5 PM		180	PL$_{13}$		chopped clams 8.2 kg	Water exchanged. Checked bottom for leftover food.
23	22.3 AM 23.2 PM		180	PL$_{14}$		chopped clams 8.2 kg	Water exchanged. Checked bottom for leftover food.
24	22.4 AM 23.1 PM		180	PL$_{15}$		chopped clams 8.5 kg	Average body weight 5.7 mg. Growth slow. Water exchanged. Checked bottom for leftover food.
25	22.4 AM 23.3 PM		180	PL$_{16}$		chopped clams 8.3 kg	Water exchanged. Checked bottom for leftover food.
26	22.3 AM 23.8 PM		180	PL$_{17}$		chopped clams 9.0 kg	Water exchanged. Checked bottom for leftover food.
27	23.3 AM 24.3 PM		180	PL$_{18}$		chopped clams 9.0 kg	Water exchanged. Checked bottom for leftover food.
28	24.0 AM 25.8 PM		180	PL$_{19}$		chopped clams 6.0 kg	No feeding at night for harvesting preparation. Water exchanged.
29				PL$_{20}$			Average weight 8.5 mg. Harvested 980,000 postlarvae.
						TOTAL Nutrients - KNO_3 650 g: K_2HPO_4 65 g: K_2SiO_4 32.5 g. Artemia cysts - 4.4 kg chopped clams - 124.7 kg	
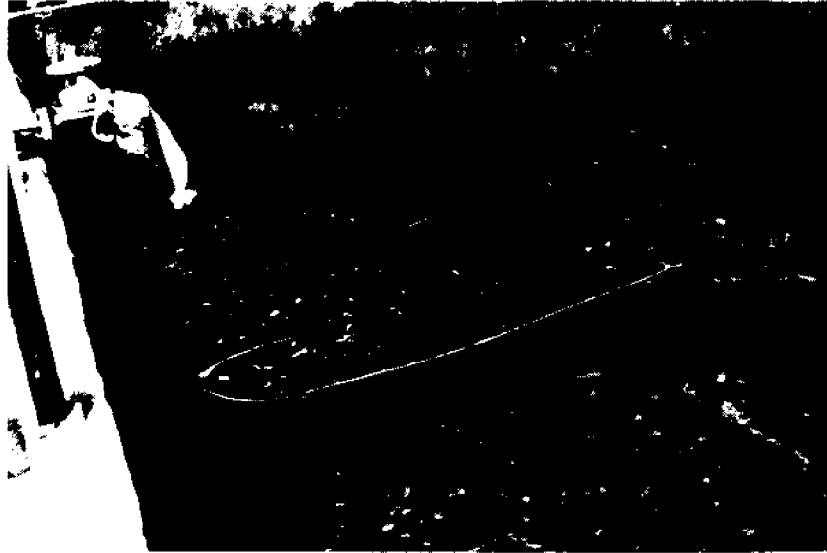
Figure 15. The initial water level in the culture tank. As nauplii hatch, the aeration is increased (large airstones used here). The water level is about 70-80 cm in depth. The tank bottom is visible before the phytoplankton bloom.

Figure 16. The postlarval stage culture with 20% of the water exchanged daily. Water is added through a large mesh plankton net bag (350 microns). Note the protein foam on the water surface and the strong aeration in the water of 180 cm depth.

HARVESTING

The time of the day for harvesting the postlarvae depends on the
arrangements made for shipping time. It is crucial to minimize the time
from harvesting to the time of release in the farming area; keep in mind
that shipping time is only a part of the time that the larvae will
be stressed. Thus, the harvesting operation demands intensive labor and
usually requires additional temporary manpower.

The harvesting method is dependent upon the culture system used.
The following procedures relate to the large-tank culture methods des-
cribed in this manual.

(1) Discontinue feeding about ten hours before the harvest.

(2) Using the siphons, drain the tank slowly through the water
exchange filters to about 30 cm depth. It will be necessary to start the
siphons in a large tank during the night to reduce the water level in
preparation for a shipment the following morning.

(3) Observe the aeration carefully during the harvest. As the tank is
drained the reduced water level will permit the aeration to increase.
Inevitably some bottom material will be stirred up; however, unnecessary
agitation of bottom materials should be avoided as old molts and debris
will be harvested with the postlarvae (Fig. 17).

(4) A framed net (about 1 m X 0.5 m) can be used to collect the
postlarvae when the water has been drained to about 30 cm. However,
with this method bottom debris is more likely to be stirred up and
collected with the larvae. The best harvesting technique utilizes the
drain valve and sump of the culture tank as shown in Figure 1. The dam

Figure 17. The harvested postlarvae (PL_{20}). In addition to the postlarvae, note the cast off molts.
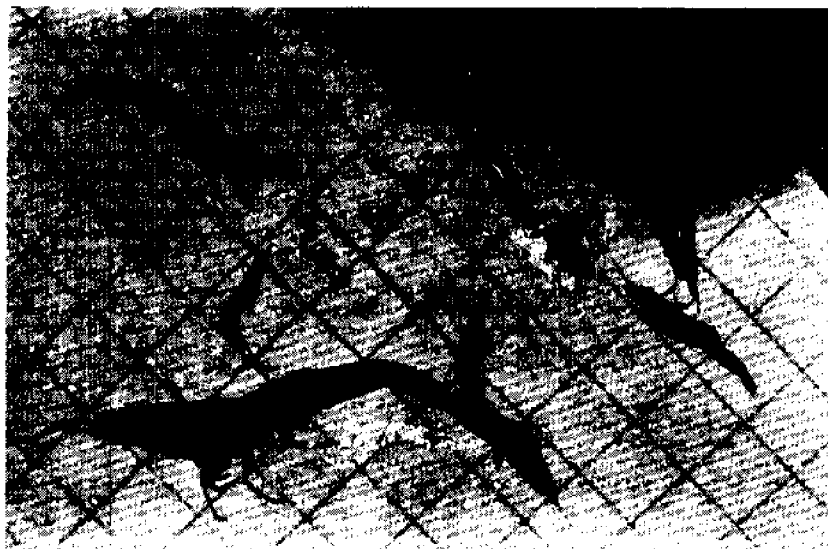

Figure 18. Samples of postlarvae (PL_{20}). Note the size variation in the sample. Such size variation in the final production indicates that management of the culture was not good. (Each scale unit equals 0.5 cm.)

boards are placed at the sides of the sump and the level of the seawater in the sump adjusted. The drain valve is opened, slowly at first, until the sump is filled to overflowing. A basket-like net is then placed in the sump under the valve and the drain valve opened further. When the net is sufficiently full of shrimp larvae, it is removed to the shipping tanks.

(5) As the tank is drained, seawater is used to flush out those larvae remaining in any pools of water.

(6) At this point, the condition of the bottom of the tank should be noted. If the bottom is clean and relatively free of debris, the feeding routine has been proper. If a sludge-like material has been accumulated on the bottom, especially at the deeper part of the tank, overfeeding and mismanagement of the culture are indicated.

(7) Finally, as another measure of the success of the culture technique, a sample of the larvae should be inspected for any significant variation in larval sizes (Figs. 17, 18). If the size variation is great, the conditions of the culture were not what they should have been. A nearly uniform size distribution in a culture results from good culture management techniques.

SHIPPING POSTLARVAE

In may situations, the larval rearing facilities are located at considerable distances from the farming or "grow-out" areas. It is, of course, vitally important to ensure as great a survival rate as possible during the shipment of the postlarvae. Any conditions that may cause stress to the shrimp during harvesting, packing, shipping or releasing should be avoided. Stresses in handling will also be reflected by mortality of poor growth after planting in the "grow-out" area.

The method employed for shipping will vary with the particular circumstances, i.e., size and age of the postlarvae, distance, transportation available, etc. If only a short distance is to be covered (total time to release of less than 10 hours), a land vehicle is most efficient. During warm seasons and especially during daytime shipments, refrigerated vehicles should be used. Cooler weater or night shipments frequently do not require such refrigerated carriers. Long distance shipments should be handled by air carriers and the shrimp should be packed in such a manner as to maintain cool water temperatures. Should the need arise, a great number of nauplii packed in plastic bags of chilled seawater may be shipped over long distances. Since they do not feed and are much smaller in size, and because this stage is of long duration, shrimp nauplii are much easier to ship than are the postlarvae.

Reduced temperatures are essential to successful shipments. If the seawater in which the shrimp are packed is allowed to warm, the increased metabolism of the larvae will deplete the oxygen and increase the molting rate and the tendency toward cannibalism. If unconsumed foods, debris

84

or molted exoskeletons are packed with the larvae, oxygen depletion will proceed at a much higher rate.

A common packing procedure for the shipment of postlarvae is outlined below:

(1). The shrimp larvae are moved from the culture tank (temperature 26 to 28°C) into a 2 to 3-ton capacity cooling tank (about 22°C). The harvesting net or basket should be suspended directly in the cooling tank. This first step produces a gradual cooling of the larvae to reduce metabolic rates during transit.

(2). Approximately 3,000 - 4,000 larvae (PL_{20}) are counted and placed in a pail containing six liters of clean, chilled (about 15°C) seawater. This pail will serve as a standard for other pails, which are similarly filled with six liters of cool seawater. After sufficient larvae to fill the standard pail have been hand counted, other pails can be filled to the same density by dipnetting larvae into them. It is advisable to keep the "standard pail" adjacent to those being filled with larvae and to stir the contents occasionally for a better estimate of the larval density.

(3). Place each six-liter pailful into a clean 25-liter plastic bag and remove or press out the air. The air should be replaced with about 15 liters of oxygen. The bags are then securely tied with rubber bands; each is then placed in a second bag, for added security against water loss, and sealed.

(4). Each bag is then carefully placed into a separate cardboard box, which is then taped shut. A rope or heavy twine (at least 1/4" diameter) is tied around the box for ease in handling but, more importantly,

to provide an air space between the boxes when they are stacked for shipment. The cool air of the refrigerated transport vehicle can then circulate among the boxes.

(5). A refrigerated vehicle should be kept between 14 and 17°C. This will insure minimum postlarval mortality during a 24-hour transport period.

Kittaka (1971) shipped twenty-day old postlarvae at 14 to 18°C at a density of 600 PL_{20} per liter. At this density, less than a one percent loss in 18 hours was reported. For 1,000 postlarvae per liter with a temperature range of 12 to 19°C, only about 3.5% were lost. Shigueno (1972a, b) successfully shipped 6,000 PL_{20} in eight liters of seawater with four liters of oxygen at an unspecified temperature. Note that tropical species may not require or tolerate chilling to 12°C; warmer water may be sufficiently cool for transport. Tabb, et al (1972) shipped P. duorarum at 18°C and Liao and Huang (1972) shipped the Formosan penaeid at 15 to 18°C.

For the shipment of a small number of shrimp or for long distance shipping, air carriers are the best. A different packing method is used for this purpose and is described as follows:

(1). The shrimp are counted, double-bagged and given oxygen as above. An appropriate packing density may also be achieved using the following aliquot method. The harvested postlarvae are placed into a 200 to 400-liter chilled tank (about 18°C) making sure that little debris or molts also have been transferred. With stirring and strong aerating, the shrimp are evenly distributed in this tank; a one-liter aliquot is removed and counted. From this density estimate, the

appropriate volume of water for each shipping bag can be calculated.

(2). Ice cubes are placed into heavy-duty plastic bags (about 200 g per bag); the bags are sealed and wrapped in a thin layer of newspaper.

(3). Four such wrapped bags are placed in the corners of a styrofoam shipping container (standard size used for shipping tropical fish).

(4). The ice bags are partitioned off from the center of the container by inserting cardboard across the corners.

(5). Oxygen is supplied as previously described (see #3, p. 85). The large bag containing the chilled larvae is securely positioned in the center of this iced styrofoam container.

(6). The styrofoam lid is added, taped in place, and the entire unit placed into a cardboard box.

(7). A rope will provide security and ease of handling but is not required to provide a means for cool air circulation.

Other transportation techniques have been tried, some successfully. Kittaka (1971) used a live box for transportation by boat. Twenty-day postlarvae in a 2.4-ton live box with open circulation (not chilled) suffered a 13% mortality when transported at densities of 24 per liter, yet only a 2% loss at 13 per liter; the shipment time was 24 hours. The Shibushi Seedling Center of the Seto Inland Sea Culture Fisheries Association used a 10-ton live box stocked with 120 postlarvae per liter (Anonymous, 1969). Aeration and circulation were maintained and five kilograms of minced Tapes clams were fed over the 28-hour period. There was very little mortality at 22 to 23°C, and the larvae were healthy when released.

Finally, an attempt was made to transport shrimp postlarvae
packed on wooden frames of flannel material soaked in chilled seawater.
The shrimp were chilled and carefully arranged on the soaked flannel
cloth stretched on the frames (33 cm X 53 cm). The frames were stacked
and placed inside plastic bags charged with oxygen. After 18 hours
in transit, 80 - 90% survival was reported (Anonymous, 1969).

Upon arrival at the final destination, all temperature and salinity
adjustments must be made with care. A rapid rise in temperature at this
stage will cause an oxygen depletion. For this reason, the transfer
to the new environment must not be delayed. Thermal equilibrium
involves suspending the individual plastic bags of larvae in the new
environment and allowing sufficient time for the two water masses to
come to equal temperatures. After thermal equilibrium has been reached,
salinity adjustments should be made by gradually opening the bags to
admit the new seawater; salinity adjustments should not be difficult
as the postlarvae are relatively euryhaline. If, upon arrival, the
postlarvae are not active, they should not be released directly into
the pond, but should first be retained in a small enclosed area of the
pond.

LITERATURE CITED

Akazawa, K. 1973. Shubyo ryosan ni okeru shiikusui no kakuhan sochi to sono koka ni tsuite. [The culture water stirring system and its effect on the mass seedling production]. Saibai Kiken [Culture Technology Research], 2(1):49-55 (in Japanese).

Anonymous 1969. Kuruma-ebi no shubiyo yuso — Shibushi jigyojo kara Kochi-ken Usa-shi e. [Transportation of Penaeus japonicus seedlings — from Shibushi production center to Usa-city, Kochi-ken]. Saibai Gyogyo [Culture Fisheries], 6(4):23-26 (in Japanese).

Bae, S. 1969. Kuruma-ebi yosei no jiryo koka zikken. [An experiment on feed efficiency of Penaeus japonicus larvae]. Saibai Gyogyo [Culture Fisheries], 7(2):36-43.

Bookhout, C.G. and J.D. Costlow, Jr. 1970. Nutritional effects of Artemia from different locations on larval development of crabs. Helgölander wiss. Meeresunters., 20(1-4):435-442.

Bowen, S.T. 1962. The genetics of Artemia salina. I. The reproductive cycle. Biol. Bull., 122(1):25-32.

Brown, A., Jr. 1972. Experimental techniques for preserving diatoms used as food for larval Penaeus aztecus. Proc. natl. Shellfisheries Assoc., 62:21-25.

Brown, A., Jr. and D. Patlan. 1974. Color changes in the ovaries of penaeid shrimp as a determinant of their maturity. Mar. Fish. Rev., 36(7):23-26.

Cook, H.L. 1969. A method of rearing penaeid shrimp larvae for experimental studies. FAO Fish. Rep., (57)3:709-715.

Cook, H.L. and M.A. Murphy. 1969. The culture of larval penaeid shrimp. Trans. Amer. Fish. Soc., 98(4):751-754.

Cook, H.L. and M.A. Murphy. 1971. Early developmental stages of the brown shrimp, Penaeus aztecus Ives, reared in the laboratory. Fish. Bull., 69(1):223-239.

Dobkin, S. 1961. Early developmental stages of pink shrimp Penaeus duorarum from Florida waters. Fish. Bull., 61(190):321-349.

Ewald, J.J. 1965. The laboratory rearing of pink shrimp, Penaeus duorarum Burkenroad. Bull. mar. Sci., 15(2):436-449.

Fujinaga, M. 1967. [A proposal on the denomination of the number in the seed production of fishes and shellfishes.] Aquiculture, 15(1): 1-2 (in Japanese).

Fujinaga, M. 1969. Kuruma shrimp (Penaeus japonicus) cultivation in Japan. FAO Fish. Rep., (57)3:811-832.

Furukawa, I. 1972. Kaiyo kobo ni yoru Kuruma-ebi shubiyo no tsukurikata. [Seedling production method of P. japonicus by use of marine yeast.] Fish Culture, 9(9):38-42 (in Japanese).

George, M.J. 1970a. Synopsis of biological data on the penaeid prawn Metapenaeus dobsoni (Miers, 1878). FAO Fish. Rept., (57)4:1335-1357.

George, M.J. 1970b. Synopsis of biological data on the penaeid prawn Metapenaeus affinis (H. Milne-Edwards). FAO Fish. Rept., (57)4:1359-1375.

Griessinger, J.M. 1975. Maturation and spawning in captivity of penaeid prawns, Penaeus merguiensis de Man, P. japonicus Bate, P. aztecus Ives and Metapenaeus ensis de Man. Proc. 6th Ann. Workshop World Maricult. Soc. (abstract).

Guillard, R.R.L. and J.H. Ryther. 1962. Studies on marine planktonic diatoms, I. Cyclotella nana Hustedt and Detonula confervacea (Cleve) Gran. Can. J. Microbiol., 8:229-239.

Hara, K., T. Hiyama, T. Ouchi, and S. Tamura. 1969. Korai-ebi shubiyo no ishoku horyu ni tsuite. [On the transplantation of Penaeus orientalis.] Yamaguchi Inland Sea Fish. Expt. Sta. Contr., 18(1):1-9 (in Japanese).

Hirata, H. and T. Wada. 1969. Suiei puru no riyo ni yoru kuruma-ebi no shubiyo seisan to teisei seikatsu ni haitta kuruma-ebi yosei no kyujiryo ni tsuite. [Penaeus japonicus seedling production in large swimming pool, and the feeding of postlarvae after settling.] Saibai Gyogyo [Culture Fisheries], 7(2):44-49 (in Japanese).

Hudinaga, M. 1941. On the nauplius stages of Penaeopsis monoceros and Penaeopsis affinis. Proc. sci. Fish. Assoc., Tokyo, 8:3-4.

Hudinaga, M. 1942. Reproduction, development and rearing of Penaeus japonicus Bate. Jap. J. Zool., 10(2):305-393.

Hudinaga, M. and J. Kittaka. 1966. Studies on food and growth of larval stage of a prawn, Penaeus japonicus, with reference to the application to practical mass culture. Bull. Plankt. Soc. Japan, 13:83-94 (in Japanese).

Hudinaga, M. and J. Kittaka. 1967. The large-scale production of the young Kuruma prawn, Penaeus japonicus Bate. Bull. Plankt. Soc. Japan, Commemoration Number of Dr. Y. Matsue's Sixtieth Birthday, December 1967, pp. 35-46.

Hudinaga, M. and M. Miyamura. 1962. [Breeding of the "Kuruma" prawn (Penaeus japonicus Bate).] J. oceanogr. Soc. Jap., 20th Anniversary volume, pp. 694-706 (in Japanese).

Imamura, S. 1974. Taiwan san kuruma-ebi no sairanyo oya-ebi to shite no riyo kachi ni tsuite. [On the value of utilization of Formosan Penaeus japonicus as the spawning gravid females.] Saibai Kiken [Culture Technology Research], 3(1):77-85 (in Japanese).

Imamura, S. and T. Sugita. 1972. Kuruma-ebi shubiyo ryosan kizutsu
    kaihatsu kenkyu: Jinkoteki yuki gentakubutsu o shiyo sita shoki
    shiiku. [Penaeus shrimp seedling mass-production technology develop-
    ment studies: early rearing of larvae using the artifical organic
    suspended matters.] Saibai Kiken [Culture Technology Research],
    1(2):35-46 (in Japanese).

Ishida, M. 1967. Komenuka ni yoru kuruma-ebi zoea-ki no shiiku ni tsuite.
    [The culture of Penaeus zoeae on a diet of rice bran.] Fukuoka-ken
    Buzen suisan Shikenjo, Kenkyu Gyomu Hokoku [Fukuoka Prefect. Buzen
    exp. Sta., Res. Project Rept.], 1966:87-88 (in Japanese).

Ito, T. 1960. On the culture of mixohaline rotifer Brachionus plicatilis
    O. F. Muller in the seawater. Rept. Fac. Fish. Pref. Univ. Mie,
    3(3):708-740.

Johnson, M.C. and J.R. Fielding. 1956. Propagation of the white shrimp,
    Penaeus setiferus (Linn.) in captivity. Tulane Stud. Zool.,
    4:175-190.

Kittaka, J. 1971. Kuruma egi no yoshoku kizutsu. [Techniques of prawn
    culture.] In: T. Imai (Ed.), Senkai kanzen yoshoku, senkai yoshoku no
    shinpo. [Through culture in shallow seas, progress in shallow sea
    culture.] Tokyo, Koseisha Koseikaku Publ. Co., pp. 344-408 (in Japanese).

Krantz, G.E. and J. Norris. (MS). Culture of pink shrimp, Penaeus duorarum,
    at Turkey Point Experimental Mariculture Laboratory. Proc. 6th
    Ann. Workshop World Maricult. Soc.

Kurata, H. 1967. [Notes on the brine shrimp eggs.] Aquiculture, 14(4): 205-
    219 (in Japanese).

Kurata, H. 1973. [Variation and mechanisms of survival of the seeds shrimp
    sown on the tidelands.] Rep. Fish. Res. Invest. sci. Fish. Agen.,
    Japan. Gov., No. 15, pp. 71-84 (in Japanese, with English abstract).

Kureha, N. and T. Nakanishi. 1972. Shibushijigojo no 2800-ton okata suiso
    ni uoru kuruma-ebi no shubiyo seisan no tsuite. [On the Penaeus
    japonicus seedling production by using 2800-ton capacity large size
    tank at Shibushi Seedling Center.] Saibai Kiken [Culture Tech-
    nology Research], 1(1):41-46 (in Japanese).

Lares, L.B. 1974. Description de los estados larvales de Penaeus
    brasiliensis Latreille, criados en laboratorio. [Description of
    the larval stages of Penaeus brasiliensis Latreille, reared in
    the laboratory.] Publ. Com. Org. III Conf. N.U. Der. del Mar.
    Cuad. Az., 7, Caracas, 31 pp.

Lee, T.Y. 1968. Studies on the gonad development in Metapenaeus joyneri
    (Miers) under various feeding regimes. Publ. mar. Lab., Pusan Fish.
    Coll., 1:19-28.

Lee, B.D. and T.Y. Lee. 1968. Larval development of the penaeidean shrimp
Metapenaeus joyneri (Miers). Publ. Haewundae mar. Lab., 1:1-18.

Lee, B.D. and T.Y. Lee. 1970. Studies on the rearing of larvae and
juveniles of Metapenaeus joyneri (Miers) under various feeding
regimes. Publ. mar. Lab., Pusan Fish. Coll., 3:27-35.

Liao, I.C. 1973. [Note on the cultured spawner of Red-tailed prawn Penaeus
penicillatus Alcock.] JCRR Fish. Ser. (15):59-65 (in Chinese with
English abstract).

Liao, I.C. and T.L. Huang. 1972. Experiments on the propagation and culture
of prawns in Taiwan. In: T.V.R. Pillay (Ed.), Coastal aquaculture
in the Indo-Pacific region. London, Fishing News (Books) Ltd.,
pp. 328-354.

Maeda, Y. 1968. Kuruma-ebi no shubiyo seisan. [The seedling production of
Penaeus shrimp.] Fish Culture, 5(2):64-70 (in Japanese).

Maekawa, K. 1961. Seto-Naikai, tokuni Yamaguchi-Ken engan ni okeru gyogyo
no tosei kanri to shigen baiyo ni kansuru kenkyu. [Studies on
controlled fisheries management and resources cultivation in
Seto Inland Sea, especially in the Yamaguchi-Ken coastal waters.]
Yamaguchi-Ken Naikai Suisan Shikenjo Chosa Kenkys Hokoku
[Yamaguchi-Ken Inland Sea Fish. Expt. Sta. Res. Contr.], 11(1):1-483
(in Japanese).

Maekawa, K. 1970. Korai-ebi no zoyosoku to sono Hiyoka. [The culture of
Penaeus orientalis and its evaluation.] Fish Culture,
7(2):22-24 (in Japanese).

Matsunaga, S. 1973. Kuruma-ebi shubyo seisan ni okeru oya-ebi shiyo no genzo
to mondai ten. [The use of gravid females present conditions
and problems in the Penaeus japonicus seedling production.]
Saibai Kiken [Culture Technology Research], 2(2):39-49.

Menon, M.K. 1952. The life history and bionomics of an Indian penaeid
prawn Metapenaeus dobsoni. Proc. Indo-Pacific Fish. Coun.,
3(II):80-93.

Miyamura, M. 1967. Kuruma-ebi. [Penaeus japonicus]. In: N. Kawamura (Ed.).
Yogyogaku Kakuron. [A treatise on fish culture.] Tokyo,
Koseisha Koseikaku Publ., pp. 727-752.

Mock, C.R. 1974. Larval culture of penaeid shrimp at the Galveston
Biological Laboratory. NOAA Tech. Rep., NMFS CIRC-388:33-40.

Mock, C.R. and M.A. Murphy. 1971. Techniques for raising penaeid shrimp.
Proc. 1st Ann. Workshop World Maricult. Soc., pp. 143-156.

Mock, C.R. and R.A. Neal. 1974. Penaeid shrimp hatchery systems.
FAO/CARPAS Symposium on Aquaculture in Latin America, CARPAS/6/74/SE29,
9 pp.

Nakanishi, T. and S. Kureha. 1971. Shoyu kasu biryushi no katsuyo no ginmi. [Tests on utilization of granulated soy sauce cake.] Saibai Gyogyo [Culture Fisheries], 7(3&4):39-48 (in Japanese).

Oka, M. 1967a. Studies on the propagation of a prawn, Penaeus orientalis, and its culture. II. Mass production of its seeds and culture. Aquiculture, 15(2):7-32.

Oka, M. 1967b. Studies on Penaeus orientalis Kishinouye. V. Fertilization and development. Bull. Fac. Fish. Nagasaki Univ., (23):71-87 (in Japanese, with English summary).

Oka, M. 1970. Kourai-ebi (taisho-ebi) no saibiyo kara yoshoku made. [From seedling production to the farming of Penaeus orientalis.] Fish. Culture, 7(2):34-39 (in Japanese).

Oshima, Y. (Ed.) 1969. Suisan Yoshoku hando buku. [Fisheries aquaculture handbook.] Tokyo, Suisan-sha Pub. Co., 535 pp. (in Japanese).

Oshima, Y. 1972. Setonaikai saibai Gyogyo no genzo to knogo no kadai. [The present status of the Seto Inland Sea Culture fisheries and its future problems.] Setonaikai Saibai Gyogyo Kyokai [Seto Inland Sea Culture Fisheries Association], 29 pp.

Perez-Farfante, I. 1969. Western Atlantic shrimps of the genus Penaeus. Fish. Bull., 67(3):461-591.

Pinto, L.G. and J.J. Ewald. 1974. Desarrollo larval del camaron blanco, Penaeus schmitti Burkenroad, 1936. Bol. Cent. Invest. Biol., Univ. Zulia, No. 12, 61 pp.

San Feliu, J.M., F. Munoz and M. Alcaraz. 1973. Techniques of artificial rearing of crustaceans. Stud. Rev. GFCM, No. 52, pp. 105-121.

Setoguchi, I., H. Shiihara and S. Fujita. 1974. Kuruma-ebi hogoikusei no 2, 3 no mondaiten. [A couple problems on the protective farming of Kuruma shrimp.] Saibai Kiken, 3(1):71-76.

Shigeno, K. 1965. Subyo seisan-yo shisetsu no shomondai. [The problems of the installation of seedling production facilities.] Aquiculture, Spec. Issue No. 4, pp. 19-23.

Shigeno, K. 1969. [Problems on prawn culture (Penaeus japonicus Bate).] Kagoshima Suisan shikenjo Kiyo. [Memoirs Kagoshima Fish. Expt. Sta.], No. 8, 93 pp. (in Japanese).

Shigueno, K. 1972a. Problems of prawn culture in Japan. Overseas Technical Cooperation Agency, Tokyo, Japan, 37 pp.

Shigueno, K. 1972b. Problems of prawn culture in Japan. In: T.V.R. Pillay (Ed.). Coastal aquaculture in the Indo-Pacific region. London, Fishing News (Books) Ltd., pp. 282-312.

Shokita, S. 1970. A note on the development of eggs and larvae of
Penaeus latisulcatus Kishinouye reared in an aquarium.
Biol. Mag. Okinawa, 6(8):34-36 (in Japanese with English abstract).

Spotte, S.H. 1970. Fish and invertebrate culture, water management in
closed systems. New York, Wiley Interscience, 145 pp.

Tabb, D.C., W.T. Yang, Y. Hirono, and J. Heinen. 1972. A manual for
culture of pink shrimp, Penaeus duorarum, from eggs to postlarvae
suitable for stocking. Miami, Florida, Sea Grant Special
Bulletin No. 7, University of Miami, 59 pp.

Takano, H. 1968. On the diatom Chaetoceros calcitrans (Paulsen) emend. and
its dwarf from pumilus forma nov. Bull. Tokai Reg. Fish. Res. Lab.,
55:1-7.

Tuma, D.J. 1967. A description of the development of primary and secondary
sexual characters in the banana prawn, Penaeus merguiensis de Man
(Crustacea: Decapoda: Penaeidae). Aust. J. mar. Freshwat. Res.,
18(1):73-88.

Villaluz, D.K. and A. Villaluz. 1971. Reproduction, larval development and
cultivation of sugpo, Penaeus monodon Fabricius. Paper read at
Marine Science Seminar, Xavier Univ., Cagayan de Oro City,
Philippines, June 17-20, 1971, 37 pp.

Villaluz, D.K., A. Villaluz, B. Ladrera, M. Sheik and A. Gonzaga, 1969.
Reproduction, larval development, and cultivation of sugpo
(Penaeus monodon Fabricius). Philip. J. Sci., 98(3-4):205-236.

Villaluz, D.K., A. Villaluz, B. Ladrera, M. Sheik, and A. Gonzaga. 1971.
Reproduction, larval development and cultivation of sugpo
(Penaeus monodon Fabricius). Mindanao State University--
National Science Development Board assisted Research Project
No. 2.156, Second Annual Report, 33 pp.

Williamson, D.I. 1969. Names of larvae in the decapoda and Euphausiacea.
Crustaceana, 16(2):210-213.

Yang, W.T. and N.C. Alon. in press. Studies on the culture of a rotifer
(Brachionus plicatilis Muller) for use as a larval food organism.
I. Simplified culture method using Torula yeast diets and EDTA.

Yang, W.T. and N.C. Alon. in press. Studies on the culture of the rotifer
(Brachionus plicatilis Muller) for use as a larval food organism.
II. Effect of sterilization and concentration of food on
population growth.