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AQUACULTURE EXTENSION MANUAL NO. 17
MAY 1990

R. S.J. Gapasin
C.L. Marte



AQUACULTURE DEPARTMENT
SOUTHEAST ASIAN FISHERIES DEVELOPMENT CENTER

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**MILKFISH
HATCHERY
OPERATIONS**

R. S.J. Gapasin
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Aquaculture Extension Manual No. 17

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FOREWORD

The Philippines is at the center of distribution of milkfish in the Indo-Pacific region. It is no surprise thus that the hardy *bangus* is both a national symbol and the *raison d'etre* of much of the country's tradition in aquaculture and fisheries.

The Aquaculture Department (AQD) of the Southeast Asian Fisheries Development Center (SEAFDEC) has had over a decade of research work on milkfish, from the development of broodstock technology that led the Philippine Government to establish in 1980 the *National Bangus Breeding Program*; to the spontaneous spawning of broodstock in captivity, first in 1983 and thereafter, yearly during the breeding season; to implementation in the collection of spawned eggs; and to the rapid progress in larval rearing in 1986 to the present.

But these strides were quickly overshadowed by the fast-paced prawn industry, until the economic forces in 1989 caught up with the prawn boom and in turn restored - and even increased - the demand for, and the market price of, finfish including milkfish.

That the technology for milkfish hatchery operations has come of age is signaled by the numerous orders for *bangus* eggs received regularly at SEAFDEC/AQD. Somehow the economic misfortune of those involved in prawn hatchery and pond operations has to be alleviated. This manual offers them and the multitude other fish farmers a viable alternative - one which targets not only the foreign market but answers as well the need to augment the protein food on the table of Filipinos.

F.J. Lacanilao
Chief
SEAFDEC/AQD

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CONTENTS

FOREWORD **iii**

INTRODUCTION **1**

PURPOSE OF THIS GUIDE **1**

ESSENTIAL FACILITIES

 A. Tanks **3**

 B. Equipment **6**

PRODUCTION OF NATURAL FOOD

 A. *Chlorella* Culture **7**

 • Outdoor Mass Culture **7**

 • Indoor Back-Up Culture **10**

 B. *Brachionus* Culture **10**

PRODUCTION OF MILKFISH FRY

 A. Preparation of Hatching and Larval Rearing Tanks **11**

 B. Hatching of Milkfish Eggs **11**

 C. Stocking of Larvae **13**

 D. Larval Rearing **14**

 E. Harvesting **14**

ACKNOWLEDGMENT 16

SELECTED REFERENCES 16

ANNEXES

- A. Materials and Supplies 17
- B. Preparation of Liao and Huang's Medium 18
- C. Preparation of Culture Water 18
- D. Chlorination Procedure for Disinfection 19
- E. Determination of *Brachionus* Feeding Level 20
- F. Determination of *Artemia* Feeding Level 21
- G. Disinfection and Hatching of *Artemia* Cysts 22
- H. Packing and Transport of Eggs 23

INTRODUCTION

Sexual maturation and spontaneous spawning of milkfish [*Chanos chanos* (Försskal)] reared in floating net cages have been attained at the Igang (Guimaras) Substation, Southeast Asian Fisheries Development Center (SEAFDEC) Aquaculture Department and project sites of the Bureau of Fisheries and Aquatic Resources' (BFAR) National Bangus Breeding Program (NBBP) at Masinloc, Zambales; Alaminos, Pangasinan; Calape, Bohol; and Sta. Cruz, Davao del Sur. Techniques of collection and transport of spawned eggs as well as larval rearing have also been developed, thus mass production of milkfish fry is now possible.

Captive milkfish broodstock undergoes first sexual maturation and spawning in five years and thereafter spawns yearly during the breeding season. The period during which milkfish eggs are available therefore coincides with the months when wild fry are relatively abundant. However, the supply of wild fry is often unpredictable and the catch in recent years has apparently diminished. Further, the recent trend toward semi-intensive culture is expected to create a heavier demand for fry which may not be met by the supply from traditional sources. Hatchery production of fry can stabilize the supply of seeds and eventually promote increased production of milkfish, an important food fish in the Philippines.

PURPOSE OF THIS GUIDE

This manual is intended primarily for practicing prawn hatchery operators who would like to diversify their operations to include larval rearing of milkfish. We have therefore considered the following assumptions in its preparation:

- Availability of an operational prawn hatchery, i.e., equipped with the essential facilities;
- Availability of experienced prawn hatchery technicians.

Topics such as site selection, hatchery design and construction, broodstock management, spawning and egg collection are therefore not dealt with. Only those procedures important in the daily operations of a milkfish hatchery are described in detail.

We relied on our experience with a small-scale milkfish hatchery with a fry production of 60,000 per run of 21 days. Nevertheless, we are confident that the same procedures only need to be modified depending on available resource for large-scale production.

2 Milkfish Hatchery Operations

Naturally spawned milkfish eggs may be secured from SEAFDEC Aquaculture Department and from the NBBP project sites of BFAR in Alaminos, Pangasinan; Calape, Bohol; and Sta. Cruz, Davao del Sur.

This is but a first attempt at a procedural guide to the operation of a milkfish hatchery. We anticipate that the technology here packaged will in the long run be improved upon by the hatchery operators themselves and other end-users, especially if such modifications will mean higher efficiency, productivity, and economic returns. We therefore welcome comments, suggestions and other feedbacks for consideration in future revisions of this manual.

ESSENTIAL FACILITIES

A. Tanks

A milkfish hatchery needs larval rearing tanks, culture tanks for rotifer (*Brachionus*) and green algae (*Chlorella*), and hatching tanks for the brine shrimp (*Artemia*). A volume ratio of 1 ton larval rearing tank to 3 tons algal and rotifer tank is recommended. Tanks should be easily drained through a harvesting canal. A layout of a typical milkfish hatchery is shown in Figure 1. A prawn hatchery operator may need only a slight modification, i.e.; addition of algal and rotifer culture tanks to come up with the recommended ratio of 1:3.

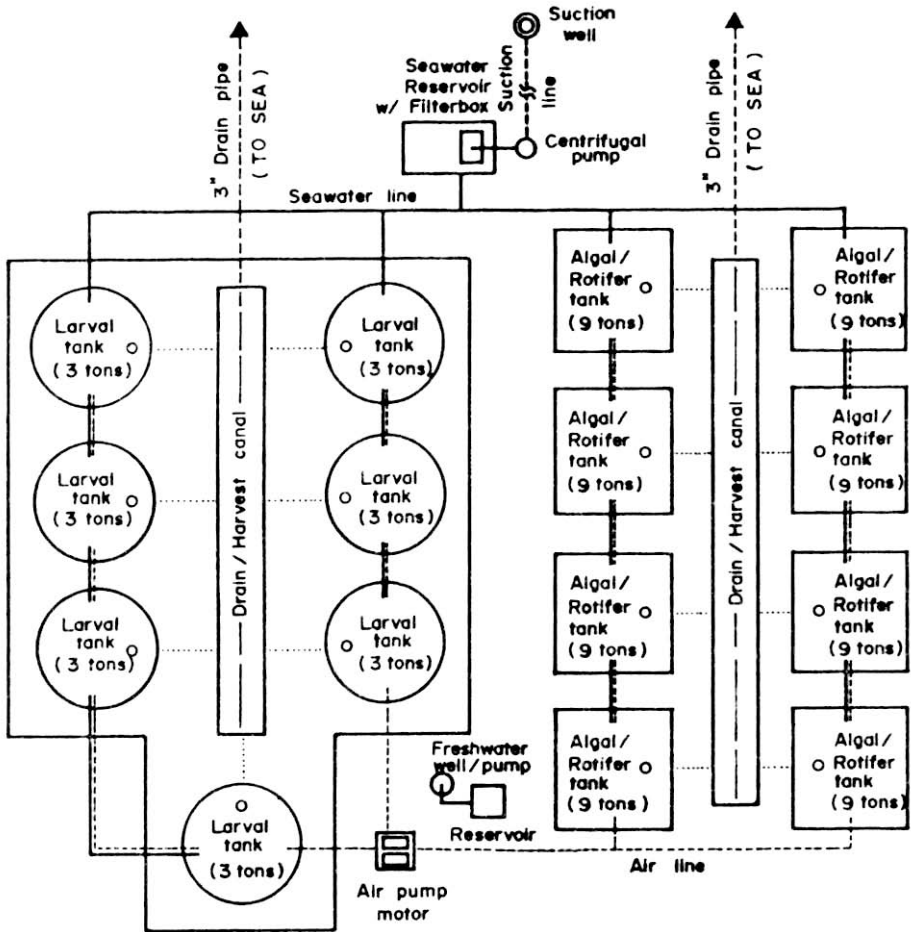


Fig. 1. Layout of a typical small-scale milkfish hatchery.

1. Larval Rearing Tank

Circular (2-3-meter diameter) canvas or concrete tanks (3-5-ton capacity) with an airstone at the center may be used. Tanks of smaller volumes are preferred for easy management. Larval rearing tanks should be placed under a shade to protect the larvae from the glare and heat of direct sunlight and to deter growth of diatoms that contribute to poor water quality.

2. Algal/Rotifer Tank

Square (3x3x 1 meter), rectangular (3.5x2.5x 1 meter) or circular (4-meter diameter) canvas or concrete tanks may be used for mass production of *Chlorella* and *Brachionus*. To maximize tank usage, tanks for algae are also used to culture rotifer.

3. Brine Shrimp Hatching Tank

A cylindro-conical plexiglass, transparent conical fiberglass, or plastic carbuoy may be used in hatching *Artemia* cysts (Fig. 2). The capacity of the hatching container varies depending on the amount of *Artemia* to be hatched. A plastic carbuoy usually has a capacity of 10 liters.

4. Gravity Sand Filter

A wooden, fiberglass, or concrete sand filter box (1x1x1 meter) is positioned above the seawater storage tank. The filter medium is generally composed of layers of fine sand, coarse sand, gravel or coarse stones interspersed with charcoal, and shell or coral pieces (Fig. 3). Filtered seawater directly flows into the storage tank by gravity.

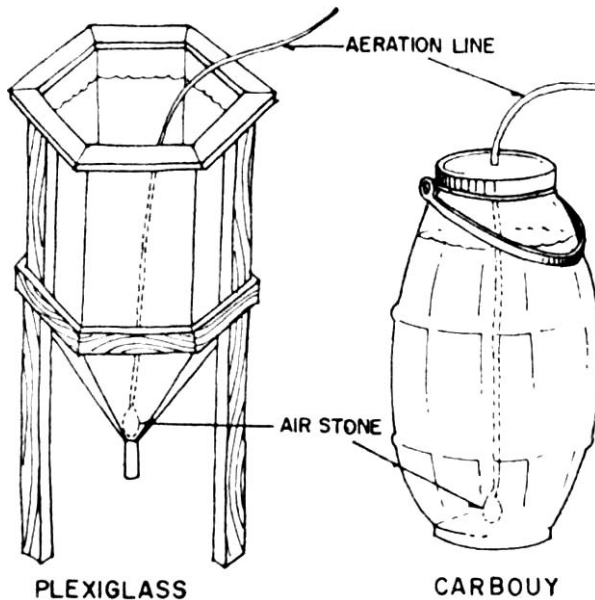


Fig. 2. Containers for hatching *Artemia* cysts.

5. Seawater Storage Tank

A seawater tank with a capacity of at least 50% of the total volume of culture tanks is recommended. Storage tank should be elevated so that filtered seawater can be distributed to all tanks by gravity flow (Fig. 3).

6. Fiberglass Tanks

- 1-ton capacity (4 units)
- 500-liter capacity (4 units)
- 250-liter capacity (5 units)
- 100-liter capacity (3 units)

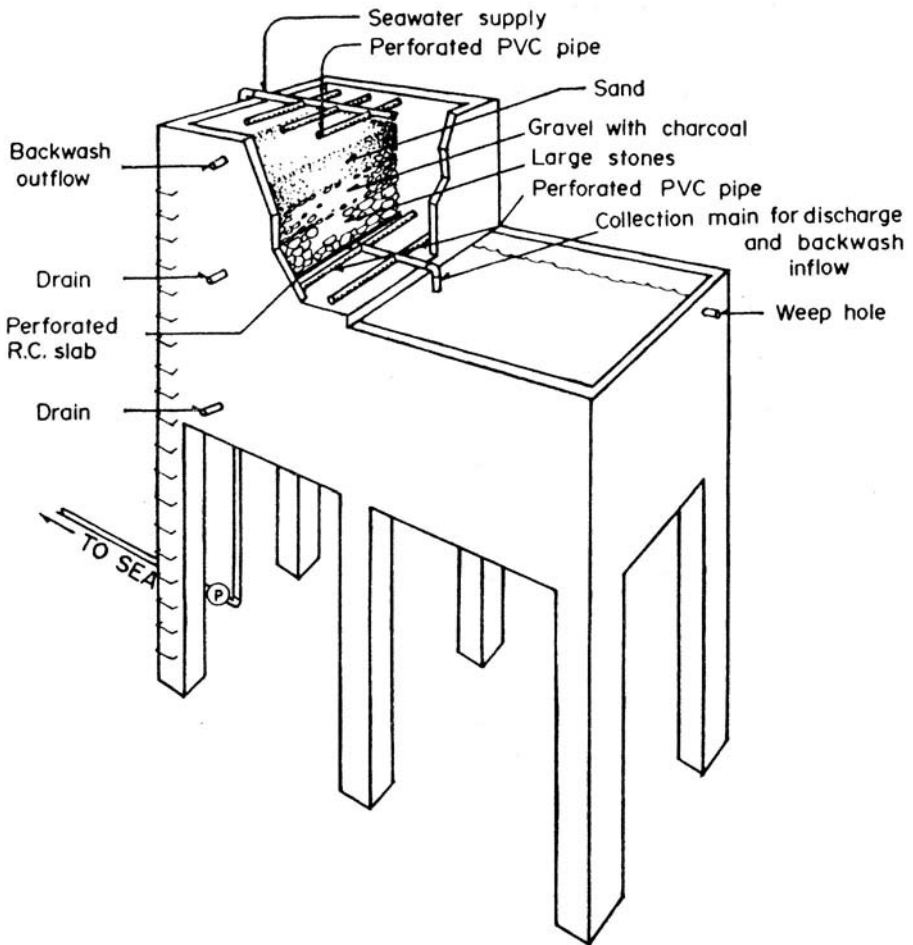


Fig. 3. Elevated seawater reservoir with cut-out diagram of gravity sand filter.

B. Equipment

Essential components of a milkfish hatchery are pumps for air and seawater. Spare pumps should be provided in case of breakdown.

1. *Seawater centrifugal pump* (2 HP) for pumping prefiltered seawater from the suction well to filter tank and reservoir. A schematic diagram of a suction well is shown in Figure 4.

2. *Air blower* (1.5 HP) provides aeration to all tanks.

3. *Submersible pump* (1/3 HP) for mass transfer of algae to rotifer tanks.

4. *Freshwater pump* (1/3 HP) draws freshwater from a shallow well for hatchery use.

5. *Standby generator* (5 KVA) should be available in case of power failure.

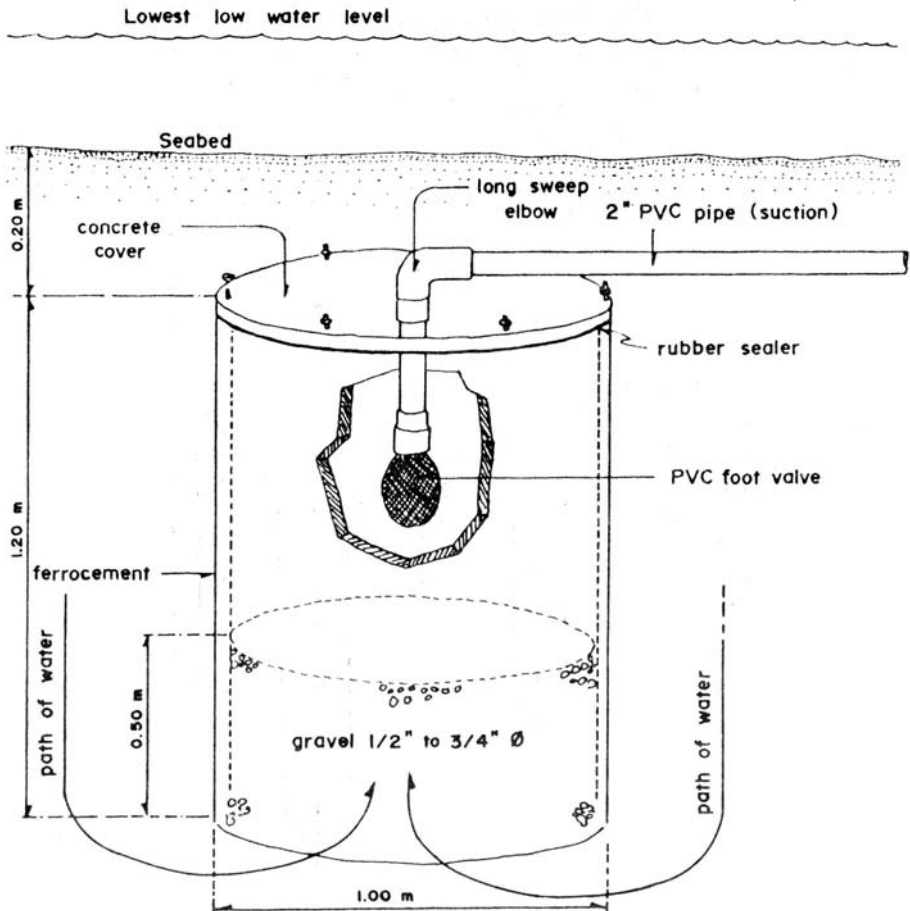


Fig. 4. Suction well for prefiltered seawater.

6. *Stereomicroscope* for estimating density of rotifer, egg, and larval samples.

7. *Weighing scale* (preferably top-loading type):

- i) 1-kilogram capacity, 10-gram sensitivity - for weighing *Artemia* cysts.
- ii) 10-kilogram capacity, 50-gram sensitivity - for weighing fertilizers.

A checklist of materials, supplies and optional equipment is given in Annex A.

PRODUCTION OF NATURAL FOOD

The food organisms used for rearing milkfish larvae are the unicellular green alga *Chlorella*, the rotifer *Brachionus*, and the brine shrimp *Artemia*. Green algae aid in maintaining good water quality in the larval rearing tank and serve as food for *Brachionus*. Milkfish larvae are fed with rotifers throughout the whole rearing period. Brine shrimp nauplii are fed on the 15th day to the 21st day when larvae are usually harvested.

Chlorella and *Brachionus* starters are obtained in limited quantities from laboratories or other hatcheries. Thus mass culture of these food organisms has to be started at least one month before larval rearing commences. Small-scale indoor culture of these food organisms in fiberglass tanks precedes large-scale culture in canvas or concrete tanks.

The procedure for culturing starters of *Chlorella* and *Brachionus* in indoor facilities and then in outdoor tanks follows. Figure 5 illustrates programming of both culture operations. Figure 6 shows the basic scheme for outdoor mass culture following the "daily tank-transfer" method.

It is important to clean thoroughly before use all fiberglass and canvas tanks. Scrub with sponge and detergent, rinse well, and sun dry.

A. *Chlorella* Culture

Obtain four 10-liter *Chlorella* starter cultures. Three starters will be used for outdoor culture; the fourth is for indoor back-up culture. *Chlorella* is cultured first before *Brachionus*.

Outdoor Mass Culture

1) Set up three 100-liter fiberglass tanks in open air in a makeshift shelter with translucent plastic roofing.

2) Fill up the first tank with 20 liters filtered seawater. Introduce the first 10-liter *Chlorella* starter. Make sure the starter and seawater in the tanks have the same salinity and temperature.

(Cultures using the second and third *Chlorella* starters will be started on the second and third days, respectively, following steps 2-9).

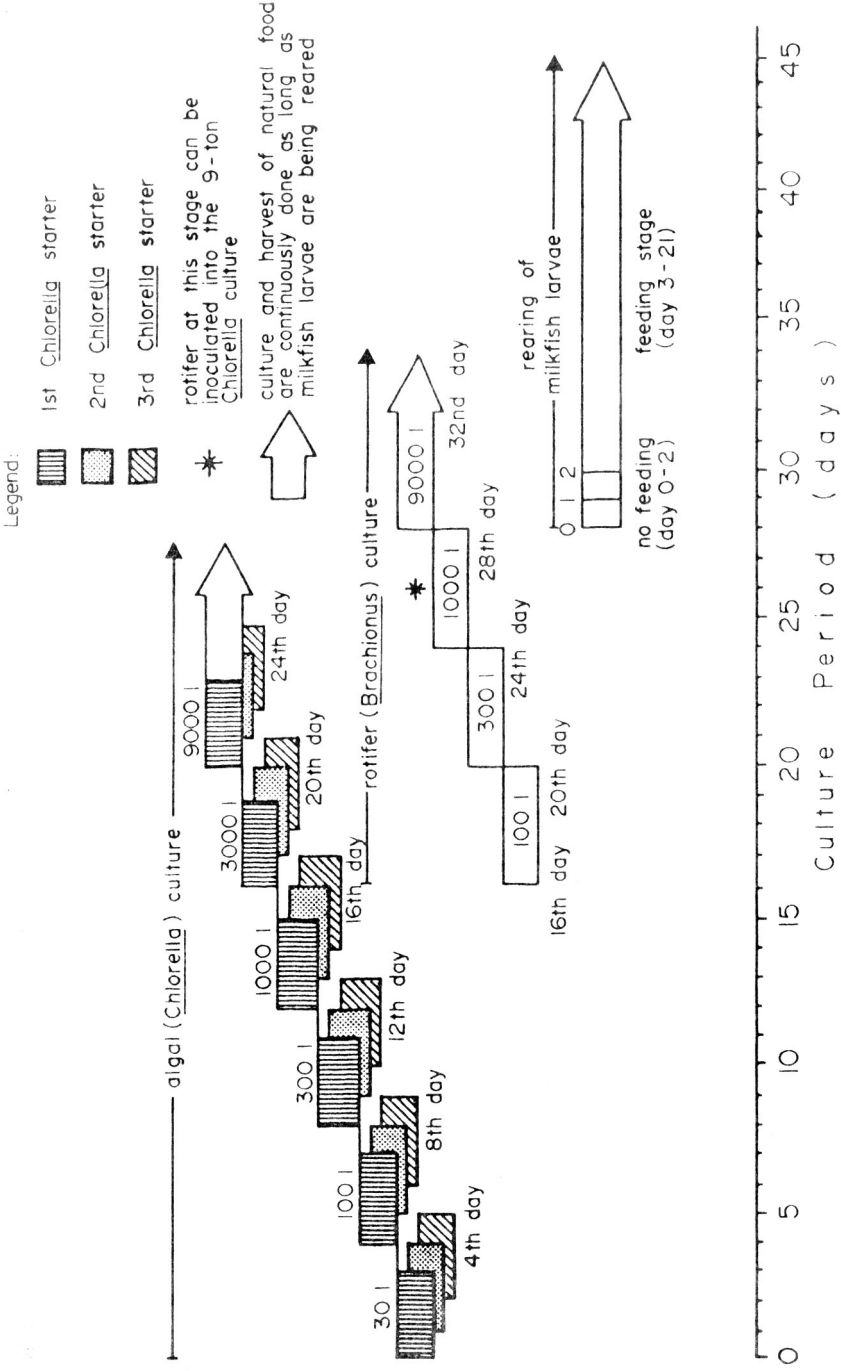


Fig. 5. Programming culture of natural food for milkfish hatchery operations.

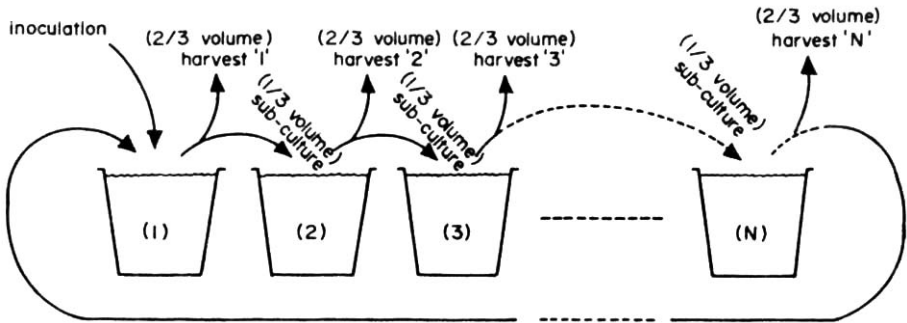


Fig. 6. Daily tank-transfer method for culturing *Chlorella* and *Brachionus*.

3) Fertilize. In a pail containing small volume of seawater, dissolve the following commercial fertilizers:

Ammonium phosphate (16-20-0) = 15 milligrams/liter

Urea (46-0-0) = 15 milligrams/liter

Ammonium sulfate (21-0-0) = 100 milligrams/liter

Add dissolved fertilizers into each of three 30-liter cultures. Aerate moderately.

4) Under normal outdoor conditions, the culture reaches peak density in 3-4 days after seeding and usually appears green to dark green.

5) On the 3rd or 4th day of culture, add 70 liters filtered seawater into the first 100-liter culture tank. Fertilize (*see* step 3).

6) After 3-4 days, transfer the 100-liter culture into the 500-liter fiberglass tank using a submersible pump. Add 200 liters filtered seawater. Fertilize.

7) When culture appears green to dark green, transfer each 300-liter culture into a 1-ton fiberglass tank using a submersible pump. Add 700 liters filtered seawater into the tank. Fertilize.

8) After 3-4 days, large-scale outdoor culture of *Chlorella* can be started. Using a submersible pump, transfer the 1000-liter culture into a 9-ton outdoor tank. Add Purex[®] (sodium hypochlorite) solution (10 milliliters/ton) to deter growth of diatoms. Then add 2000 liters filtered seawater. Fertilize.

Small-scale culture of *Brachionus* may also be started (Fig. 5). Refer to Section B (page 10) for procedures.

[®] Mention of brand names in this manual does not mean endorsement of product.

9) When culture appears green to dark green, add Purex solution (10 milliliters/ton). Then add 6000 liters filtered seawater. Fertilize. On the 3rd or 4th day, transfer 3000 liters of the culture as starter for the second 9-ton tank. Into the 6000 liters *Chlorella* left in the first tank, introduce *Brachionus* scaled-up from 1 ton (Fig. 5).

10) Culture and harvest on a daily basis (Fig. 6).

Indoor Back-Up Culture

Divide the fourth 10-liter *Chlorella* starter culture into three plastic carbuoys. Add 6 liters chlorinated seawater into each carbuoy. Place carbuoys in the makeshift shelter. Fertilize using Liao and Huang's Medium (modified). If technical grade chemicals are not available or too expensive, commercial grade (agricultural) fertilizers may be used. For preparation of Liao and Huang's Medium (modified) or substitute fertilizer mixture, refer to Annex B. Use chlorinated water to deter growth of diatoms. For preparation of chlorinated water, refer to Annex C.

During the night, provide cultures with artificial light. Use 2-3 40-watt flourescent lamps.

Maintain these starter cultures by repeating the procedure. These cultures serve as back-up should outdoor cultures collapse.

B. *Brachionus Culture*

Mass culture of rotifer may be started on the 24th day when culture of *Chlorella* in 9-ton tanks has reached optimum density (Fig. 5). *Brachionus* in 1-ton culture can be directly seeded into the 9-ton *Chlorella* culture for mass-scale production. If additional tanks are available, *Brachionus* may be cultured separately from *Chlorella*.

Obtain two 10-liter *Brachionus* starter cultures. Use the first for mass culture. The second culture serves as back-up.

1) Using a submersible pump, fill up one 250-liter fiberglass tank with 100 liters *Chlorella* from 1-ton fiberglass cultures. Introduce the first *Brachionus* starter culture (initial density, 15-20 individuals/milliliter). Make sure the *Brachionus* and *Chlorella* cultures have the same temperature and salinity. Allow 3-4 days for *Brachionus* to reach peak density (100-200 individuals/milliliter) or until culture turns pale or lose its dark green color.

2) Harvest. Using a filter bag (pore size, 60 microns), concentrate *Brachionus* from the 250-liter fiberglass tank. Place concentrated *Brachionus* into a plastic pail (8-10-liter capacity). Aerate moderately.

3) Using a submersible pump, fill up the 500-liter fiberglass tank with 300 liters *Chlorella*. Introduce the concentrated *Brachionus*.

4) Allow 3-4 days for *Brachionus* to reach peak density, then do total harvest.

5) Using a submersible pump, fill up the 1-ton fiberglass tank with

1000 liters *Chlorella*. Introduce concentrated *Brachionus*. Allow 3-4 days for *Brachionus* to reach peak density.

[At this stage in *Brachionus* culture, larval rearing tanks can be stocked with newly hatched milkfish larvae (Fig. 5)].

6) Harvest *Brachionus* in the 1-ton culture.

7) Introduce concentrated *Brachionus* into the 9-ton *Chlorella* culture tank.

8) On the 3rd or 4th day, again totally harvest *Brachionus*. Feed two-thirds of the harvested *Brachionus* to milkfish larvae. Use the other one-third as starter for the next culture.

9) Repeat above procedures with the succeeding tanks. Figure 6 illustrates programmed culture and harvest of *Chlorella* and *Brachionus* known as "daily tank-transfer".

PRODUCTION OF MILKFISH FRY

Milkfish eggs are collected from broodstock cages between 6:00 and 8:00 A.M. These are in an early stage of embryonic development and should be transported immediately to the hatchery. Hatching occurs in 14 to 16 hours after collection. Hatchery and larval rearing tanks therefore have to be prepared at least one day before eggs are obtained.

A. *Preparation of Hatching and Larval Rearing Tanks*

1) Thoroughly scrub with scrubbing pad or nylon brush and detergent the 500-liter fiberglass hatching tanks and 3-ton larval rearing tanks. Rinse well with seawater or freshwater.

2) Fill up the tanks, preferably with freshwater, and disinfect by chlorination. Refer to Annex D for procedure.

B. *Hatching of Milkfish Eggs*

1) Fill up hatching tank with filtered seawater. To estimate the number of hatching tanks needed, allow a density of 300 eggs/liter for optimum hatching. Eggs are packed in double-layered oxygenated plastic bags usually at a density of 8,000 eggs/liter of transport water. Refer to Annex H for packing procedures.

2) Upon arrival of eggs at the hatchery, let the plastic bag float in the hatching tank for 15 minutes to allow temperature in the bag to equilibrate with the temperature in the hatching tank. Allow small amounts of water in the hatching tank to intermix slowly with water in the transport bag before gently pouring the content of the bag into the hatching tank.

12 Milkfish Hatchery Operations

3) Stock not more than 300 eggs/liter. Aerate moderately to prevent aggregation of eggs.

4) Wait for about 15-30 minutes, then stop aeration. Gently swirl the water and wait until water motion stops. Siphon out unfertilized and dead eggs at the bottom of the tank.

5) Flow-through filtered seawater into the hatching tank for 1-2 hours (Fig. 7). Provide moderate aeration.

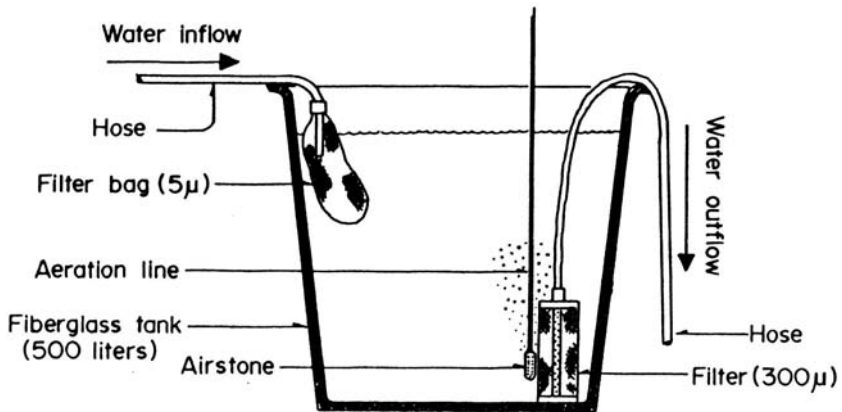


Fig. 7. Flow-through set-up for hatching milkfish eggs.

6) For estimating the number of viable eggs, collect samples using a 1.5-meter long PVC pipe (diameter, 2 inches) at five different points (4 at the sides, 1 at the center) of the hatching tank. Drain these samples into a pail and label. Using a plankton net sieve (pore size, 300 microns), strain eggs from the pail while pouring the water directly into a graduated 4-liter plastic beaker to estimate the volume of water sample. Pipette out a small quantity of eggs, deposit into a modified Sedgewick-Rafter counting chamber (Fig. 8), and count the number of eggs under a stereomicroscope. Do this until all the strained eggs have been counted. Estimated egg count (EEC) is computed as follows:

$$EEC = \frac{\text{No. of eggs counted}}{\text{Total water volume from PVC sampler}} \times \text{Water volume of hatching tank}$$

7) Milkfish eggs at 28-29°C usually hatch within 24-26 hours after spawning or 14-16 hours after collection. When hatching is completed, siphon out egg cases, dead eggs and other debris following steps 4 and 5 above. Estimated larval count (ELC) is computed using the formula

$$ELC = \frac{\text{No. of larvae counted}}{\text{Total water volume from PVC sampler}} \times \text{Water volume of hatching tank}$$

8) Hatching rate (HR) can be determined using the equation

$$HR (\%) = \frac{ELC}{EEC} \times 100$$

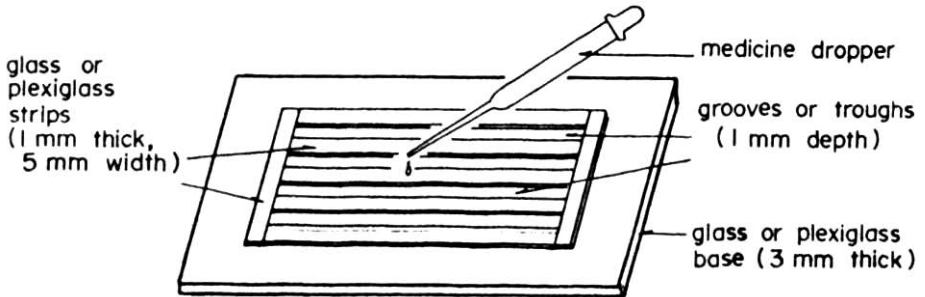


Fig. 8. Modified Sedgewick-Rafter counting chamber.

C. Stocking of Larvae

To obtain an optimum survival of 30-40%, rearing tanks are stocked at a density of 30 larvae/liter.

1) Fill up the rearing tank with 2250 liters filtered seawater (75% of the total volume, in case different tank capacity is used). Add "green water" (use *Chlorella* culture at its optimum density) at a ratio of 1 liter for every 5 liters of seawater (750 liters for 2250 liters seawater, if 3-ton tank is used).

2) Compute for the number of larvae to be stocked into the rearing tank using the equation

$$\begin{matrix} \text{Desired number} \\ \text{of larvae to be} \\ \text{stocked in the} \\ \text{rearing tank} \end{matrix} = 30 \text{ larvae/liter} \times \text{Total tank water volume}$$

3) After determining number of hatched larvae, reduce water volume in the 500-liter hatching tank to 100 liters. Compute for the water volume to be transferred from the hatching tank into the rearing tank by following the example below.

14 Milkfish Hatchery Operations

Assuming an ELC of 180,000 in 100 liters in the hatching tank and 90,000 larvae are to be stocked in a 3-ton rearing tank, the volume of water with newly hatched larvae to be transferred (x) is computed as follows:

$$\frac{100 \text{ liters}}{x} = \frac{180,000 \text{ larvae}}{90,000 \text{ larvae}}$$

$$x = 50 \text{ liters}$$

If 8-liter plastic pails are used, then about 6 pails of water with larvae would be needed.

D. Larval Rearing

The schedule to be followed for water management and feeding during larval rearing is illustrated in Figure 9.

1) Maintain mild aeration during the first week of rearing. Should water in rearing tanks turn pale, add enough *Chlorella* to restore the green-to-light-green color. *Chlorella* should be added preferably in the morning before feeding the larvae.

2) Starting on day 2 until day 14, add *Brachionus* at 10-15 individuals/milliliter. From day 15 to day 21, reduce *Brachionus* count to 0.5 individual/milliliter. For computation of *Brachionus* feeding level, see Annex E.

3) *Artemia* nauplii at 0.5 individual/milliliter are fed from day 15 to day 17. Increase feeding level to 1 individual/milliliter from day 18 until harvest (day 21). The procedure for computing *Artemia* feeding level is given in Annex F. The steps in the disinfection and hatching of *Artemia* cysts are described in Annex G.

4) Siphon out wastes and uneaten food in the tank bottom every morning from day 2 until day 21.

5) Change 30% of the water volume every morning from day 2 until day 14. Change 50-70% of the water from day 15 to day 21.

E. Harvesting

Milkfish larvae on day 21 are at about the same developmental stage as wild-caught fry and are ready to be harvested. The procedures for harvesting, estimating and packing larvae to be sold or transported for stocking in nursery ponds or tanks follow those practised by prawn hatchery operators and fry gatherers and dealers.

1) Drain water in the rearing tank to about one foot deep.

2) Using a small basin, scoop larvae and transfer into a big plastic basin.

3) To estimate the number of harvestable larvae, count the number of larvae in a basin of known water volume. This serves as a standard on

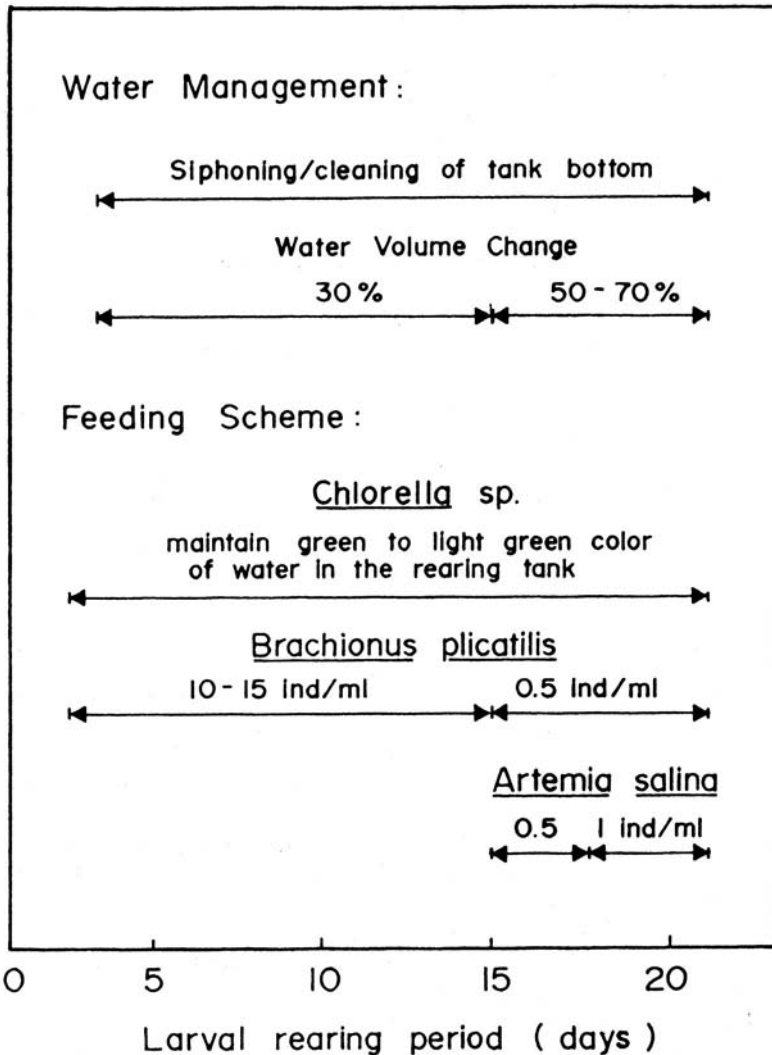


Fig. 9. Water management and feeding scheme for rearing milkfish larvae (after Marte, 1987).

which all estimates are based. Distribute larvae evenly in other basins containing the same volume of seawater as the standard basin. Compare visually whether the other basins contain more or less the same number of larvae as for the standard basin.

4) Pack larvae in double-layered oxygenated plastic bags containing 8-15 liters of seawater at a loading rate of 300 larvae/liter. Packing and transport procedures are similar to those for spawned eggs (Annex H).

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ANNEX A. Materials and Supplies.

A. Natural Food Starters

1) *Chlorella* starter - This may be obtained from research institutions or from other hatcheries.

2) *Brachionus* starter - Like *Chlorella*, it can be secured from nearby hatcheries or from research institutions.

3) *Artemia* cysts - These are available from leading aquaculture supply stores. Quality *Artemia* strains with high hatching efficiency are preferred.

B. Chemicals

1) Fertilizers - These are used for mass production of *Chlorella*.

a) Potassium nitrate (KNO_3) or

Sodium nitrate (NaNO_3) crystals

b) Sodium phosphate ($\text{Na}_2\text{HPO}_4 \cdot 12\text{H}_2\text{O}$) crystals

c) Ferric chloride ($\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$) crystals

d) Urea (46-0-0)

e) Ammonium phosphate (16-20-0)

f) Ammonium sulfate (21-0-0)

2) Others for cleaning and disinfecting tanks, hatchery glassware, pails, etc.

a) Chlorine granules

b) Sodium thiosulfate

c) Sodium hypochlorite (Purex) solution

d) Detergent

3) Supplies

a) Plastic carbuoy - 10-liter capacity (10 units)

b) Plastic or rubber hose - 2-inch diameter

c) Plastic tubing - 0.25-inch diameter

d) Beakers - plastic, 4-liter capacity (2 pieces)

glass, 1-liter capacity (2 pieces)

100- milliliter capacity (2 pieces)

e) Sieve - plankton net (pore size, 300 microns) for straining egg and larval samples.

- f) Harvesting bag - plankton net (pore size, 60 microns) for harvesting rotifers.
- g) Filter bag - bolting cloth (pore size, 5 microns) for filtering sea water.
- h) Pasteur pipettes (glass) with rubber bulbs or medicine droppers (12 pieces)
- i) Sedgewick-Rafter counting chamber
- j) Plastic pails - 8-10-liter capacity (6 units)
- k) Plastic basins - 20-30-liter capacity (12 units)
5-liter capacity (6 units)

4) Optional Equipment

- a) Dissolved oxygen meter (YSI Model 51B)
- b) Refractometer (Atago or American Optical brand)
- c) Thermometer
- d) Weighing scales (preferably top-loading type):
 - i) 1-kilogram capacity, 10-gram sensitivity - for weighing *Artemia* cysts.
 - ii) 10-kilogram capacity, 50-gram sensitivity - for weighing fertilizers.

ANNEX B. Preparation of Liao and Huang's Medium (modified).

1. Weigh out the following technical grade chemicals:

Potassium nitrate (KNO ₃) or	
sodium nitrate (NaNO ₃).....	100 grams
Sodium phosphate (Na ₂ HPO ₄ ·12H ₂ O).....	10 grams
Ferric chloride (FeCl ₃ ·6H ₂ O).....	3 grams

2. Dissolve the chemicals in 1 liter of distilled or chlorinated water.

3. Use 1 milliliter of fertilizer solution for every 1 liter of *Chlorella* culture.

4. Keep solution in a refrigerator (15-20°C) to lengthen storage period.

If technical grade chemicals are not available or too expensive, commercial fertilizers can be used. For every 1 liter of *Chlorella* culture, dissolve the following fertilizers:

Ammonium phosphate (16-20-0).....	15 milligrams
Urea (46-0-0).....	15 milligrams
Ammonium sulfate (21-0-0).....	100 milligrams

ANNEX C. Preparation of Culture Water.

A. Chlorination

1. Fill up reservoir (250-liter or 0.25-ton capacity) with filtered sea water.
2. Treat sea water with 7 ppm hypochlorite solution. Commercial

chlorine granules contains 70% sodium hypochlorite).

Example:

$$(7 \text{ ppm}/0.7) \times 0.25 \text{ ton} = 2.5 \text{ grams chlorine granules}$$

3. Weigh out 2.5 grams chlorine granules.
4. Fully dissolve chlorine granules in small amount of freshwater.
5. Pour and mix hypochlorite solution into the reservoir containing filtered seawater.
6. Let stand for 12-24 hours.

B. Neutralization

1. Weigh out the same amount of sodium thiosulfate as that of chlorine granules, i.e., 2.5 grams.
2. Dissolve first in small amount of freshwater.
3. Add the sodium thiosulfate solution into the chlorinated seawater. Let stand for 1-2 hours.
4. Seawater can now be used as culture medium.

ANNEX D. Chlorination Procedure for Disinfection.

- 1) Determine capacity (water volume) of tank to be disinfected.
- 2) To obtain a 200 ppm solution, calculate the amount of chlorine powder or granules to be used from the following information:

$$\begin{aligned} 1 \text{ gram}/1000 \text{ liters of water} &= 1 \text{ ppm} \\ 1 \text{ milligram}/\text{liter of water} &= 1 \text{ ppm} \end{aligned}$$

Example:

For a 3.0-ton (3000-liter) capacity tank, determine the amount of chlorine granules to obtain a 200 ppm solution.

$$\begin{aligned} \text{If } 1 \text{ gram}/1000 \text{ liters} &= 1 \text{ ppm, then } 200 \text{ gram}/1000 \text{ liters} \\ &= 200 \text{ ppm.} \end{aligned}$$

$$\begin{aligned} \text{Therefore;} \\ \frac{200 \text{ grams}}{x} &= \frac{1000 \text{ liters}}{3000 \text{ liters}} \end{aligned}$$

$$\begin{aligned} x &= 600 \text{ grams of chlorine granules to be} \\ &\text{dissolved in } 3000 \text{ liters of water to make} \\ &200 \text{ ppm solution} \end{aligned}$$

- 3) After computing for the amount of chlorine granules needed, weigh out the desired amount. Dissolve this in a small amount of fresh water before adding to the tank to be disinfected. Mix well.

4) Let stand overnight. Drain water the following day. Clean the tank by scrubbing with sponge and detergent. Rinse thoroughly and let dry.

ANNEX E. Determination of *Brachionus* Feeding Level.

The required amount of *Brachionus* to be fed can be calculated from the general formula

Required volume of concentrated *Brachionus* to be fed (liters) =
$$\left[\frac{\text{Desired } Brachionus \text{ feeding density (B)} - \text{Existing } Brachionus \text{ density in tank (C)}}{\text{Density of concentrated } Brachionus \text{ (A)}} \right] \div 1000$$

A - Density of Concentrated *Brachionus*

1) Harvest *Brachionus* stock. Concentrate into a 8-1 plastic pail (see *Brachionus* Culture, p. 10). Feed with enough *Chlorella*. Provide moderate aeration.

2. Pipette out 1 milliliter of concentrated *Brachionus* stock and transfer into a beaker filled with 100 milliliters seawater.

3) Take 1 milliliter subsample and deposit into a modified Sedgewick-Rafter counting chamber (Fig. 8). Count the number of *Brachionus* under a stereomicroscope. Do this five times. Disregard the highest and lowest counts. Get the average of the three remaining counts.

4) Multiply the average count by 100. The product is the *density of the concentrated Brachionus stock*.

Example:

Given counts of 35, 30, 40, 49 and 20, average count is 35, disregarding counts 49 and 20. Density of concentrated *Brachionus* is computed as follows:

$$\begin{aligned} A &= (35 \text{ individuals/milliliter}) (100 \text{ milliliter}) \\ &= 3,500 \text{ individuals/milliliter} \end{aligned}$$

B - Desired *Brachionus* Feeding Density

1) Consult Figure 9 for daily feeding level of larvae (number of individuals/milliliter). Multiply this particular feeding level by the volume of water in the larval rearing tank. This is the *desired Brachionus feeding density level*.

Example:

For 7-day old larvae, feeding level is 10 individuals/milliliter. Water volume in the tank is 3 tons (3,000 liters = 3,000,000 or 3.0×10^6 milliliters). Desired *Brachionus* feeding level is calculated as follows:

$$\begin{aligned} \mathbf{B} &= (10 \text{ individuals/milliliter}) (3.0 \times 10^6 \text{ milliliters}) \\ &= 3.0 \times 10^7 \text{ individuals} \end{aligned}$$

C - *Existing Brachionus Density in Rearing Tank*

1) Using a 30-50-milliliter plastic container, take water samples from rearing tank, preferably at the center.

2) Do Step A.3. The product of *average value* multiplied by the volume of water in the larval rearing tank is the *existing Brachionus density*.

Example:

Given counts of 10, 5, 4, 3 and 6, average count is 5, disregarding counts 10 and 3. Average count is expressed as 5 individuals/milliliter. If water volume in the rearing tank is 3 tons, *existing Brachionus density* is computed as follows:

$$\begin{aligned} \mathbf{C} &= (5 \text{ individuals/milliliter}) (3.0 \times 10^6 \text{ milliliters}) \\ &= 1.5 \times 10^7 \text{ individuals} \end{aligned}$$

After deriving values for **A**, **B** and **C**, simply plug in these values into the general formula given above (page 20). Resulting value is the volume (in liters) of concentrated *Brachionus* to be fed to milkfish larvae.

ANNEX F. Determination of *Artemia* Feeding Level.

Compute as follows for the quantity (grams) of *Artemia* cysts to be hatched (X):

$$X = \frac{\text{Feeding rate} \times \text{Volume of larval rearing tank (milliliters)}}{\text{Hatching efficiency of } Artemia \text{ cysts}}$$

NOTE:

One gram of commercial *Artemia* is made up of approximately 300,000 cysts. If hatching efficiency by weight is 50%, then 300,000 cysts would produce about 150,000 *Artemia* nauplii (150,000 nauplii/gram cysts).

Example:

Hatching efficiency = 150,000 nauplii/gram cysts

Feeding rate = 0.5 individual/milliliter (Fig. 9)

Volume of larval rearing tank = 3,000,000 milliliters (3 tons)

Substituting values for the formula above,

$$X = \frac{0.5 \text{ individual/milliliter} \times 3,000,000 \text{ milliliters}}{150,000 \text{ nauplii}}$$

= 10 grams of *Artemia* cysts to be hatched

ANNEX G. Disinfection and Hatching of *Artemia* Cysts.

A. Disinfection

- 1) Determine amount of *Artemia* cysts to be hatched (*see* Annex F).
- 2) Weigh out desired amount of *Artemia* cysts.
- 3) Soak cysts in 5-8 liters of freshwater in plastic pail for 5 minutes. Add 20-30 milliliters sodium hypochlorite solution (Purex).
- 4) Aerate vigorously for 30-45 minutes.
- 5) Wash cysts 4-5 times with freshwater or until "chlorine odor" is removed completely.

B. Hatching

- 1) Transfer and hatch cysts in conical plexiglass or plastic carbuoys containing filtered seawater (Fig. 2). Density should be 1-2 grams of *Artemia* cysts per 1 liter of water.
- 2) Aerate vigorously. Aeration should come from the bottom of the water to keep the cysts in suspension. In 22-26 hours, 90% of the *Artemia* cysts should have hatched. Stop or remove aeration.
- 3) Cover upper half of the hatching tank or container with a black cloth or opaque plastic sheet.
- 4) Let stand for 15-20 minutes.
- 5) Into a clean pail, siphon out nauplii crowding near the bottom of the hatching tank by using a rubber or plastic hose (0.25-inch diameter, 1-meter long). Wash with filtered seawater for 1-2 minutes. Feed to milkfish larvae.

ANNEX H. Packing and Transport of Eggs

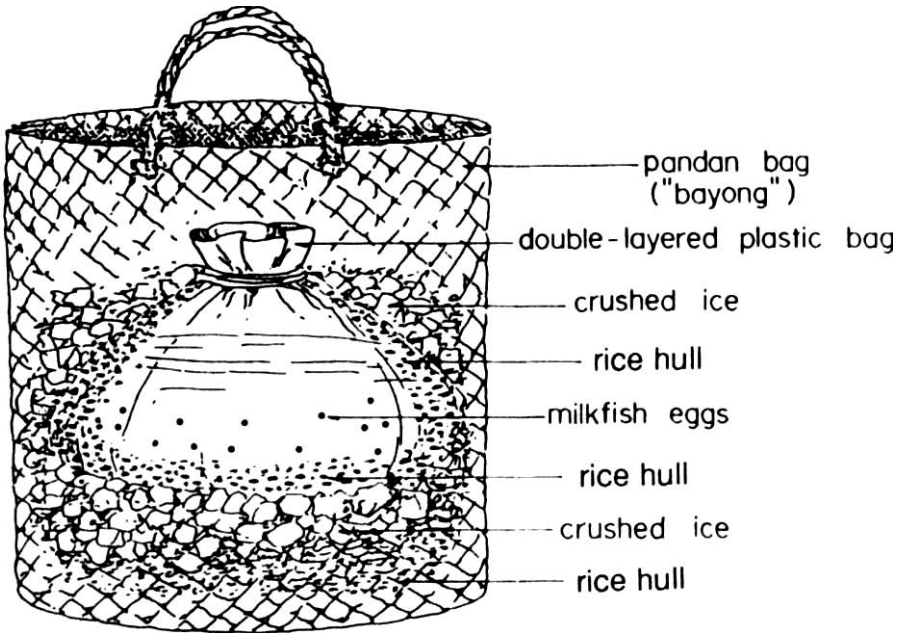
Eggs should be handled carefully and transferred as soon as possible to the hatching tanks. When transport to the hatchery site takes more than one hour, it is advisable to start packing and transport procedures 6 hours *after* spawning, that is, when eggs are already at a more advanced stage of embryonic development. About one hour *before* transport, eggs are packed following these steps:

1) Turn off aeration and gently swirl the water once or twice in order to concentrate dead eggs to the bottom of the container. Quickly siphon out these dead eggs by using a rubber or plastic tubing.

2) Slowly drain to one-half the volume of water suspending the collected eggs. This is done by siphoning out the water with the nozzle of the tube wrapped with screen net or filter so that live eggs are excluded.

3) Layer as many pandan bags (*bayong*) as needed with 2-3 inches of rice hull, 2 liters of crushed ice, and another layer of rice hull in that order.

4) Into each *bayong* so prepared, set a double-layered plastic bag containing 15 liters of seawater with a salinity of 20-35 ppt. Note that milkfish eggs tend to sink in seawater with salinities of 25 ppt or less; these eggs are *not* dead and will float when transferred to higher salinities.



Bayong bag for transporting milkfish eggs.

24 Milkfish Hatchery Operations

5) Using a fine scoop net (pore size, 0.6-0.8 millimeter), gently concentrate a scoopful of eggs.

6) Gently scoop out with a beaker 150 milliliters of eggs from the net and quickly transfer into the bag of water in the *bayong*. About 100 milliliters has approximately 60,000 eggs. Never place more than 150 milliliters of eggs into each bag of 15 liters seawater in the *bayong*.

7) Fill to inflate each plastic bag with oxygen and seal tightly with rubber bands.

8) Place half a liter of crushed ice and some rice hull on top of each plastic bag of eggs.

9) *Bayong* bags must be kept under shade during transport, but make sure water temperature in the bags does not go down to 20°C or lower throughout the trip. The ideal water temperature in the *bayong* is 28°C for at least 2 hours.

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