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Technology Series No.2 **A Prototype Warm Water Shrimp Hatchery**

by

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PROTOTYPE WARM WATER SHRIMP HATCHERY

INTRODUCTION

The completion of the life cycle of many commercially important warm water shrimps in captivity, such as the tiger shrimp (*Penaeus monodon*) and the white and banana shrimps (*P. indicus* and *P. merguensis*), has greatly enhanced mass production of shrimp fry under hatchery conditions. Hatchery-bred fry have been found to show comparable growth and survival rate in grow-out shrimp ponds with those collected from the wild. The shortage, and inconsistent supply of wild fry coupled with increasing areas of shrimp farms have made the shrimp hatchery an indispensable facility to meet the growing fry requirement of the shrimp industry.

Although various techniques have been developed, existing techniques in shrimp seed production as a result of more than 50 years of research studies have yet to be refined, packaged and tested in different environment conditions into an appropriate technology that can be disseminated to the shrimp growers.

Shrimp hatchery technology that has been established in developed nations may not be economically appropriate to developing nations. Hence, testing of any developed technology is essential to ensure its technical and economic viability in the country concerned.

A successful hatchery usually depends on a number of conditions; among the more important ones are right choice of site, effectiveness and efficiency of operational management.

In designing a shrimp hatchery, it is essential to consider the production target of a species or a number of species and the level of financial inputs. In the case of the tiger shrimps, the production target is very much limited by the supply of the spawners. This limits the production capacity of the hatchery. In the case of the white or banana shrimps for which spawners are easily available, tank capacity can be much higher. This prototype hatchery, however, was especially designed for the seed production of warm water shrimps especially the tiger shrimps, white and banana shrimps. The design of the prototype hatchery takes into consideration the technical advantages of the large and small tank hatchery systems to maximize hatchery facilities for the economic production of the target species. In essence, the prototype hatchery has the flexibility of operating at the desired level of operation dictated by the availability of spawners.

The present technology series attempts to describe the prototype warm water shrimp hatchery established and operated by SEAFDEC Aquaculture Department in collaboration with FAO since 1982. The techniques used in the operation of the hatchery have been refined, repeatedly tested and are now packaged for further testing in different environmental conditions elsewhere in the Philippines and other tropical countries.

HATCHERY DESIGN

The hatchery is sited near the seashore where relatively clean, clear and unpolluted seawater is easily available by means of a mechanical pump. In the tropical climate where temperature fluctuation is relatively small, the hatchery can be operated all year round.

The size of the hatchery is dependent on the production target. The number and sizes of various holding tanks. Water and air delivery systems can be computed and designed accordingly. The basic facilities in a shrimp hatchery include (a) holding tanks (b) filtration systems (c) water, air and power supply systems.

The warm water hatchery is so designed to provide a production capacity of 10 million shrimp fry which is sufficient to stock at least 2000 hectares of traditional shrimp farms (5000/ha) or no less than 50 hectares of modern shrimp farms using high stocking rate (e.g. 20/m²).

Holding tanks

The holding tanks of the shrimp hatchery are used for various purposes such as for maturation, spawning, rearing and storage. The number and size for the various types of holding tanks of the hatchery are computed based on the production target per hatchery run. The hatchery in the tropical conditions can be operated all year round with 7–8 operational runs a year. As the annual production target is approximately ten million twenty five day-old postlarvae, the tank facilities should be designed to provide a production capacity of 1.4 million postlarvae per run.

Research studies have shown that:

- A nursery tank can produce 3000–5000 twenty five day-old postlarvae per cubic meter of water with 50% mortality from P₁ to P₂₅.
- A larval rearing tank can produce 30,000–50,000 one day-old postlarvae per cubic meter of water with a survival rate of 30–40% from nauplii to a day-old postlarvae in 10–12 days from hatching.
- A wild spawner produces an average of 400,000 eggs.
- An ablated spawner produces an average of 200,000 eggs.
- Optimal stocking rate in maturation tank is 5–6 individuals/cubic meter.
- The ratio of tank capacity for larval food to that of shrimp larvae is 1:10.

The size and number of the various holding tanks are computed as follows:

Type of tank	Shape	Total Capacity (tons)	Unit Capacity (tons)	Number of Units
Maturation	circular	48	12	4
Spawning	circular	2.5	0.25	10
Larval rearing	circular	30	2.5	12
Nursery	rectangular	400	40	10
Live food culture	circular	30	1	30
Water storage	circular	50	1	1

The detail specifications for each category of the holding tanks are illustrated in **Figs. 1** to **5**

Water storage and filtration tank

When filtered water is used, the water storage tank should at least 10% of the capacity. Hence, approximately a 50-ton water storage holding tank equipped with sand and gravel filtration system will be needed (Fig. 6). A reversing filter is used and can be easily maintained by backflushing.

Aeration system

Roots blower with capacity to generate an air pressure about 0.2 – 0.3 kg/m³ and a volume of 4 – 5 liters/m²/minute is adequate for tank less than 2 meters deep. At least one stand-by roots blower is needed. The airstones used are usually round and are made of Carborundum grinding stone. Such air stone can aerate 5 cubic meters of water. PVC pipes are used for air delivery. In this prototype hatchery, the capacity of root blower should be 7.5–10 horsepower with 2 inches wide outlet.

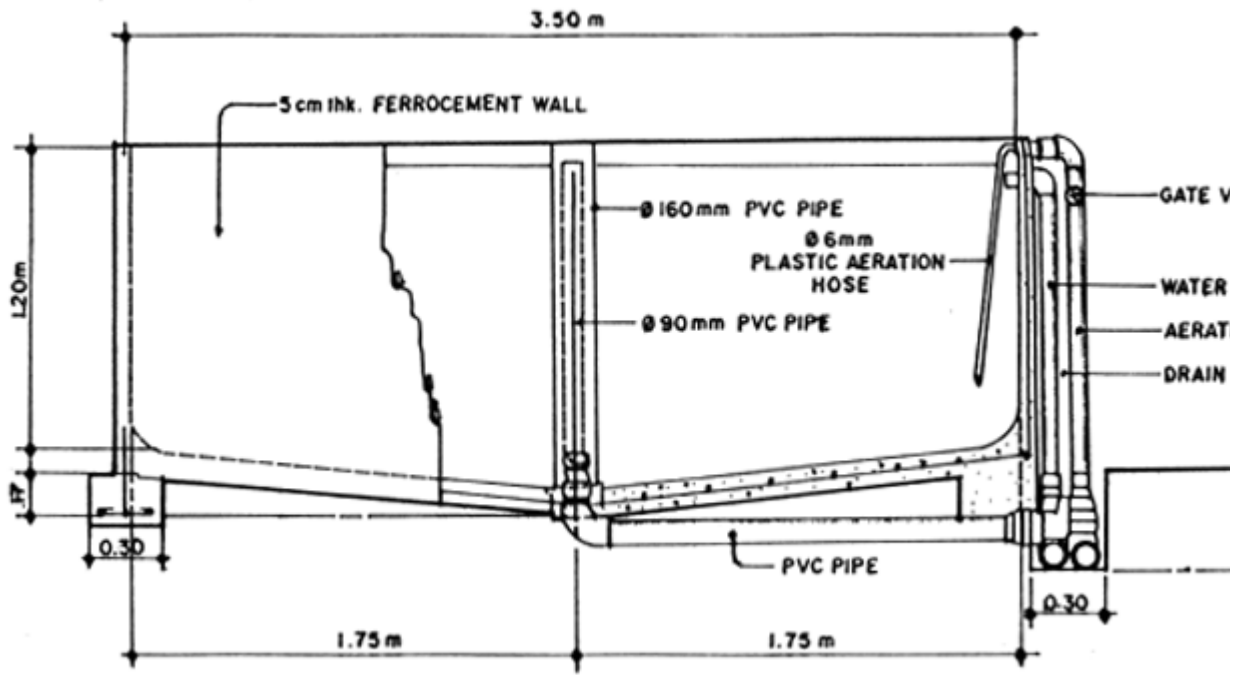


Fig. 1. Cross sectional detail of a 12-ton ferrocement broodstock tank.

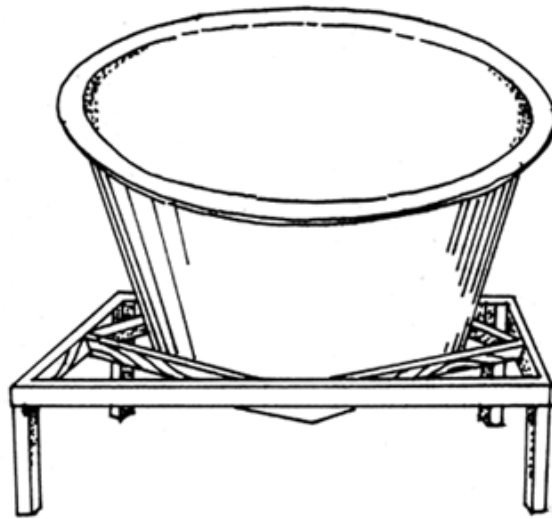
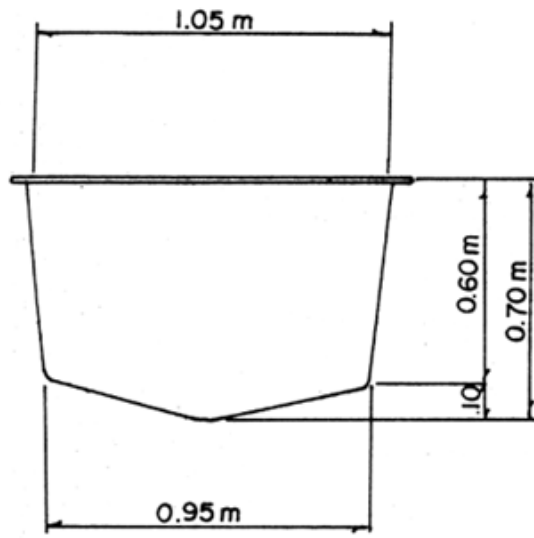


Fig.2. 250 liter fiberglass spawning tank.

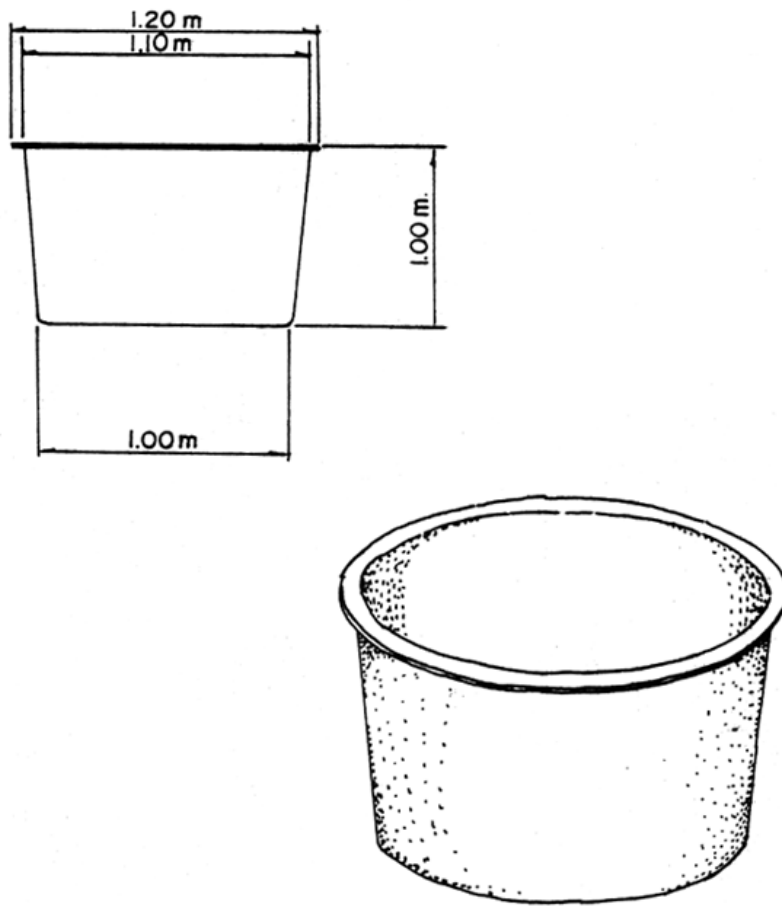


Fig. 5. 1-ton fiberglass tank utilized for algae culture

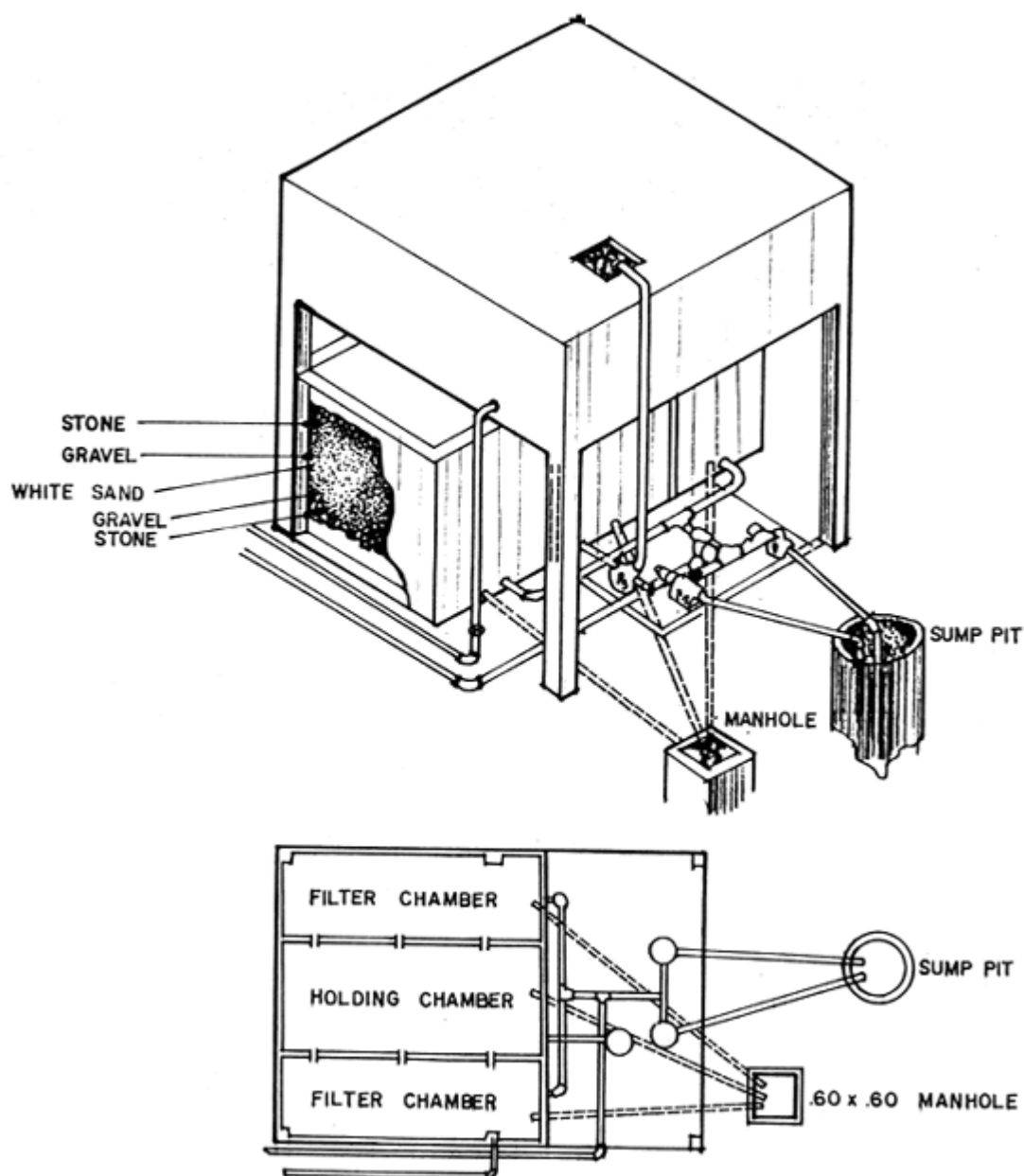


Fig. 6. Water Storage and Filtration Tank

Stand-by generator

Continuous aeration is extremely crucial in ensuring survival of larvae in high density. Any prolonged power interruption would seriously affect the culture organisms in the tank. Thus, it is essential to have a stand-by generator installed with automatic alarm or switch system whereby any power disconnection will automatically switch on the generator thereby providing the necessary power. A hatchery can be operated in an area with no supply of electricity since a marine pump and aerator can be driven directly by handy generators. However, it is more economical to have a hatchery in areas where electricity is available.

Seawater supply and piping system

Seawater is drawn directly from the sea by a marine pump through PVC piping to the overhead filter tank and stored in the reservoir. If the seawater is too turbid, the water is first pumped into a sedimentation tank before passing through the filtering system. Water from the storage tank is gravity-fed to the various tanks in the hatchery (**Fig. 6**).

The marine pump used depends on the volume requirement, pumping time and the total head. For a medium scale hatchery, it should have a discharge rate of approximately 1 ton/minute. A marine pump with 10 HP capacity will adequately meet this requirement.

Layout

In addition to the basic tank facilities, a functional hatchery should be complemented with a small room for algal starter, storeroom, office and all other auxilliary facilities. Hatchery layout depends on the topographic characteristics of the site and the environmental conditions. The layout of the hatchery which covers a total floor area of 2,000 sq.m. is illustrated in **Fig.7**.

HATCHERY OPERATION AND MANAGEMENT

The operational activities of a shrimp hatchery can be grouped into three broad categories, viz: those of preparatory phase, rearing operation and routine management. (All activities must be efficiently executed and systematically coordinated so as to attain the set production goal).

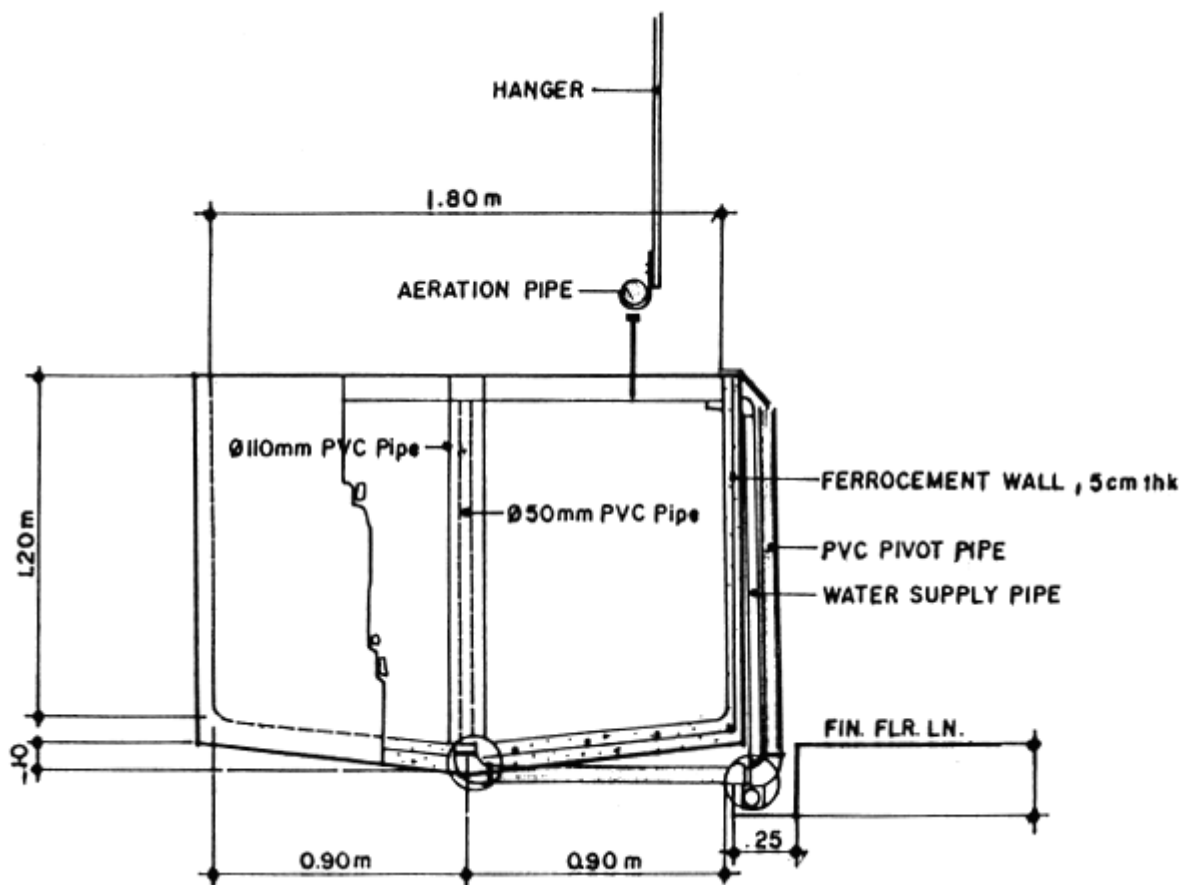


Fig.3. Cross sectional detail of 2.5-ton ferrocement larval rearing tank.

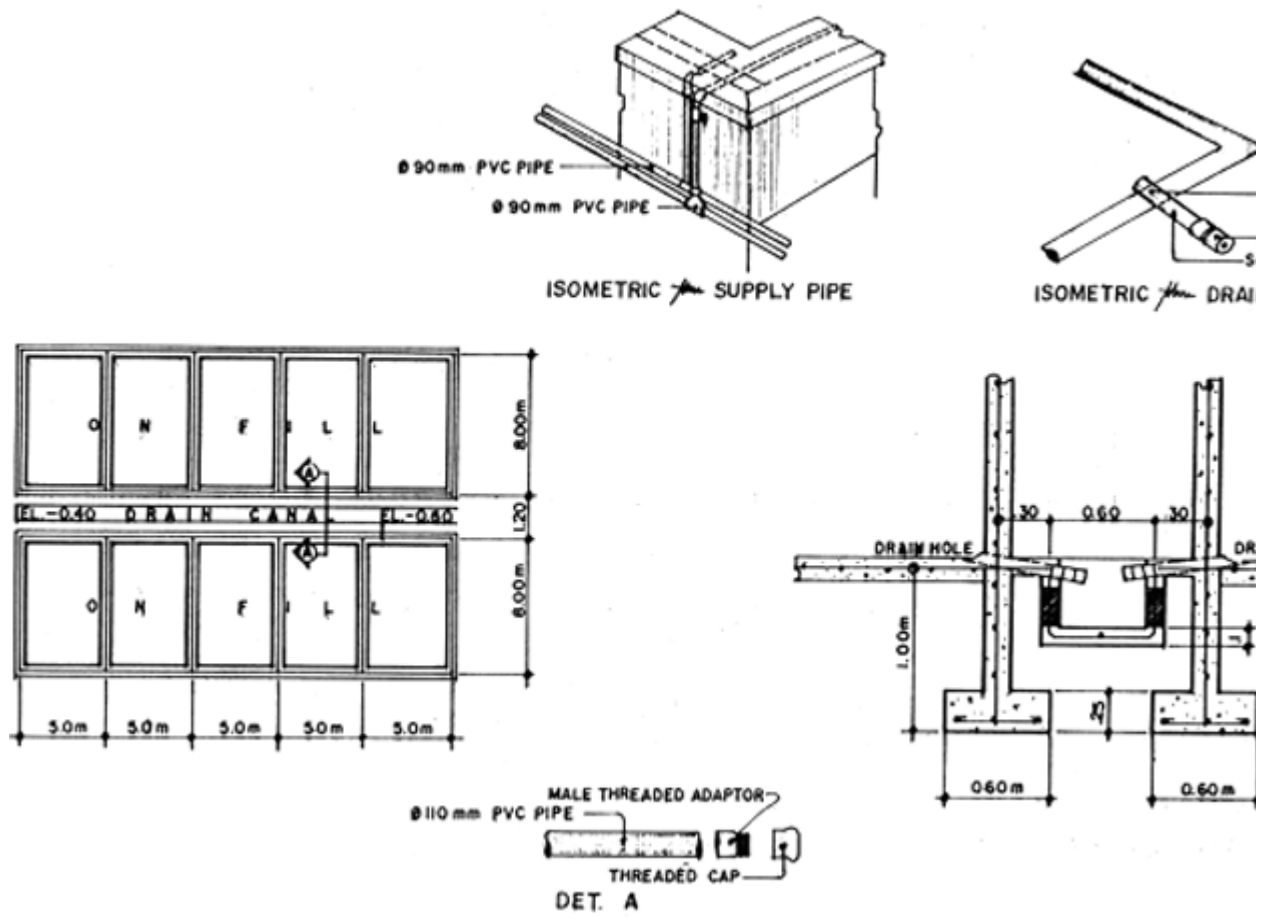


Fig. 4. Detail of 40-ton concrete nursery tanks.

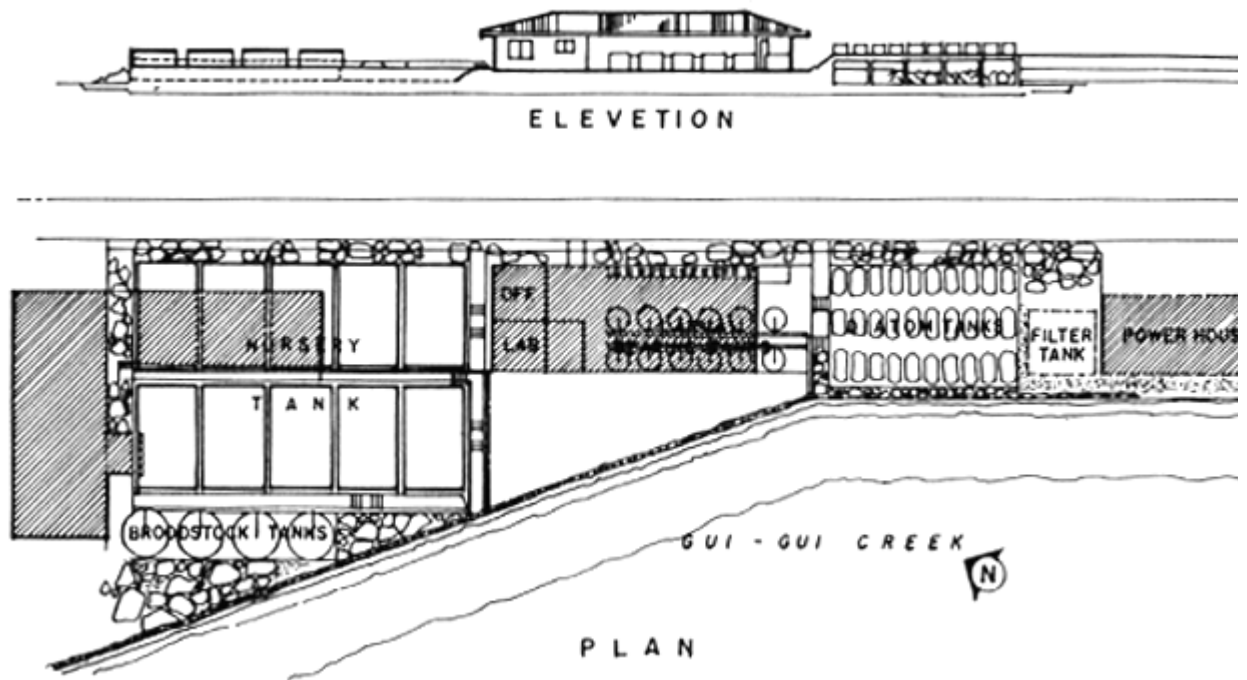


Fig. 7. Layout of medium scale hatchery.

I. Preparatory Phase

This is the initial phase of hatchery operation. All activities under this phase are as important as the rearing operation. Under this phase, all hatchery facilities must be adequately prepared for the maturation, spawning, hatching and larval rearing operation. Conditioning of broodstock is necessary prior to ablation for maturation. Proper selection of wild and ablated spawners and treatment if necessary should be done. The live food organisms must be ready in sufficient quantity when the larvae start feeding.

Facilities

For a newly constructed hatchery, tank facilities especially concrete ones must be adequately conditioned with alum (potassium aluminum sulphate) which would age the tanks and protect them from leakages. The newly constructed tanks are first filled with sea or freshwater and small pieces of alum are broadcast at a rate of 1 kg/m³ and allowed to stay for about a week until the pH of the water in the tank is fully stabilized.

For an operational hatchery, all tanks used must be properly cleaned with freshwater, dried and exposed to the sun for at least one day if possible prior to stocking. After each run, the tanks are disinfected with 12% sodium hypochloride for 24 hours. (12% sodium hypochloride, 2 liters/cubic meter of seawater).

It is important to ensure that the air from the aeration units is oil free and the air pressure is adjustable. Generally, root blowers are designed for oil free operation unlike air compressors and rotary air blowers. Air filters at the inlet and outlet pipes are therefore adequate to filter the air. The water supply system should guarantee good water quality during operation. Periodic checking during and after rearing operation should maintain the variation of the physico-chemical parameters of the water to allowable limits for living organisms. As a matter of practice, disinfection of the sand filters is often done monthly with 12% sodium hypochloride solution to reduce organic contamination due to bacterial decomposition.

Broodstocks and spawners

Sufficient broodstocks must be maintained to provide the necessary quantity of spawners for supplying the targeted quantity of seeds. When wild spawners are scarce especially for P. monodon. It is essential to develop sufficient broodstocks to supplement in time of shortage of wild spawners. A shrimp can be induced to mature by ablating one of its eyestalks (Fig. 3).

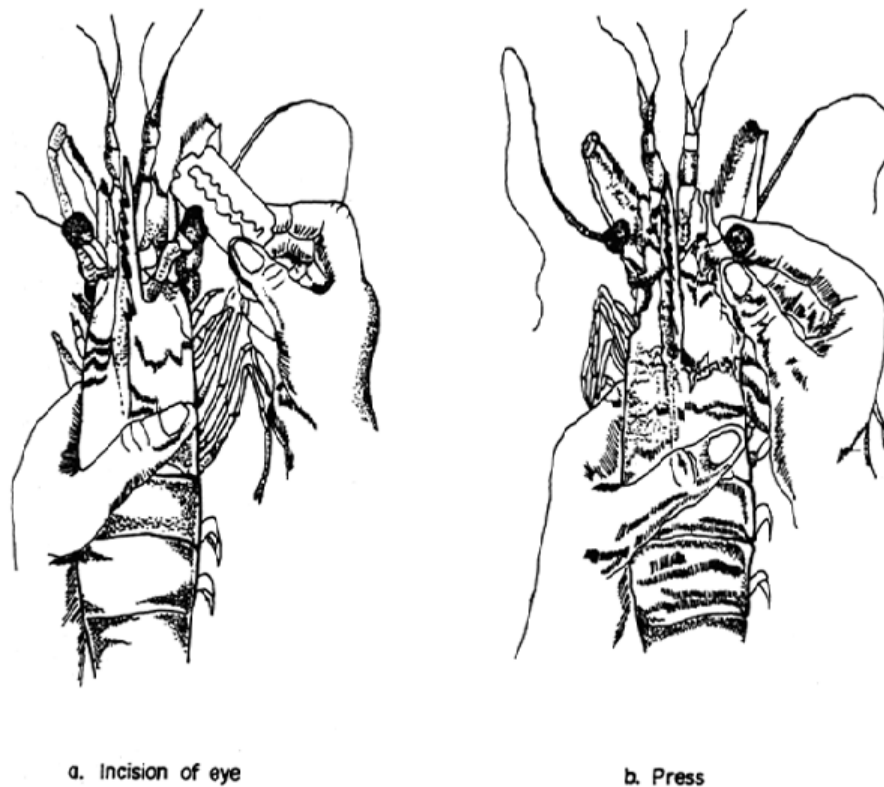


Fig. 8. Eyestalk ablation

Broodstocks collected from the wild undergo the following treatment in the hatchery:

- Conditioning - they are acclimatized in holding tanks for 4–7 days. Each tank is adequately aerated with 60% of its water replaced daily. Once the animals have recovered from transport stress, they are further induced to molt by decreasing the salinity of the water by about 4–5 ppt for 2 days. The salinity is then raised to the normal level. Mating takes place soon after molting.
- Induced maturation - the shrimps are ablated 2–3 days after molting or when the shell is completely hardened. They are stocked in maturation tanks at a sex ratio of 1:1. Only suitable females are used for induced maturation based on the following criteria: (a) complete appendages (b) presence of spermatophore in the thylecum of females and (c) size at least 100 grams. After ablation, the broodstocks are fed with high protein diet at 10% body weight. The diet consists of squid, mussel or oyster meat or pellet feeds. A flow through system is usually applied to maintain the water quality.

Ablated or wild females attaining maturity stages 3 and 4 (**Fig. 9**) are transferred to the spawning tanks which is sufficiently covered and left undisturbed till after spawning.

Larval feeds

One of the key factors ensuring success in shrimp hatchery production is the timely supply of the needed food organisms in sufficient quantity. The hatchery should have an algal culture room to maintain pure stocks of the needed live feeds such as Chaetoceros, Skeletonema, Tetraselmis, Chlorella. The algal stocks can be easily maintained in the standard culture media (**Appendix 1**). While a pure rotifer Brachionus sp. stock must also be maintained throughout the year.

Mass production of larval feeds must closely synchronize with the various procedures

shown in **Fig. 10** which should be closely followed to mass produce sufficient quantity of the desired species. The steps suggested in the figure is based on results of numerous trials and are found to be reliable procedures based on the knowledge of the growth pattern of each species and economic production cost of live foods using different quality of fertilizers. Technical procedures in the cultivation of diatoms, rotifers and the hatching of the *Artemia* eggs are briefly outlined in Figs. 11 to 13.

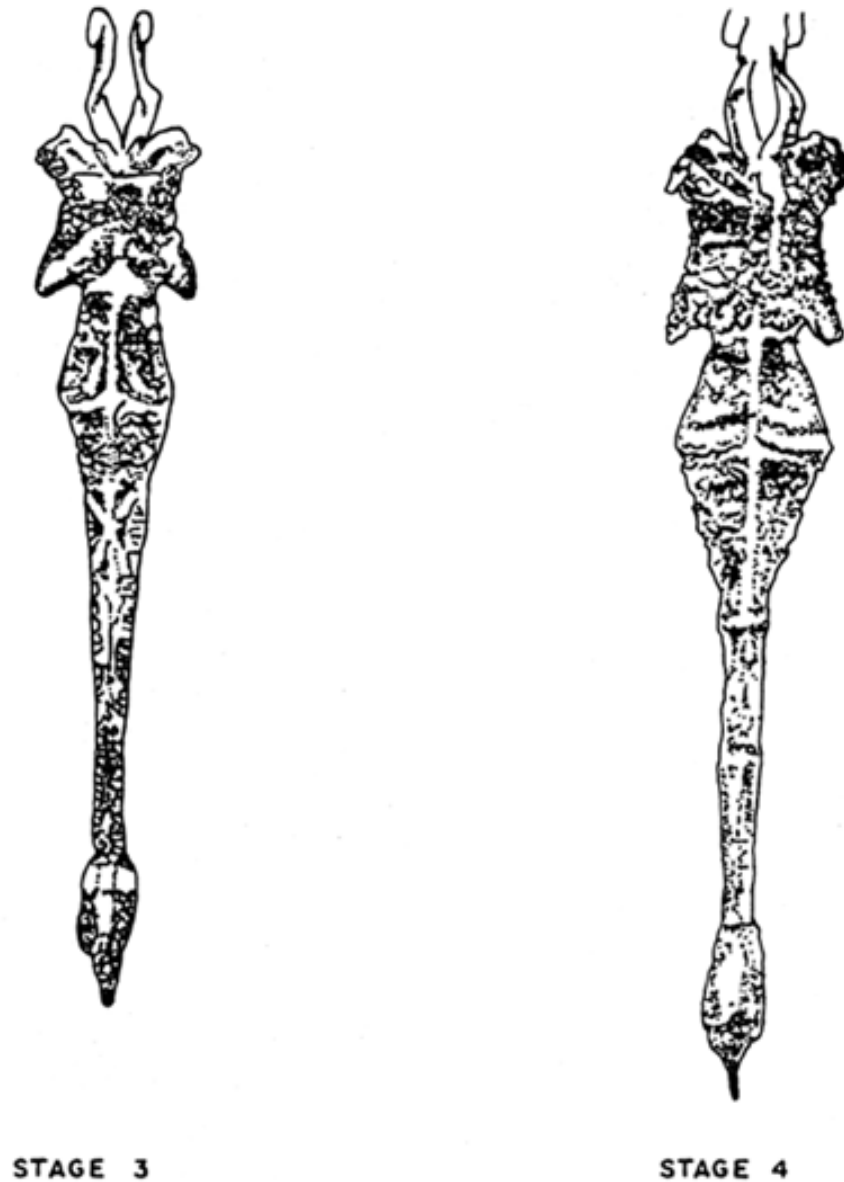


Fig. 9. Spawner with mature gonad.

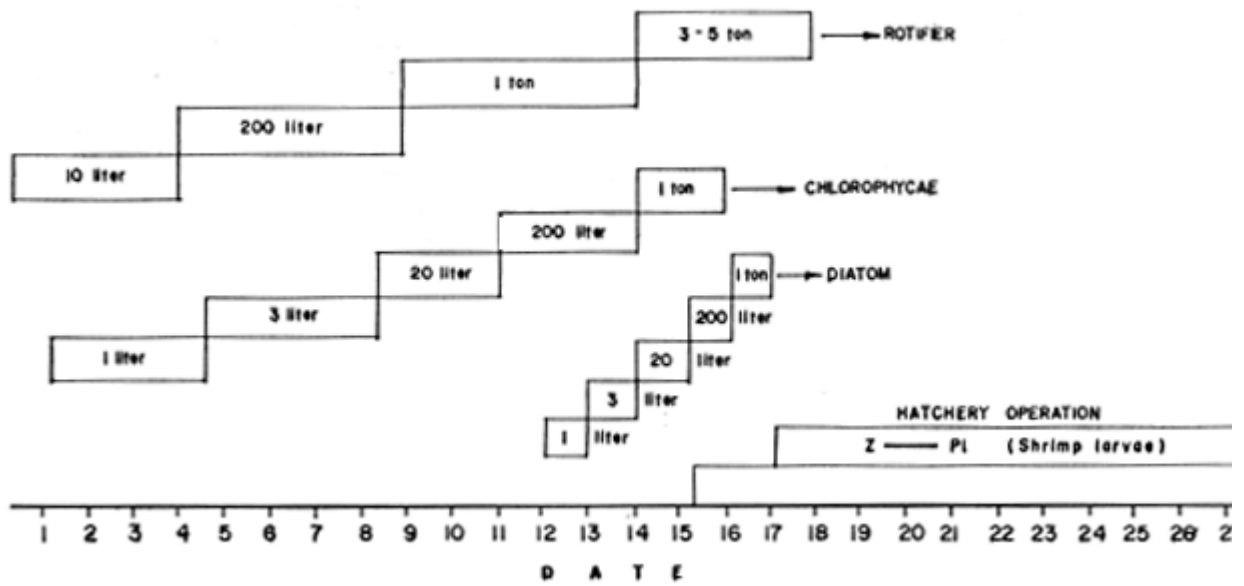


Fig. 10. Programming of Natural Food Culture for Hatchery

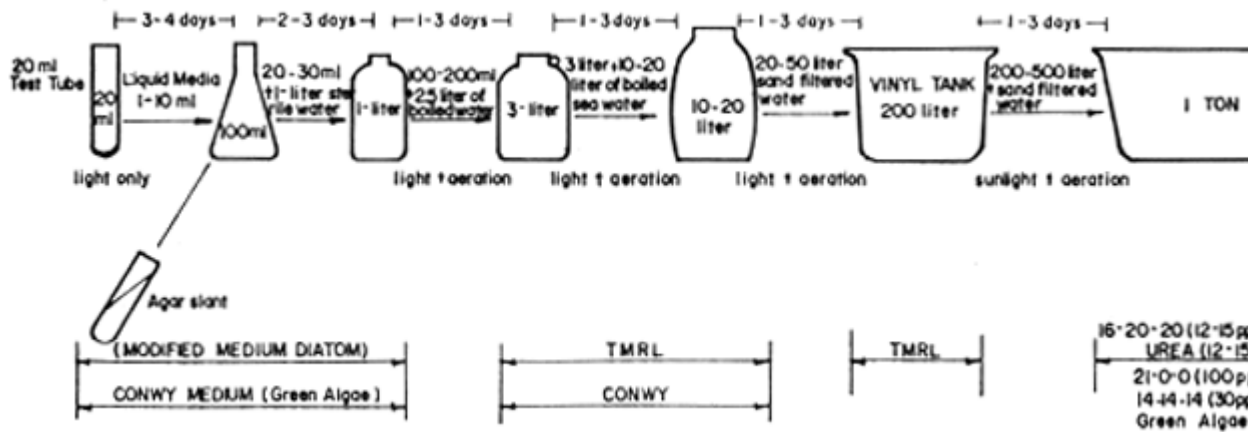


Fig. 11. Flow Chart for Mass Culture of Phytoplankton

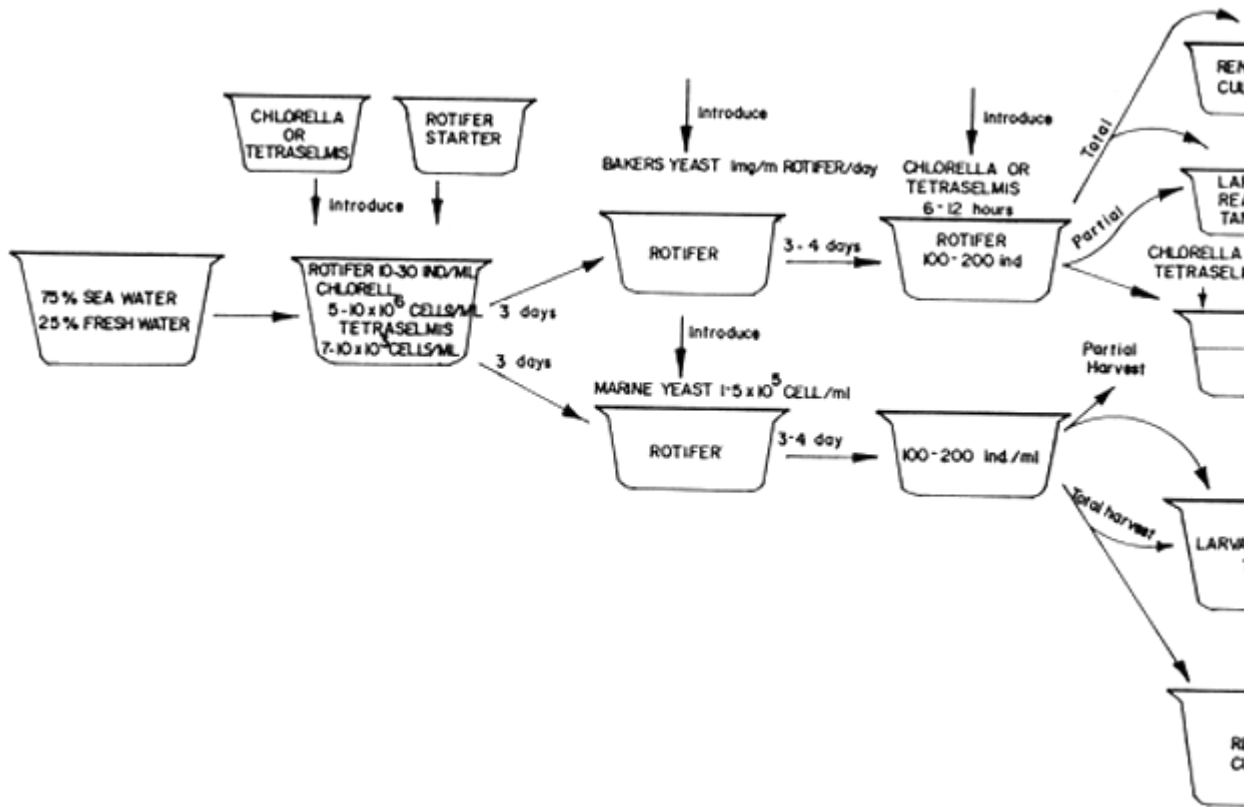


Fig. 12. Flow Chart of Rotifer Culture

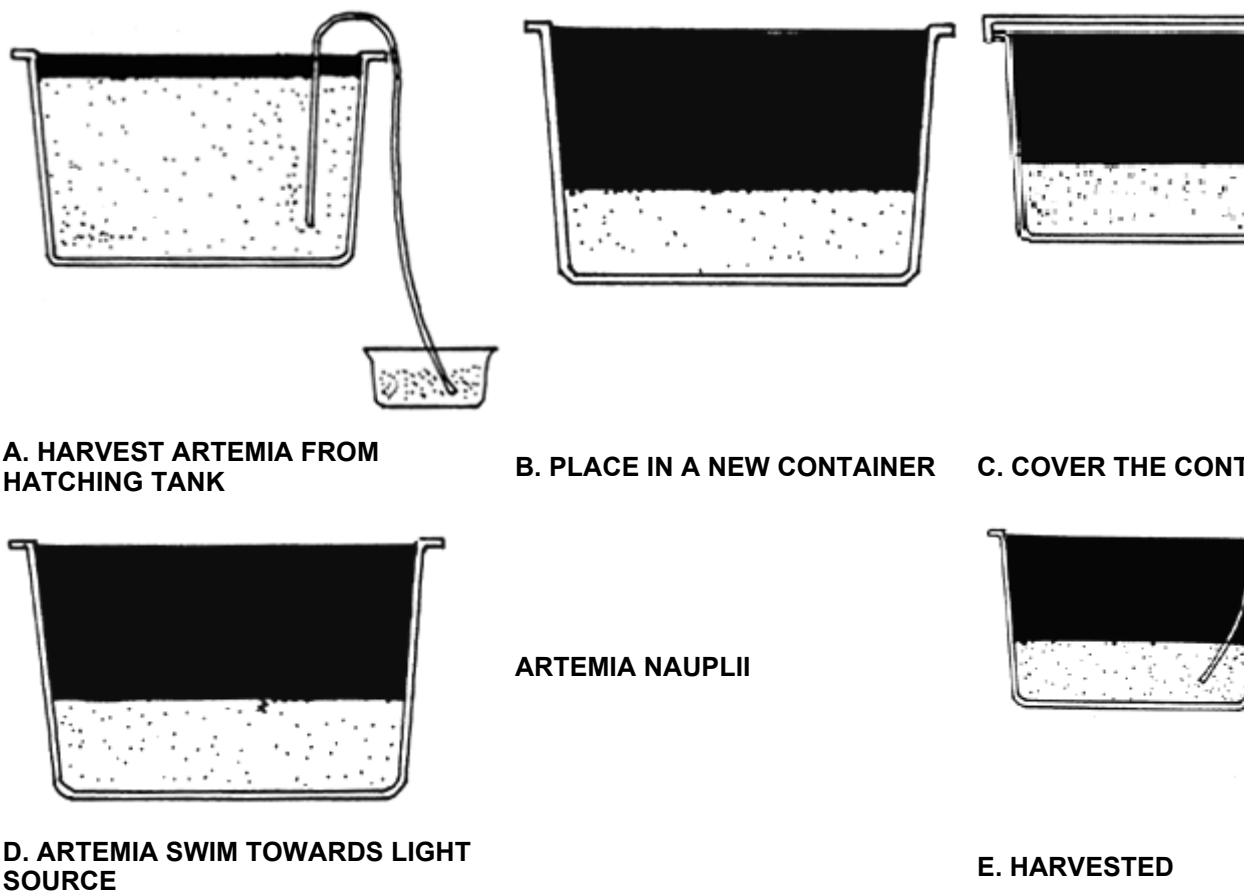


Fig. 13. Harvesting of Artemia

II. Larval Rearing Operation

After spawning, the animals are removed from the tank the following morning. The eggs are then cleaned either by siphoning them into the egg collectors or draining 2/3 of the water through a filter net that effectively retains the eggs within the tank. After draining, the scum in the tank is then removed using a scoop net with mesh size bigger than that of the shrimp eggs. The tank is then filled up with water.

From the spawning tank, sample of eggs are counted to determine the number of eggs spawned per female. In normal condition, fertilized eggs hatch within 12–15 hours. The hatching rate is measured by assessing the number of hatched nauplii. Nauplii are then transferred directly into the 40-ton tanks if the number of nauplii is between 0.5 million to 1 million. They are then reared directly in the large tank up to the twenty-fifth post larval day. On the other hand, if the number of nauplii is less than 0.5 million, they are stocked in 2.5 tons indoor tanks at a density of 100–150 larvae/liter. The larvae are reared either to the third mysis stage (M_3) or one-day old (P_1) postlarvae. They are then transferred to the outdoor 40-ton nursery tanks for further nursing.

LARVAL REARING IN SMALL INDOOR TANKS

After hatching, the newly hatched nauplii are stocked at a density of 100–150 nauplii per liter in the 2.5-ton larval rearing tanks with fresh filtered seawater filling up to 3/4 of tank capacity. No feed is required at the nauplius stage since the nauplius still utilizes its yolk as food. However, diatom are inoculated immediately after stocking to ensure availability of feed when the nauplii molt into the Protozoa stage.

Protozoa stage

This is a critical stage of larval rearing. The larvae at this stage start feeding on external food and feed on minute and easily digested microscopic algae such as Skeletonema costatum, Chaetoceros sp. and Tetraselmis sp. The optimal feeding of phytoplankton in the tank is 50,000 cells/ml for Skeletonema or Chaetoceros and 10,000 cells/ml for Tetraselmis.

Failure in diatom bloom may happen especially during rainy months. As alternative feed, marine yeast, soya bean milk, fertilized oyster eggs or fine particles of egg custard may be used. However, the molting period of the animal may be delayed when fed with such diets.

On the first day of protozoa 1, about 100 liters per cubic meter of fresh filtered seawater is added and on the succeeding protozoa 1000 litres/m³ of water is replaced daily until the animal reach the mysis stage. Henceforth, approximately 30% of the water should be changed daily but the required density of food organisms must be maintained.

Mysis stage

The larvae at this stage will start feeding on rotifers (Brachionus plicatilis) or the brine shrimp nauplii (Artemia salina). The number of rotifers or brine shrimp nauplii required depends on the density of shrimp larvae being reared. Each mysis larvae consumes about 100–200 rotifers or 20–50 Artemia nauplii per day or a standard ratio of about 5 grams dry Artemia cysts is required per m³ of rearing water.

During this stage, the tank bottom is already filled with dead organisms and must be siphoned out daily. Once the larvae reach the first day of postlarval stage (P_1), they can be transferred to the bigger nursery tanks.

One day before transferring the postlarvae to the outdoor tanks, the nursery tanks should be first filled up with fresh filtered seawater to allow adequate blooming of diatoms. The

postlarvae is stocked at a density of 15–20 larvae/liter.

LARVAL REARING IN LARGE NURSERY TANKS

The initial water level in the 40-ton nursery tanks during stocking is 100 cm. The nauplii density is usually about 20–50/liter. Immediately after stocking, diatom starters are inoculated to ensure bloom of the desirable species. Technical grade fertilizers can be used directly to enhance algae growth. The fertilizers used are:

KNO_3	3 ppm
Na_2HPO_4	0.3 ppm

It is pertinent to monitor the types and density of algae in big tanks to ensure that the optimal density is maintained.

During the protozoa stage, about 10–20 cm of fresh filtered seawater is then added daily. However, the amount of water added is dependent on the diatom growth. When diatom density is below the desired level in the culture tank, more cultured diatoms and fertilizers are added to accelerate algal bloom. On the other hand, over-blooming of algae should also be controlled by shading or by draining out a portion of water and replenish with fresh seawater.

Mysis stage

The same operational procedure for rearing of mysis stage used in small tank system is employed when conducting in the outdoor nursery tanks. After the water level in nursery tank has been filled to its full rearing capacity, approximately 30% of the water is changed daily.

Postlarvae stage

Early postlarval stages (P_1 - P_6) are fed with brine shrimp nauplii at a rate of 100–200 per postlarvae per day.

Once the postlarvae reaches the sixth day (P_6), they are fed with finely minced mussel or cockle meat or larval pellet feed while gradually decreasing dependency on brine shrimp nauplii which eventually stopped at P_9 . Beyond this stage, the larvae are fed solely on minced mussel or cockle meat or artificial diets 3 to 4 times daily. In the meantime, polyethylene nets were provided as a substrate for the larvae.

Good water quality should be strictly maintained especially during this phase of larval rearing. While 30–40% of the rearing water is being changed daily, efforts must be made to ensure saturation of dissolved oxygen concentration in the water and low concentration of ammonia (0.1 ppm). Hence, regular siphoning of tank bottom to remove excess feeds, metabolic wastes and dead algae is an important and routine hatchery function. When 50% of the rearing water has been drained, continuous flow-through of fresh seawater is maintained for 2–3 hours. Such flow-through operation enables the suspended (solid) particles to be drained out and water clarity maintained.

Sometimes, when over-blooming of diatom becomes uncontrollable or the sediment accumulation at the tank bottom is too thick, which deteriorate water quality rapidly, it becomes necessary to transfer the larvae to another well-prepared tank.

Some operational procedures have become a routine management activity. It is highly important to ensure no drastic fluctuation of environmental conditions. Efforts must be made

to maintain stable condition in the tank and the water quality maintained within the allowable limits:

- * Salinity 30–32 ppt
- * Dissolved oxygen saturated

While hatchery techniques are gradually being perfected hatchery operation is still highly dependent on the experience and skill of the hatchery operators/technicians. Mechanical control of diatom blooms, for example, has yet to be developed and is still dependent on the technical experience of the hatchery operators. Regulation of optimal food density at different stages of larval development remains an important operational strategy. Supplementary feeds in time of shortage of natural food organisms become a management decision and the types and quality of supplementary feed used varies with the operational situation. Fertilized oyster eggs, soybean cake, soybean curd, egg custard and encapsulated egg yolk are possible supplementary feeds but they should not be used as a total substitute (**Fig. 14**). The importance of maintaining diatoms should be emphasized in regulation and maintenance of water quality if supplementary feeds are the only option.

ROUTINE HATCHERY MANAGEMENT

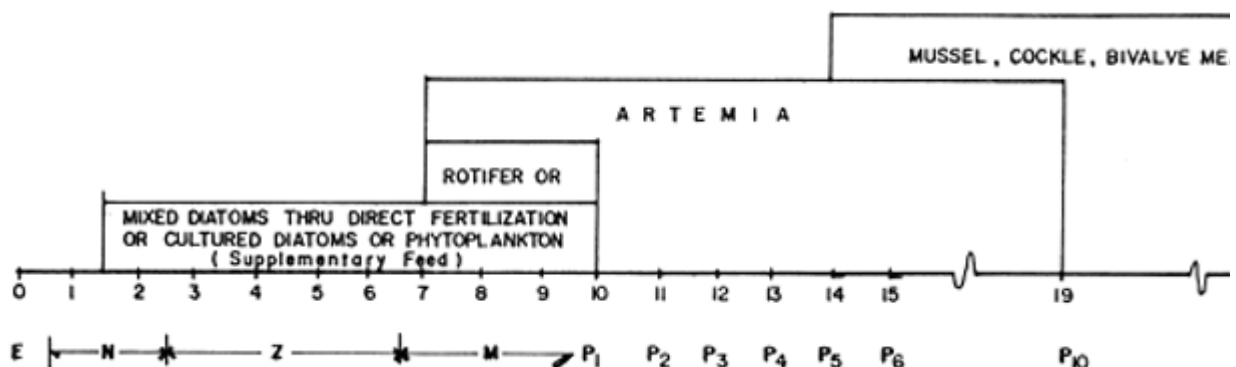
Management is a very important operational function of a hatchery. Some of the operational procedures have become daily routines. In shrimp hatchery, such routine activities include (a) maintenance of water quality (b) regulation of feeds and feeding schemes at different stages of larval growth and (c) monitoring of the environmental parameters in relation to survival rate and growth.

Maintenance of water quality

Good water quality is essential throughout the hatchery operation. It is also very important to minimize drastic fluctuation of environmental parameters such as water temperature, salinity or dissolved oxygen content of water.

For the prototype hatchery, the major parameters are maintained within the following allowable limits:

- * Salinity 30–32 ppt
- * Dissolved oxygen content supersaturated
- * Water temperature 26 – 31°C
- * pH 7.5 – 8.5
- * NH_3^- 0.1 ppm
- * NH_1 1.5 ppm



	FERTILIZER	KNO ₃	3.0 gm./m ³
N NAUPLIUS		NA ₂ HPO ₄	0.3 gm/m ³
Z PROTOZOEAE	DIATOM/PHYTOPLANKTON	SKELETONEMA COSTATUM	40,000– 50,000 Cell ^s /ml
M MYSIS		TETRASELMIS SP.	10,000 Cell ^s /ml
P POST LARVAE		CHAETOCEROS SP.	40,000– 80,000 Cell ^s /ml
	SUPPLEMENTARY FEED	FERTILIZED EGGS OF OYSTERS	
		SOYBEAN CAKE	
		SOYBEAN CURD	
		EGG CUSTARD	10 mg / larva / day
		EGG YOLK	
		DRY ACETES	10 mg / larva/ day
		FRESH ACETES	50 mg/ larva/ day

Fig. 14. Feed and Feeding Scheme for Shrimp Larvae

Monitoring of environmental parameters

In any functional hatchery, regular monitoring of basic environmental parameters such as temperature, salinity, dissolved oxygen, ammonia and nutrient levels is a routine basic operational function. Equipment for determination of these parameters are easily available in the market.

While the above activities are operational routines, the environmental data should be carefully analyzed to detect development of undesirable changes of environmental conditions.

HARVESTING AND TRANSPORTATION

The juveniles in the nursery tanks can be harvested when they reach 21 to 25 days old. The larvae at this stage have reached not only the preferred size of shrimp growers but also are more sturdy to withstand stress due to transportation.

During harvest, the water level in the nursery tank is gradually reduced to about $\frac{1}{3}$ of tank depth and then drained out through the outlet pipe and with a bag net installed at the tip of the pipe. The larvae that go with the water are collected at the bag net. This method is efficient enough to harvest all the larvae.

The postlarvae can be transported live in tanks or plastic bags following the standard packing and transportation procedure for shrimp larvae. Very often, they are conveniently transported in polyethelene bags provided with oxygen. The bag (60 × 40 cm) is first filled with 6–8 liters of fresh seawater and then packed with 3,000–5,000 postlarvae. They are then placed in styrofoam boxes. Temperature inside the bag can be reduced to about 20–24°C by crushed ice mixed with sawdust placed at the bottom of the box and on the top of the polyethelene bag. Under such condition, the larvae can be kept alive for more than 12 hours.

FINANCIAL ANALYSIS

Financial analysis shows that hatchery operation is economically viable if production target can be reached (Table 1), considering the hatchery to be operating at 10 million tiger shrimp fry per year based on the existing production capability, a net income of 77 centavos per peso investment can be obtained fully demonstrating its commercial viability. Operating cost is fairly high (66.8%), almost half of which are spent on the procurement of *Artemia* eggs. The hatchery is fully dependent on electricity supply which constitutes 13.5% of the total cost. Since the hatchery is heavily relying on the skill and experience of the operators, salary forms about 8.5% of the total cost or 12.8% of operating cost. Interest rate forms a sizeable expenditure (20.2%) because of the high bank interest rate.

The hatchery operation is still profitable if it is operated at 80% capacity. The income over total cost is about 55%. However, when operated at half its capacity, the profit margin will go below 8.5%, rendering hatchery operation less lucrative.

ITEM	VALUE (PESOS)
A. Income	
<i>P. monodon</i> larvae (PL 20–21) at 0.35 pesos/pc × 10 million	<u>3,500,000</u>
Sub Total A	3,500,000
B. Fixed Cost/tax	
Land cost (100,000 pesos × 20% interest)	20,000 (1.0%)
Hatchery construction ¹ (1,000,000 pesos × 10% depreciation)	100,000 (5.1%)
Equipment (500,000 pesos × 20% depreciation)	100,000 (5.1%)
Interest (2,000,000 pesos × 20%)	400,000 (20.2%)
Property tax (1.5%)	1,500 (0.07%)
Sales tax (1% of income)	35,000 (1.8%)
Sub Total B	<u>656,500 (33.2%)</u>
C. Operating Cost	
<i>P. monodon</i> spawners	160,000 (8.1%)
Fertilizer/chemical	20,000 (1.0%)
Feed	15,000 (0.71%)
<i>Artemia</i>	600,000 (30.4%)
Electricity	276,000 (13.9%)
Labour	168,000 (8.5%)
Fuel and oil	10,000 (0.5%)
Materials and supply	10,000 (0.5%)
Maintenance	20,000 (1.0%)
Miscellaneous	40,000 (1.5%)
Sub Total C	<u>1,319,000 (66.8%)</u>
D. Total cost (B + C)	1,975,500
E. Net operating income (A - C)	2,181,000
F. Net income (A-B-C)	1,524,500
G. Income over total cost	<u>77.17%</u>

¹ See Appendix 2

Table 1. The projection of cost and return of a prototype warmwater hatchery based on operation at full capacity in the Philippine conditions.

Appendix 1

THE CULTURE MEDIA AND THEIR PREPARATION

I. Allen-Neison "Miguel seawater"

Solution A

KNO_3 20.2 g/100 ml.H₂O

Solution B

$\text{Na}_2\text{HPO}_4 \cdot 12\text{H}_2\text{O}$ 4.0 g

$\text{CaCl}_2 \cdot 6\text{H}_2\text{O}$ 4.0 g

HCl conc. 2.0 g

FeCl_3 (melted) 2.0 ml

Distilled water 80.0 ml

Add 2 ml. solution A to 1 liter seawater and 1 ml. of solution B to 1 liter of seawater.

II. Conwy Medium (Walne, 1974)

A. Sodium Nitrate	100 g
EDTA, disodium salt	45 g
Boric Acid	33.6 g
Sodium Phosphate, monobasic	20 g
Ferric Chloride, 6-hydrate	1.3 g
Manganous Chloride, 4-hydrate	0.36 g
Trace Metal Solution*	1 ml
Vitamin Mix**	100 ml
Distilled water (to make)	1000 ml

(Note: use 1 ml Conwy medium/liter of seawater)

* Trace Metal Stock Solution

Zinc Chloride 2.1. g

Cobalt Chloride.

6-hydrate 2.1 g

Ammonium Molybdate,

4-hydrate 2.1 g

Copper Sulfate 2.0 g

Distilled water 100 ml.

(Note: acidify with 1N HCl until solution is clear)

**Vitamin Mix

Vitamin B₁₂ 10.2 mg

Vitamin B₁ 20.0 mg

Distilled water 200 ml

III. TMRL Enrichment (Liao & Huang, 1970)

KNO_3 100 g

$\text{Na}_2\text{HPO}_4 \cdot 12\text{H}_2\text{O}$ 10 g

$\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$ 3 g

Na ₂ SiO ₃ ·9H ₂ O	1 g
Distilled water	1000 ml
(Note: Use 1 ml TMRL/liter of seawater)	
IV. Enrichment for Outdoor Cultures	
16-20-0	12 g
Urea 46-0-0	12 g
21-0-0	100 g
(Note: add above nutrients to 1 ton seawater)	
V. Modified F Medium (modified from Guillard & Ryther, 1962)	
A. N-P Stock (500×)	
Sodium nitrate	42.07 g
Sodium Phosphate, monobasic	5.00 g
Distilled water (to make)	1 liter
B. Sodium Metasilicate Stock (500 x)	
Sodium metasilicate	15.0 g
Distilled water (to make)	1 liter
C. Ferric Chloride Stock (500 ×)	1.45 g
Distilled water (to make)	1 liter
D. EDTA Stock (1000 ×)	
EDTA, Disodium salt	10.00 g
Distilled water (to make)	1 liter
E. Vitamin stock (1000×)	
Thiamine HCl	0.2 g
B ₁₂	10.0 ml*
Biotin	10.0 ml**
* B ₁₂ stock (primary)	
- B ₁₂	0.1g
Distilled water	1 liter
** Biotin primary stock	
Biotin	0.1 g
Distilled water	1 liter
F. Trace Metal Stock (1000×)	
TM primary stock A	1 ml
TM primary stock B	1 ml
TM primary stock C	1 ml
TM primary stock D	1 ml
Distilled water (to make)	1 liter
1) TM primary stock A solution	
Copper sulphate. 5-hydrate	1.96 g
Zinc sulphate. 7-hydrate	4.40 g
Distilled water (to make)	100 ml

