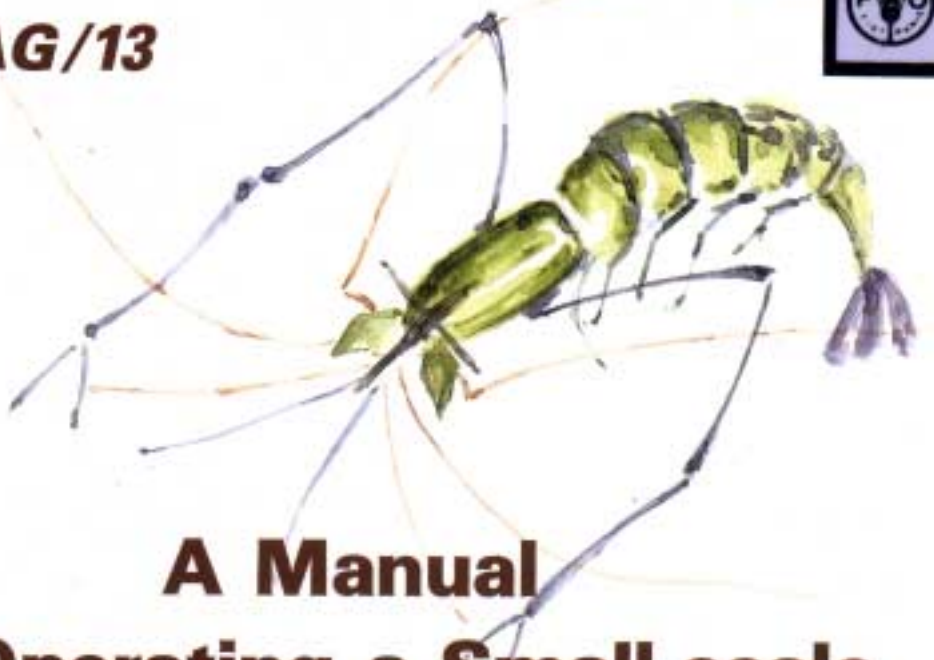
BOBP/MAG/13



A Manual for Operating a Small-scale Recirculation Freshwater Prawn Hatchery

BOBP For Fisheries Development
BAY OF BENGAL PROGRAMME

A Manual for Operating a Small-scale Recirculation Freshwater Prawn Hatchery

by

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BAY OF BENGAL PROGRAMME
MADRAS, INDIA
1993

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This manual, based on the experience of the Bay of Bengal Programme (BOBP) project in Potiya, near Chittagong, Bangladesh, is written for those interested is establishing a small-scale inland freshwater prawn hatchery using the clear water method. It is assumed that the reader has some basic knowledge of aquatic biology, but is not necessarily a degree holder in the subject. The method described is based on the use of brine obtained from salt pans. The brine is diluted with well or surface water to make up the rearing water. Such technology is widely used in commercial hatcheries in Thailand. As rifle is not always available, a simple biofilter, for recirculation of the water, is incorporated in the tank design. The biofilter greatly reduces, or eliminates, the need for water changes during the rearing cycle. The hatchery system described consists of larvae rearing tanks, mixing and brine storage tanks, Artemia incubators and supporting mechanical equipment.

Recirculation systems are becoming increasingly popular and have now been shown to give consistent production of quality post-larvae. All the necessary details for setting up such a simple system are included in this manual, which is also profusely illustrated to make what is described in the text clearer.

The Bay of Bengal Programme (BOBP) is a multiagency regional fisheries programme which covers seven countries around the Bay of Bengal — Bangladesh, India, Indonesia, Malaysia, Maldives, Shri Lanka and Thailand. The Programme plays a catalytic and consultative role : it develops, demonstrates and promotes new techniques, technologies, methodologies and ideas to help improve the conditions of small-scale fisherfolk communities in member countries. The BOBP is sponsored by the governments of Denmark, Sweden and the United Kingdom, and also by UNDP (United Nations Development Programme) and AGFUND (Arab Gulf Fund for United Nations Development Organizations). The main executing agency is the FAO (Food and Agriculture Organization of the United Nations).

This manual has not been cleared by the Government concerned or the FAO.

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A CLARIFICATION

BOBP/MAG/13 – A Manual for Operating a Small-scale Recirculation Freshwater Prawn Hatchery was written at the time the only literature available to us indicated the orange claw variety could possibly be a subspecies. Subsequently, we have come to know that both this species and the 'small' variety are actually subdominant forms which change to dominant forms when the large blue claw 'bulls' are removed from the culture pond. In this context, the section 'Subspecies of *Macrobrachium Rosenbergii*' on page 2 may be substituted as follows:

Morphotypes of *Macrobrachium rosenbergii*

Dominance patterns among males in a population of freshwater prawns leads to the appearance of three morphotypes, blue claw, orange claw and clear claw (Griessinger, *etal.*, 1991).

In a pond culture of *M. rosenbergii, 50* per cent of the males will be clear claw morphotypes and are the smallest animals in the population. The subdominant orange claw morphotypes make up 40 per cent of the males and are of intermediate size. The dominant, or blue claw morphotypes, form 10 per cent and are the largest animals in the population.

The appearance of these morphotypes is related to the culture conditions. The dominance of a few males, the blue claws, retards the growth of subdominant morphotypes. If the dominant males are removed, some of the subdominant morphotypes shift to dominant blue claws.

Females are more or less homogeneous for a given cohort and do not exhibit morphological variations due to social structure, as do the males.

*GRIESSINGER J.K., LACROIX, D. and GONDOUIN, p. (1991). *L'elevage de Ia crevette tropicale d'eau douce*.. Institute francais de recherche pour l'exploitation de Ia mer.. 372 pp.

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Contents

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Introduction

According to FAO nomenclature, freshwater paleomonids are referred to as 'prawn'; marine penaeids, metapenaeids and paleomonids are called 'shrimp'.

The giant freshwater prawn, *Macrobrachium rosenbergii,* grows to a large size in the Indo-Pacific region and is a popular aquatic food both within the region and in Europe and North America. In the early Fifties, Thai farmers started rearing freshwater prawn by collecting seed from natural waters. *M. rosenbergii* grows fast, can tolerate moderate temperature and salinity changes and can be cultured in ponds. However, due to dependence on nature for seed, production was very low.

The primary condition for intensification of any culture depends ou the availability of seed. Based on the interest shown by farmers in the culture of *M. rosenbergii* in Southeast Asia, a hatchery industry has developed.

In 1961, a Taiwanese scientist, Dr. S. W. Ling, while working at the Fisheries Research Institute in Malaysia, discovered that *M. rosenbergii,* though actually an inhabitant of freshwater, completed its larvae phase in brackishwater. In nature, *M. rosenbergii* spawns in estuaries. After spending the first month or so of their lives in these waters, the juveniles start their journey upstream.

Following this discovery, Dr. Ling reared the larvae in brackishwater and achieved success in 1962. In 1963 he produced enough prawn fry to stock culture ponds. From then on, prawn culture became popular in Malaysia. The success of Dr. Ling aroused interest all over the world. In the succeeding ten years, extensive research ensued. In 1965, research on seed production and culture of *M rosenbergii* started in Hawaii under the leadership of T. Fujimura. The 'green water' method of seed production developed through his research.

In the intervening years, many advances have been made in hatchery technology. The green water method is no longer used, having been supplemented by the clear water technique. Recirculation systems are increasingly popular and have now been shown to give consistent production of quality post-larvae.

This manual is written for those interested in establishing a small-scale inland freshwater prawn hatchery using the clear water method. It is assumed that the reader has some basic knowledge of aquatic biology, but is not necessarily a degree holder in the subject. The method described is based on the use of brine obtained from salt pans. The brine is diluted with well or surface water to make up the rearing water. Such technology is widely used in commercial hatcheries in Thailand. As brine is not always available, a simple biofilter, for recirculation of the water, is incorporated in the tank design. The biofilter greatly reduces, or eliminates, the need for water changes during the rearing cycle. The hatchery system described consists of larvae rearing tanks, mixing and brine storage tanks, Artemia incubators and supporting mechanical equipment.

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Biology of *M. rosenbergii*

Distribution

There are 150 species of *Macrobrachium* in the world, of which 49 are commercial. Twentyseven of the commercial species are found in Asia and the Pacific. Most live in freshwater. A few species live in brackishwater in the mouths of rivers.

Macrobrachium rosenbergii is found extensively in the tropical and subtropical waters of the Indo-Pacific region in Malaysia, Thailand, the Philippines, India, Shri Lanka, Bangladesh, Myanmar, Indonesia and Vietnam. They are generally found in freshwater, in ponds, rivers, lakes, ditches, canals, depressions, low-lying floodplains and river mouths. Most of the species spend their early life in brackishwater that is connected directly or indirectly with the sea. Some species complete their life cycle in freshwater, but these are not of commercial importance.

Prawns move upstream, entering lakes and even paddy fields, up to about 200 km from the sea. This type of migration is observed not only in *M. rosenbergii* but also in other species of *Macrobrachium.*

M. rosenbergii has been used in research more than any other species and has been introduced many new countries for commercial culture. Fujimura and Okamoto (1972) were successful in producing post-larvae (FL) of *M. rosenbergii* in large numbers in Hawaii in 1972. *M. rosenberg/i* is being cultured in commercial quantities in many parts of the world, including Hawaii, Honduras. Mauritius, Taiwan, Thailand and the Philippines. Farms have also been developed in Costa Rica, Israel, Malaysia, and Mexico.

Subspecies of *Macrobrachium Rosenbergii*

Due to differences in climate, weather and natural environment, many subspecies of *M. rosenbergii* have evolved. Three varieties are generally observed in nature.

Blueclaw subspecies

This subspecies grows to a large size. The ratio of claw to body length is 1.6 ±0.1. The male is territorial, its breeding behaviour is complex and growth is comparatively slow.

Orange claw subspecies

This subspecies is a little bigger than the median size of the blueclaw variety and has orange-coloured claws. The ratio of claw to body length is 1.0 ±*0.05.* The rate of fertilization of eggs is comparatively slow, but growth is fast.

Small subspecies

This is the smallest of all the subspecies with spineless claws. The ratio of claw to body length is 0.5 ±0.1. At the time of copulation, these adopt the 'snake' mating strategy. Their growth is the slowest of the three varieties.

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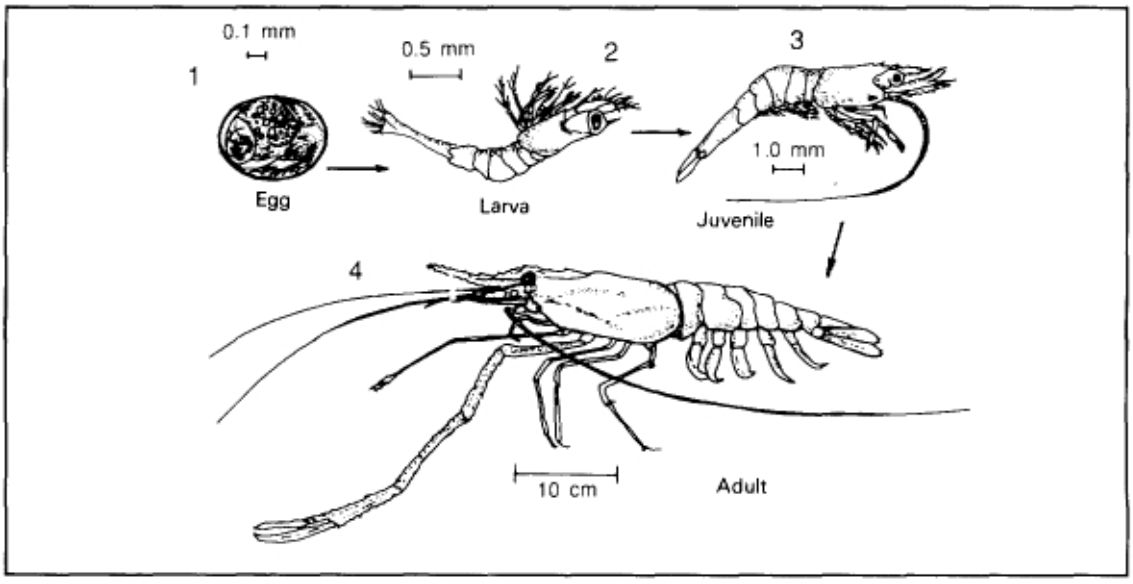
Life history

There are four stages in the life of a freshwater prawn, *viz*, egg, larva, juvenile and adult (see Figure 1). Like other crustaceans, the freshwater prawn moults. The number of moults and the durations of intermoults are not fixed, and depend on the environment, particularly temperature and the availability of food.

Fig. 1 The life cycle of *M. rosenbergii* after New and Singholka (1985)



In the natural environment, mating of *Macrobrachium* takes place all year round, although, due to environmental reasons, peak mating takes place only during certain periods of the year. A female prawn, with matured gonad, copulates just after moulting with a male prawn having a hard shell. During copulation, the male deposits a gelatinous mass, or spermataphore, on the underside of the thorax of the female, between her walking legs. The female prawn releases its eggs a few hours to a few days after copulation. The number of eggs depends on the size of the female. A fully matured female of 50-100 g can carry *50,000-100,000* eggs. But at first maturity, due to the female's small size, it lays only 5000-20,000 eggs.

As the eggs are extruded from the gonophore, they are fertilized by non-motile sperm retained in the spermatophore. The fertilized eggs are then transferred to a brood chamber on the underside of the abdominal region of the female, held in place by a thin membrane and kept aerated by vigorous movement of the abdominal appendages. Eggs are incubated in this way for 21 days and then hatch. In the laboratory, it has been observed that hatching takes place 20 days after copulation; it may even take 25-30 days if the temperature has remained below 28°C.

Ovaries frequently ripen again even while a female is carrying eggs. Immediately after hatching, the female can again release these eggs. In some cases, a female can lay eggs twice a month.

The eggs of the prawn are slightly elliptical, the longer axis being 0.6 - 0.7 mm in length. They are bright orange in colour until two or three days before hatching, when they become slate gray.

Larvae hatch during the night. Rapid movement of the female pleopods disperses the newly hatched larvae, which normally swim with their heads down and 'jump' when they contact a surface. Larvae need brackishwater to survive at this stage. Even if larvae hatch in freshwater, they will not survive if they are not put into brackishwater within two or three days. Larvae in the wild generally eat zooplankton, small insects and larvae of other aquatic invertebrates.

Larvae in a hatchery take a minimum of 26 days to metamorphose into post-larvae (PL). Post-larvae can tolerate a wide range of salinity, but freshwater is their normal habitat. And so, two to three weeks after metamorphosis, the PL move against the current and head towards freshwater canals and rivers. They abandon the planktonic habit at this stage and become omnivorous, feeding on aquatic insects and their larvae, phytoplankton, seeds of cereals, fruit, small mollusca and crustacea, fish flesh, slaughterhouse waste and animal remains. They move by crawling and generally swim with their dorsal side uppermost. They can swim rapidly.

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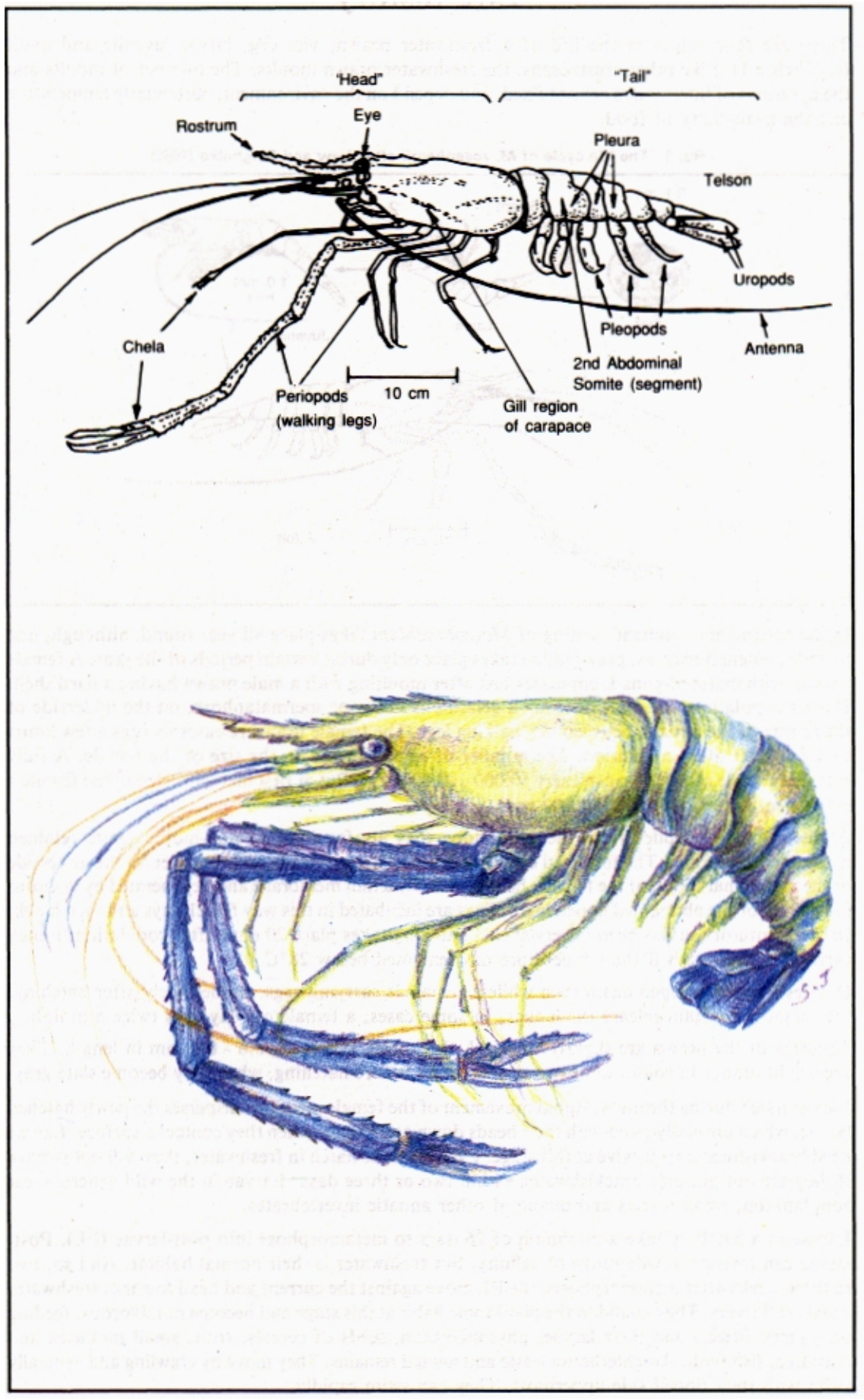
Fig. 2 Grou anatomy of M. rosenbergii (after New and Singholka 1985)



Morphology

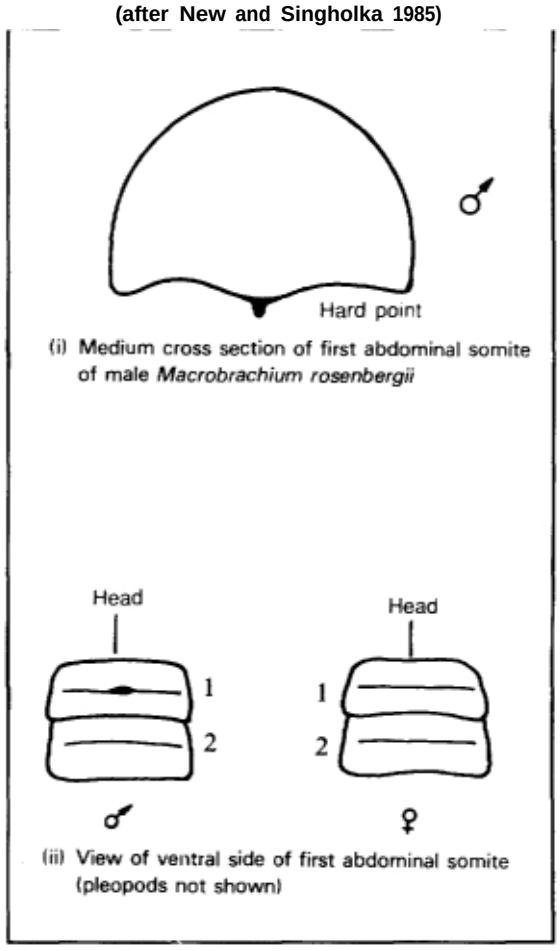
Figure 2 shows the gross anatomy of the freshwater prawn. The body is divided into segments, each with its particular appendages. These divisions are, roughly

- The 'head' (cephalothorax), which is covered by a shell or 'carapace'; and
- The tail (abdomen), which is clearly segmented.

The major appendages are

- The 'walking legs' (periopods) and pleopods;
- The antennae, for sensing their environment; and
- The maxilla and maxillipeds, to grip and chop their food.

Fig. 3. Identifying characteristics of male (♂) and female (♀) *M. rosenbergii* (after New and Singholka 1985)



Among *M. rosenbergii's* five pairs of walking legs, the second is the biggest and has a pincher (chela) at its tip. Both legs of this second pair are of the same size. Mature *M. rosenbergii* males are bigger than the females, with their cephalothorax larger and their second pair of thoracic legs comparatively longer and thicker. The cephalothorax of the male is also proportionately larger and the abdomen narrower than the female's. The genital pores of the male are situated at the base of the fifth pair of walking legs. In immature males, there is a raised hard point on the first segment of the abdominal part of the body (see Figure 3). Genital pores of the female are situated at the base of the second pair of thoracic legs. The abdominal pleura of the female are comparatively longer and the abdomen wider. The orange-coloured maturing gonad is easily visible. It is relatively easy to differentiate between deheaded freshwater prawn and marine shrimp. In freshwater prawn, the second abdominal pleuron overlaps the first and third pleura. In marine shrimp, the second pleuron overlaps only the third pleuron and is itself overlapped by the first.

Older *M. rosenbergii* juveniles and the adults are normally distinctively blue in colour. Occasionally they are brownish, with orange stripes. Brown or grey specimens are sometimes encountered. Colour seems to be related to the quality of soil and water in their environment.

Identifying characteristics

M. rosenbergii can be identified on the basis of following characteristics

- The carpus of the periopods is longer than the merus.
- **The second pair** of periopods in the male is thicker than in other species.
- There are 13 teeth in the lower part of the rostrum.
- The rostrum is long and slightly bent upward.
- Telson extends up to the end of the uropods.

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Distinguishing characteristics of male and female

Male	*Female*
The second pair of periopods is quite long and has many spines.	**The second pair is not so long and** is spineless
The genital pore is situated at the base of the fifth periopod.	The genital pores are situated at the bases of the third periopods.
The appendix masculina is situated in the second abdominal appendages.	

A key to the larval stages *of* the freshwater prawn, *M. rosenbergii*

Before metamorphosis, the larva passes through eleven distinct stages. **At the first stage** it is less than 2 mm in length, from the tip of the rostrum to the end of the telson. **At metamorphosis,** it measures about 7 mm.

The following is a simplified key to the eleven larvae stages (Uno and Soo, 1969) of *M. rosenberg/i* and is illustrated in Figure 4 (on facing page). The 'prominent characteristics' mentioned are some features which appear for the first time or only at the particular stage.

Stage	*Prominent characteristics*	*Days after hatching*
I	**Sessile** eyes	1 - 2
II	Stalked eyes	2 - 4
III	Uropods appear	4 - 7
IV	Two dorsal epigastric teeth at the base of the rostrum	7 - 12
V	Telson narrower and elongated	11 - **16**
VI	Pleopod buds appear	15 - 21
VII	**Pleopods biramous and bare**	**18** - 24
VIII	**Pleopods with setae**	**22** - 28
IX	**Endopods of pleopods with appendices internae**	**25** - **31**
X	Three or four dorsal, teeth on rostrum	28 - 33
XI	**Teeth on half of upper dorsal margin**	**31** - 50
Metamorphosis		
XII	**POST-LARVAE:** Teeth on upper and lower margin of rostrum (also behavioural changes, mainly in swimming). They are generally transparent at this stage, and have a slightly brown-coloured chromatophore on the head.	35 - 50

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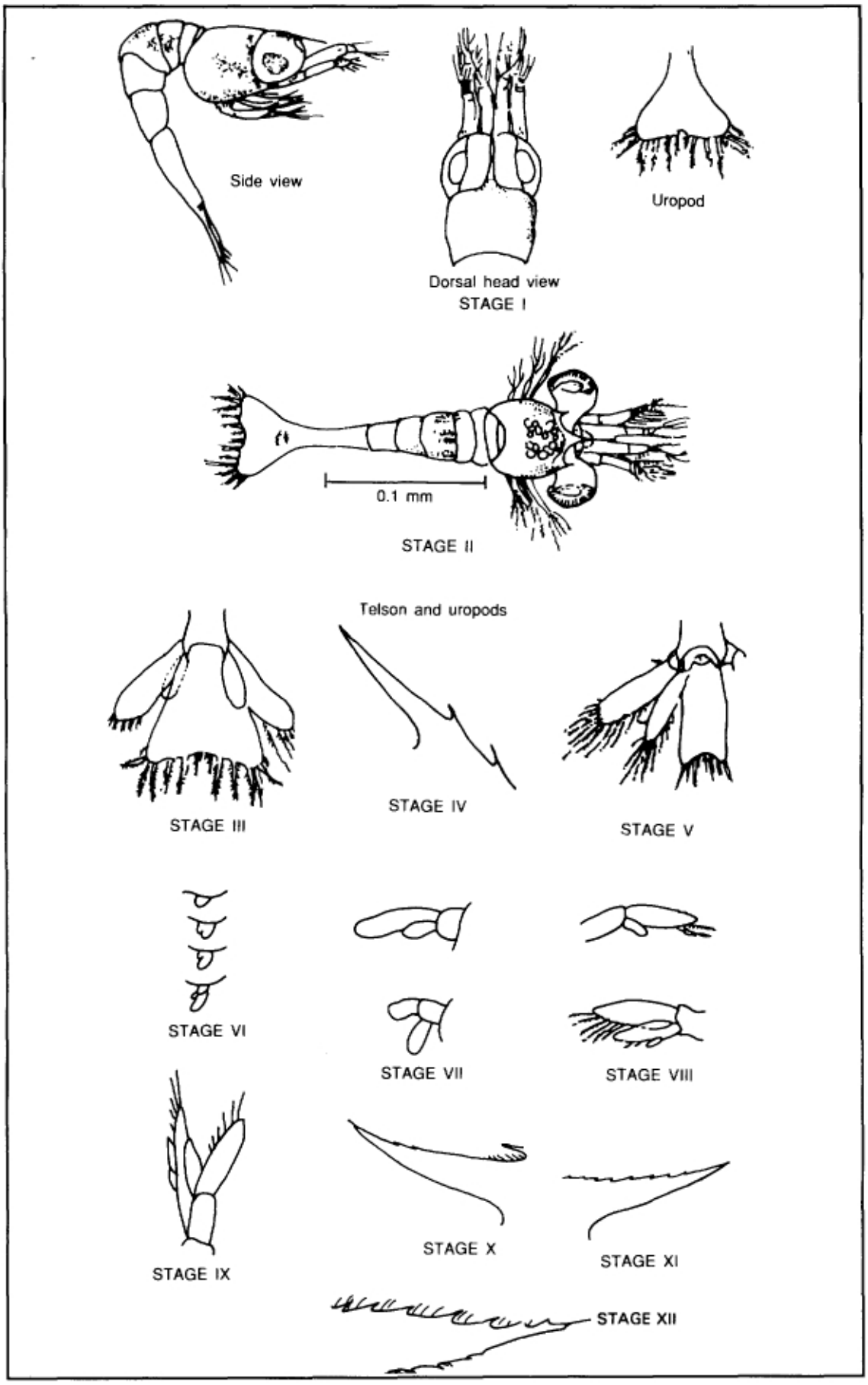
Fig. 4 Macrobrachium rosenbergii larvae. Stages 1 through 12 (after New and Singholka 1985)



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Hatchery design

This manual deals with the design and operation of small-scale, or 'backyard', hatcheries. The brine-based recirculation hatchery described may not be adaptable to large-scale production due to the considerable requirement of brine.

The definition of 'small-scale' or 'backyard' is quite flexible and depends upon the owner's production target and financial capabilities. In countries such as Thailand, where the industry is well developed, a wide variety of designs and production capacities exist. The design and construction details depend not only on the owner's financial resources, but also on his or her ingenuity.

Hatchery site selection

Factors to be considered are groundwater quality, access to brine, availability of electricity, adequacy of drainage and the availability of semi-skilled labour.

Water supply

The success of a hatchery rests on water quality, which is why the subject is stressed in this manual. No amount of remedial measures can completely overcome the problems caused by poor water quality. *Macrobrachium* hatcheries are usually established along the seacoast. However, it is possible to produce *Macrobrachium* **PL commercially in a** backyard hatchery at inland sites. Good freshwater as well as brine are required. In many areas of the world, saline- and freshwater are pumped from underground aquifers and supplied to hatcheries for prawn seed production. For hatcheries situated some distance from the sea, brine collected from salt beds in coastal areas can be stored for use the year round. Some water quality parameters for rearing water in a hatchery are shown in Table 2 (page 19).

Underground water is best for hatchery use. Deep tubewells are expensive, so a site should be chosen where shallow ground- or pond/riverwater of low iron content is available. Freshwater is best stored in an overhead tank.

Aeration during pumping can remove a major portion of the dissolved iron by precipitation, but in cases of very high iron levels, special treatment may be necessary.

Other site selection criteria

- Electric power should be 3-phase 220/440 V and supply reliable. A generator or diesel engine for the air **blower may be necessary** to cope with power failures.
- There should be good road communications for transport of brine, everyday materials and post-larvae throughout the year.
- The site of the hatchery should be near *Macrobrachium* farms supplying broodstock (at least within 16 hours' journey).
- Hatchery land should be well above sea level, in a flood/cyclone-free area.
- Adequate drainage for brine and wastewater must be provided, but drains should not discharge into paddy or other croplands.

Facility design

The plan and design of the hatchery should be prepared on the basis of production targets, weather conditions, geographical environment and situation, land conditions, availability of construction materials, skill of local labour and the availability of finance. The interior of a small demonstration hatchery built in Bangladesh is shown in Figure 5.

The hatchery building

The size of the hatchery shed depends on the number and the sizes of the tanks. Hatching tanks, larvae rearing tanks and Artemia incubators have to be accommodated. There should also be space for a small laboratory and a machine-cum-storage room. Broodstock tanks, post-larvae holding tanks and brine storage and mixing tanks may be located outside the main hatchery building, but require covers. A typical layout is shown in Figure 6.

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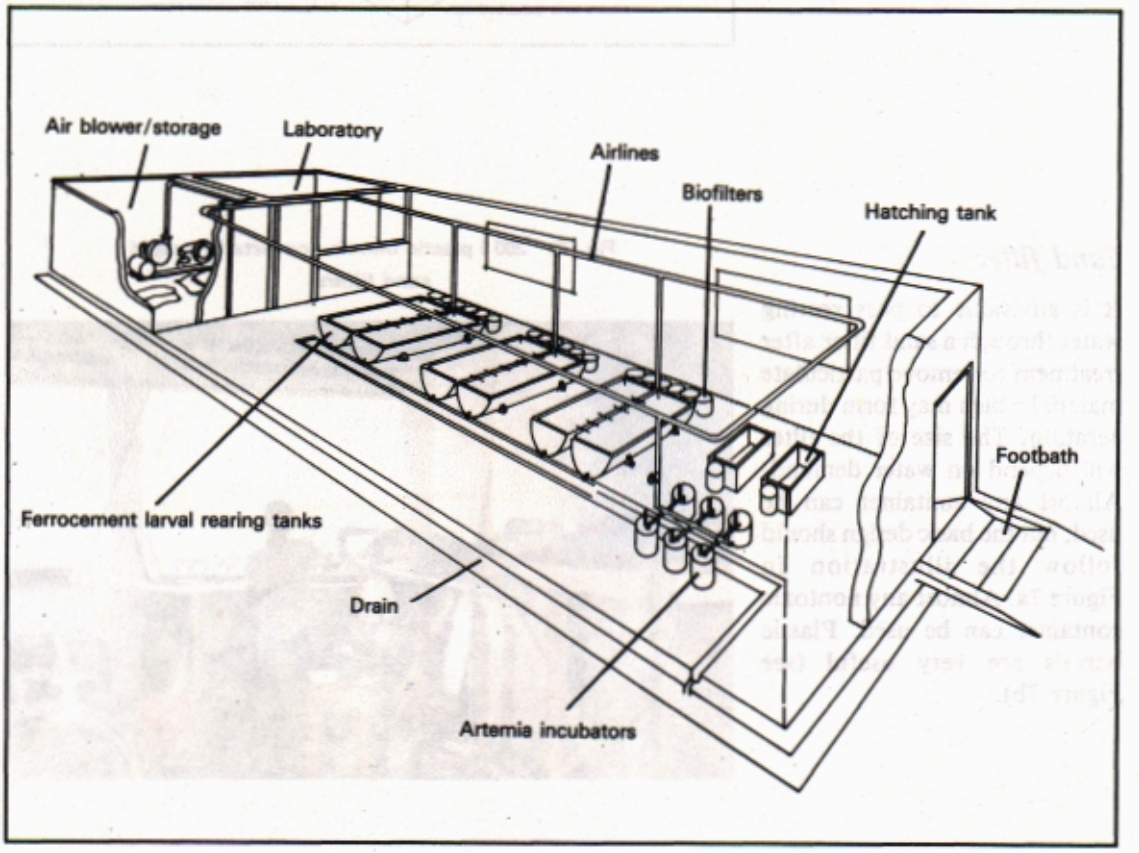
Ag. 5 Interior **of small-scale freshwater prawn hatchery.** Larval **rearing** tanks **in the foreground, brine storage and mixing tanks and sand filters In background**

Fig. 5 Sample layout for freshwater prawn **hatchery**



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A concrete structure would be preferable, but **it would prove expensive. A shed made** with galvanized **steel sheets, thatch or bamboo mat is a less expensive alternative. But whatever material is used** in constructing the shed, provision must be made for enough light and air **to enter.**

Floor

The floor of the hatchery should

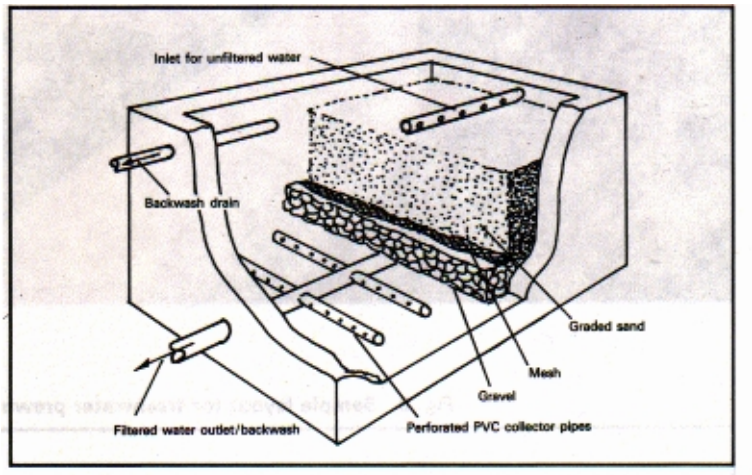
- **be cemented and smooth, to allow thorough and rapid cleaning;**
- **be provided with adequate drainage; and**
- **be strong enough to bear the weight of rearing tanks** and the water in them.

Footbaths should be placed at all entrances to the building.

Drainage

Good drainage is essential for maintaining hatchery sanitation. **Inside drains should be at least** *50* cm wide, to allow operators to reach inside them while cleaning. All drains should have removable gratings. Wastewater must discharge away from the hatchery and no water should be allowed to collect and stagnate in drains.

Fig. 7a Basic design for a rapid sand filter



Sand filter

It is advisable to pass rearing water through a sand filter after treatment to remove particulate material which **may** form during **aeration. The** size **of the filter will depend on water demand.** Almost any container can be **used, but the** basic design should **follow the illustration in Figure 7a.** Almost any nontoxic **container can be used. Plastic barrels** are **very useful** (see **Figure 7b).**

Fig. 7b 200 l plastic barrels converted to rapid sand filters

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Air system

Air supplied to a hatchery has to be oil-free, so piston-type compressors are unsuitable unless equipped with oil separators. Twin-lobe or vortex air blowers are preferable. They supply relatively large volumes of low pressure air with minimum maintenance.

A twin-lobe, or remote drive, vortex blower can be set up to be powered by an electric motor and an auxiliary diesel engine (Figure 8). The diesel engine is to power the air blower during power cuts.

The selection of a properly sized, dependable air blower is crucial to reliable hatchery operation. It is one of the most expensive components of hatchery equipment and, in some countries, has to be imported. The blower is selected according to the maximum depth of water to be aerated plus allowances for friction loss in the piping system and the pressure drop across airstones where

$$\textit{Total head (cm)} = \textit{Submergence} + HL_{pipe} + HL_{airstones}$$

Submergence represents the maximum depth to *be aerated in cm,* HL_{pipe} *the friction loss in cm* and $HL_{airstones}$ is the pressure drop across airstones, or diffusers, in cm.

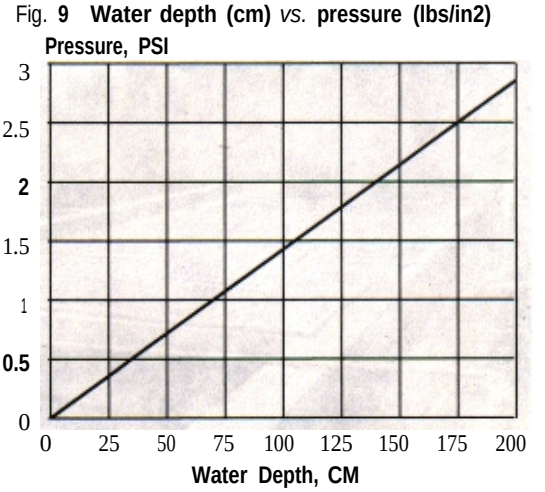
Figure 9 shows the pressure in pounds/in^2 (PSI) for various water depths (in cm). Air blower ratings are usually given in pounds/in^2. This graph can be used to help select an appropriate blower by reading off the pressure for any water depth up to 200 cm. The pressure drop across airstones depends on the pore size and air flow, and ranges from about 0.25 to 0.40 PSI, equivalent to 17.5 -34 cm water depth. Friction loss in pipes is related to air flow, pipe diameter and the length of the pipe. For the average hatchery, *25* cm can be assumed as the water depth to be added for friction loss in the air line. For example, if a hatchery has a maximum depth of 150 cm to aerate, the required pressure output of the blower would be estimated by

Total head = 150 + 25 + 18 = 193 cm

Referring to Figure 9, we fmd the blower should have an output of about 2.8 PSI. Air blower manufacturers will recommend an appropriately sized model if information on maximum water depth to be aerated, type and number of airstones and length and diameter of main air line is given.

Fig. 8. Rotary lobe air blower with auxiliary diesel power.

Fig. **9 Water depth (cm)** *vs.* **pressure (lbs/in2)**



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Electrical system

Electricity and saline water are a dangerous mixture because of the high electrical conductivity and corrosiveness of saltwater. High quality connectors, plugs and plug points must be used.

Hatching tanks, larvae rearing tanks and Artemia incubators should be provided with individual plug points. Care must be taken to ensure:

- electric lines and fittings do not come in contact with water;
- plug points are elevated well above water surfaces; and
- 'there is a ground fault fuse to protect personnel from electrocution.

An example of the calculation of power generation required for a hatchery producing 5 million PL a season is given alongside.

	KVA
5 hp blower motor	6.7
6 x 2-KVA heaters	*12.0*
Lighting	*0.5*
Submergible pump	*0.5*
	19.7

In this case, a 20 KVA generator would be required, if the heaters are turned off when the blower is started up. A generator is not necessary if auxiliary diesel power is provided for the air blower. However, *a* generator will allow continuous heater operation, as well as provide lighting during prolonged power cuts.

Tanks

A variety of tanks are required for hatchery operation and these are described below. There are a variety of construction materials that can be used, the choice depending upon availability, cost and durability. They include:

- Fibreglass: Ideal, but prohibitively expensive in some countries.
- Ferrocement: Much cheaper than reinforced concrete and can be cast into any desired shape; however, requires properly trained masons.
- Reinforced concrete: Very suitable, but also expensive.
- PlaStered brick: Easy to use, but prone to leakage without costly epoxy coating.
- Plastic-lined wooden or bamboo tanks: The cheapest, but not very durable.

Holding tanks

Round, rectangular or square tanks of 8-10 m2 and 1 m depth are suitable for holding broodstock and gravid females before they are transferred to the hatching tanks. Post-larvae may also be kept in holding tanks for a few days before sale. Draining and filling of the tank can be controlled by a standpipe, either at the centre or at either end. (see Figure 10).

Fig. **10** **Plastered brick outdoor holding tanks for broodstock, berried females and post-larvae.**

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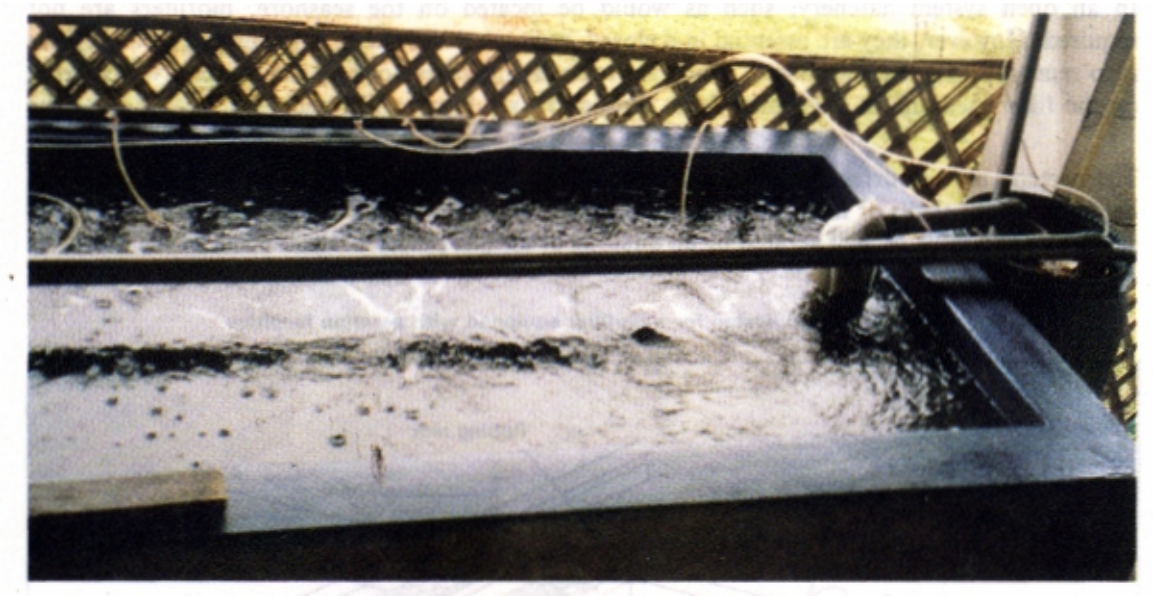
Fig. 11a 1.5 t hatching tank



Hatching tank

Tanks of any type or size, or aquaria, can be used as hatching tanks. Both portable or fixed tanks are suitable. The larvae rearing tank may also be used for hatching purposes, if necessary. Small ,tanks with conical bottoms made of cement or fibregiass are suitable (see Figure 1 la). Provision should be made fcsr regular water changes. Alternatively, a biofilter may be installed (see Figure 1 1b).

Fig. 11b 60 I biofilter for hatching tank.

Fig. 12 **Round bottom, 5 t larval** rearing **tank showing interior epoxy coating, stand pipe drain and 2 kva immersion heater**

Larvae rearing tank

Larvae can be reared in any kind of tank, round, square or rectangular. Round or conical bottom tanks are superior to flat bottom configurations.

They give better circulation and are easier to clean. The sides of the tank should be smooth and sloped towards the drain. A PVC stand pipe should be fixed with an elbow at the end to drain the water (see Figure 12).

Larvae tanks should hold at least 3-4 tonnes but should not exceed 10 t as large tanks are difficult to manage. If the tank volume is too small, diurnal temperature fluctuations will be excessive.

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In an open system hatchery, such as would be located on the seashore, biofilters are not required. However, they are essential in a closed, recirculation system. The purpose of a biofilter is to remove ammonia and nitrite, which are toxic. Removal is done by the bacteria which grow on the filter medium, as the water flows through it (see Figures 13a and 13b).

The bacteria need a surface to grow on, so the filter space is filled with 'substrate'. Substrate can be gravel, clean shells or inert plastic material, such as bottle caps. Biofilters use large quantities of oxygen and must be well aerated by an airstone or air line placed in the bottom of the biofilter.

Fig. 13a **Basic design for a biofifter equipped with aeration facilities**

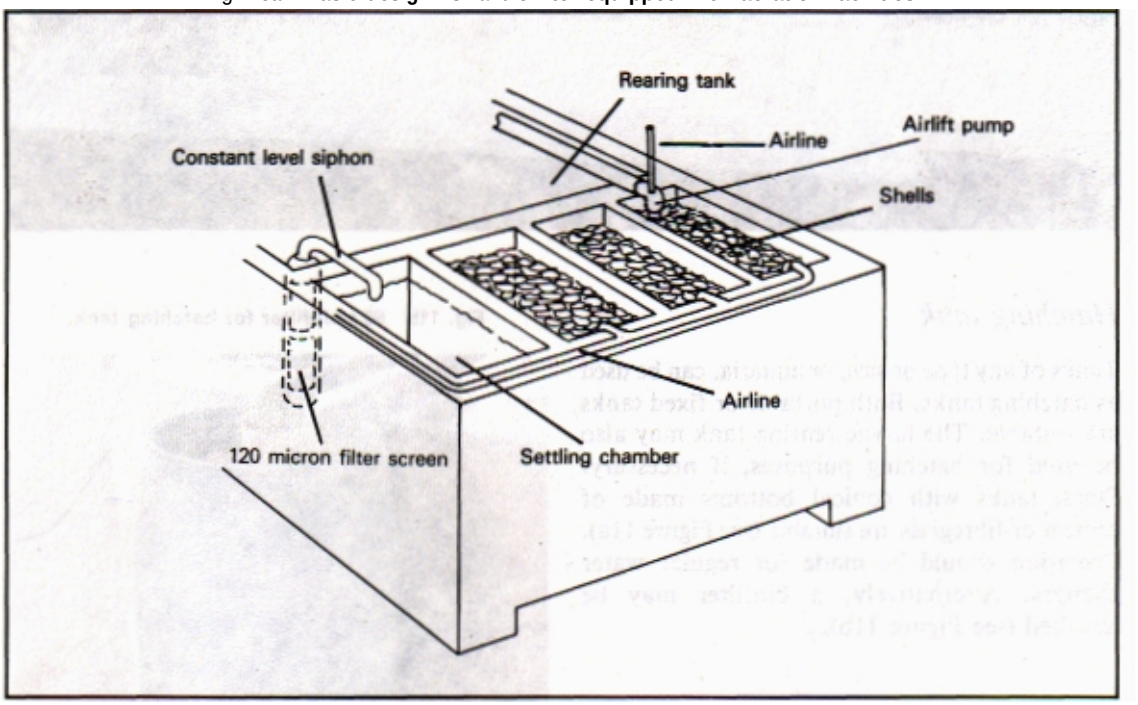

Fig. 13b Biofilter attached to larval rearing tank

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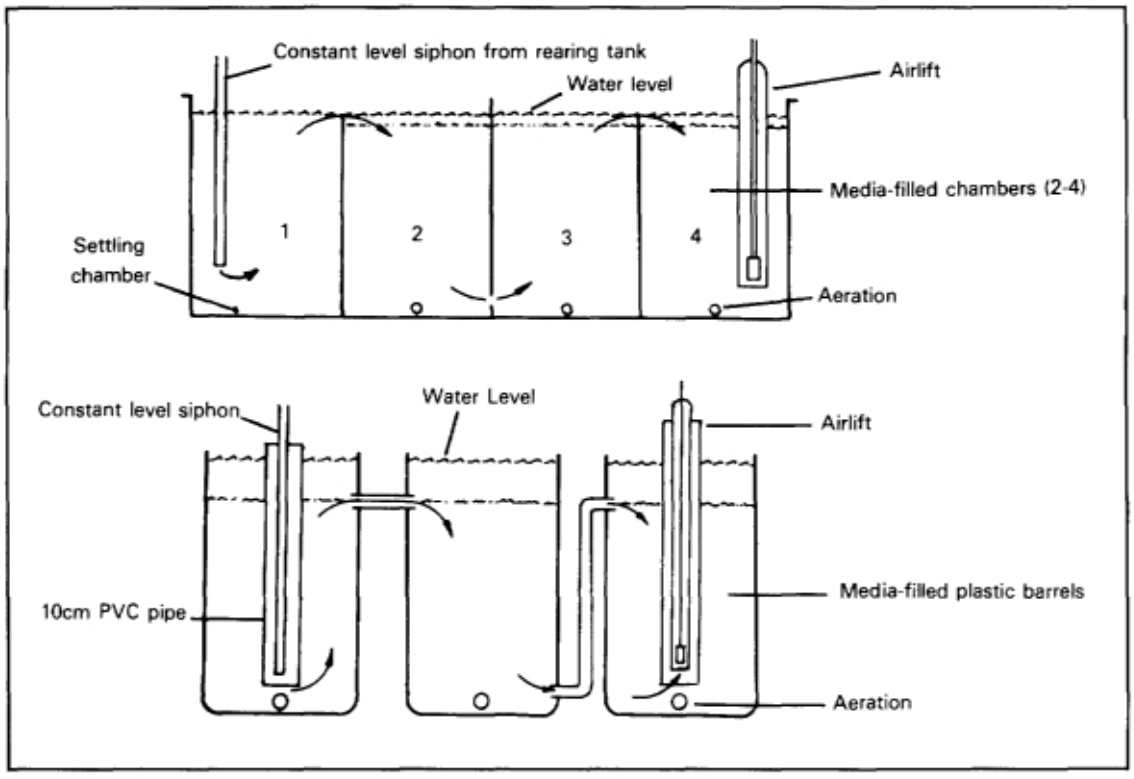
Biofilters are more efficient if they are divided into chambers with water flow as in Figure 14. There is no precise data to indicate how large a biofilter should be for larvae rearing tanks, but about 6 per cent of the tank volume seems sufficient. Water can be circulated through the biofilter using an airlift and a constant level siphon. The circulation rate may vary between 2-5 times the rearing tank volume per day.

Fig. 14 Cross-section of box type and plastic drum type biofilters



Artemia *incubator*

A cylindrical tank with a conical bottom is used for hatching *Artemia* (brine shrimp, on whose nauplii the larvae feed). A tank of 60- 75 cm diameter and 60cm height, which can hold *150-250* l of water, is generally used for *Artemia* hatching. A 60-watt light bulb should be hung Im above the incubator. The light stimulates hatching. A small window at the bottom of the tank aids in harvesting nauplii. The tank should be covered at night with a screen cloth to exclude insects.

If the incubator is made of fibreglass, the cylindrical portion should be opaque and the lower conical portion translucent.

Brine storage tank

Brine is required throughout the rearing season; in areas where brine is only seasonally available, the storage tank for brine must be large enough to accommodate an entire season's requirements.

Reinforced concrete is preferable for this tank. Brick and mortar can be used, if lined with plastic sheet to prevent leakage. An interior coat of epoxy can also be used for the same purpose.

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The size of the brine storage tank will depend on the larvae rearing tank capacity of the hatchery. An example of how to calculate the amount of brine and, hence, the size of the storage tank is given below

$$\text{Volume of brine required} = \frac{\text{(salinity of rearing water) x (volume of rearing water)}}{\text{salinity of brine}}$$

Suppose, larvae are to be reared at 12 ppt in a 5 t (5000 l) tank with 200 ppt brine being used, the amount of brine required is calculated as follows

$$\text{Volume of brine} = \frac{(12\ \text{ppt}) \times (5000\ \text{l})}{200\ \text{ppt}} = 300\ \text{l}$$

Each larvae rearing tank will, thus, require 300 l of brine and 4700 l of freshwater.

Now, suppose, the production season is 210 days, and each brood takes 40 days to complete metamorphosis. Four cycles could then be produced per season. So, each tank will require *300 1 x 4 cycles, or 1200 1 of brine.*

We also have to allow for two partial water changes, totalling 40 per cent of the FL tank volume or 5000 l x 0.4 = 2000 1.

$$\text{Volume of additional brine} = \frac{12\text{ppt} \times 2000\text{l}}{200\ \text{ppt}} = 120\ \text{l}$$

For four cycles, an additional 480 l of brine will therefore be needed, bringing the total brine requirement per tank per season to *1200 l + 480 / = 1680 l*

If the hatchery has four 5 t tanks, the brine storage tank would have to hold

$$1680 \times 4 = 6720\ \text{l}$$

An additional amount of brine will also be required for *Artemia* incubation. To estimate the brine requirement for *Artemia* required in the above example, it is assumed that the *Artemia* cysts (eggs) are stocked in the incubators at 2g/l and the salinity of incubation water is 30 ppt. From Day 1 to Day 10, the *Artemia* nauplii are fed to the larvae at a rate of 5/mi and, thereafter, until Day 40, at *2.5/ml.*

Estimate the volume of *Artemia* incubation water as follows:

$$\frac{(5\ \text{cysts/ml}) \times (5 \times 10^6\ \text{ml})}{2.50 \times 10^5 \text{cysts*/g} \times 0.8\ \text{hatching rate}} \div 2\ \text{g/l} = 62.5\ \text{l}$$

Four tanks will, therefore, require 250 l of *Artemia* incubation water per day for 10 days. After Day 10, the incubating water requirement would drop to 125 l per day for 29 days, totalling 3600 l. One cycle in the sample hatchery would, therefore, consume 6125 1(2500 l + 3625 1) of incubation water and would need:

$$\frac{6125\ \text{l incubation water} \times 30\ \text{ppt}}{200\ \text{ppt}} \quad 920\ \text{l brine}$$

A four-cycle season would, thus, need 3700 l of 200 ppt brine for *Artemia* incubation alone.

The total brine requirement for the breeding season would, thus, total 10,400 1(6720 l + 3700 1). Some additional quantity of brine should be stored to provide for emergencies, but this requirement can only be estimated on the basis of experience. An additional 1000 l should give an adequate margin of safety. Therefore, in the above example, the total brine **needed for one season of four cycles would be 11,400 l.**

Note : See pp. 24-26. The hatching rate varies from 70 to 90%, depending on quality.

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Mixing tank

Saline water and groundwater are mixed to get the required salinity of 12 ppt. The volume of the mixing tank can be equal to or double that of a larvae rearing tank. The mixing tank must have an aeration system and drain.

Water pumps

Submersible stainless steel pumps of 0.75-1.5 kw are suitable for transferring water within the hatchery. These pumps can also be used if pondwater is used as the freshwater source.

If a tubewell is the source of freshwater, the size of the pump will depend upon the depth and diameter of the well and the flow rate required.

Additional equipment

There are some basic additional items required to efficiently operate a prawn hatchery. These are listed below. Other equipment, such as electronic pH meters, are useful, but not essential.

A **refrigerator** is necessary, to keep prepared supplemental feeds, nauplii of *Artemia* and antibiotics.

A stereo microscope is sufficient to examine larvae growth and monitor health in the hatchery. A more powerful compound microscope would be required for disease diagnosis. All optical parts should be stored in a desiccator.

A refractometer is essential to measure the salinity of water. The instrument should read in ppt (0/00).

A simple beam balance is required to measure *Artemia* cysts, feed ingredients and medication.

Other apparatus and instruments, for everyday use, like beakers, conical flasks, glass jars, Petri dishes of various sizes, watch glasses, 20-1 buckets, baskets, basins, magnifying glass, test tubes, pipettes, dissection tools and a desiccator, will be needed.

Paper or liquid pH indicators, in the range pH 6-10, can be used.

An electronic pH meter is very useful and is recommended if affordable. Easy-to-use kits are available to measure ammonia, nitrite, nitrate, hardness, chlorine and iron.

Immersion heaters of high quality are the most practical solution to the problem of diurnal temperature changes, which **are** relatively great during the early and late part of the rearing season in Northern India **and Bangladesh. Larvae** are very sensitive to fluctuations of more than **±1°C.** Great care must be taken in the installation and use of these heaters. Brackishwater is very corrosive, so it should be ensured that only stainless steel or titanium is in contact with it.

Various **chemicals** are used for treatment of prawn seed, preparation of feed and pasteurizing water. The most commonly used substances are listed in Table I.

Table 1 : Commonly used hatchery chemicals and feed ingredients.

Water treatment	*Disease control*	*Feed Preparation*
Calcium hypochiorite	Chioramphenicol	Agar
Sodium bicarbonate	Tetracycline	Corn starch
Calcium oxide	Sulfamerazine	Milk powder
Sodium thiasuiphate	Farmalin	Vitamin mix
Sodium carbonate	Furanace	
Sodium EDTA		
Sodium hydroxide		

The required quantities of these chemicals will vary, of course, with the rearing capacity of the hatchery. If the example we have been using is considered, that is, a 20 t hatchery producing four cycles a year, the following would be the estimated requirements:

- **Bleaching powder:** 50 kg for one four-cycle season.
- **Sodium thiosulphate** to neutralize treated water: 5.5 kg.
- **Otherchemicaistobestocked** : 1 kgofeach.

Technical grade chemicals are sufficient for hatchery work. All antibiotics should be of veterinary grade.

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Miscellaneous items

Several other items are needed for everyday use. They include:

Nylon nets of different mesh sizes: To catch and transport mother prawn and PL.

Polythene (Netlon) **plankton nets** of different mesh sizes: To collect nauplii of Artemia, for food preparation and to change/filter the water. Mesh sizes of 120 to 200 microns are most useful.

120 **micron** screens: To retain Artemia cysts.

Plastic hose of different diameters: To siphon, clean and aerate tank bottoms and supply water.

PVC pipe and gate valves of different sizes: To control air and water flow. Brass and copper should not come in contact with rearing water in a closed system.

Saucepans, a heater, pressure cooker, spoon, strainer and knife: To prepare food.

Screens: To wash prepared feed.

A blender: To grind the steamed custard.

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Larvae Rearing

The techniques described in the following sections have been adapted from practices generally used in a brine-based recirculation hatchery established in southeastern Bangladesh in 1991. Recirculation systems for shrimp and prawn hatcheries have wide applicability wherever brine is available. Artificial sea salts could also be used in a recirculation system; **this enables prawn hatcheries to be set up almost anywhere.**

Water supply and treatment

Generally, some kind of treatment is required to render the water suitable for larvae rearing. The nature and degree of treatment depends upon the quality at the source, It bears repeating that **no amount of treatment will bring badly polluted and turbid water up to standard.**

Brine collection

Salt farms are divided into reservoirs, condensers and crystallizers. Brine can be taken from the condensers when the salinity has reached at least 180 ppt, but 220-240 ppt is preferable. Supernatant water from the crystallizers is toxic and should never be used. Brine is pumped from the condensers into plastic barrels, which are capped and transported by truck to the hatchery.

Water treatment

Underground water is ideal for hatchery operation if it fulfils the criteria mentioned in Table 2.

Table 2 : Hatchery water quality criteria

Parameter	Level
Salinity	12 - 15 ppt
Temperature	28- 31°C
pH	7.0-8.5
Nitrite nitrogen	0.1 ppm
Nitrate nitrogen	20 ppm
Chlorine	0,0
Hydrogen sulphide	0.0
Hardness	00 ppm
Iron	2 ppm

Tapwater may also be used, but it should be aerated for 24-48 hours, or passed through activated carbon, to remove chlorine. Groundwater has no oxygen and must be well aerated before use.

Dissolved iron is precipitated by aeration in the mixing tank and subsequent removal by sand filtration. Very high levels require special treatment. This can include filtration through a soda lime bed, followed by settling and sand filtration. If dissolved iron is **greater than 2ppm,** alternative sources of water or sites should be considered.

Pond or riverwater requires more elaborate treatment, but may be employed if no other source is available. Such water often contains very fine particulate organic matter and silt, making filtration difficult. Flocculation with alum at 150 ppm may be needed to ensure efficient filtration. Usually a rapid sand filter (see Figure 7) is used for this purpose.

Municipal water can be dechlorinated by activated carbon filtration. Sodium thiosulphate may be used if the water is stored in an overhead tank.

In the mixing tank, 12 ppt saline water is prepared by mixing the freshwater and brine. Table 3 shows treatment procedures for the mixed water. Bleaching powder is added to the 12 ppt brackishwater which is then aerated for 24 hrs. Excess chlorine is removed by treating the water with sodium thiosulphate. Treated water is allowed to stand several hours after thorough aeration, followed by sand filtration.

Table 3 : Culture water treatment regimen

Chlorination	*5 t water*	*10t water*	*Duration*	*Detoxification*
a. 70 % calcium hypochlorite	50 g	100 g	12 hrs static. Aerate initially for 1 hour to mix thoroughly	Mix 12 ppm sodium thiosulphate and aerate for 12-24 hrs
b. 5 to 6% sodium hypo- chlorite* (chlorox)	*½1*	*11*	*12 hrs static*	*Mix 12 ppm sodium thiosulphate and aerate* for 12-24 hrs

* Can be doubled to 10 ppm if the water is suspected to carry a heavy load of organic debris. This should be done prior to filtration, as chlorine tends to precipitate iron and flocculate organic matter.

Note If water treated with chlorine is high in organics, it is suggested that it be passed through activated carbon to detoxify chloramines after chlorination and sand filtration.

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Broodstock and spawning tank management

To get enough quality eggs, careful management of the broodstock is required.

The depth of the water in holding tanks should be 0.9-1.0 m.

Daily, in the morning and evening,. 50 per cent of the water should be changed.

Enough shelter should be provided at the bottom of the tank to reduce stress. Tank covers, or a shed, should be installed for shade.

Rainwater stimulates sexual activity of the prawn, so better results can be expected if rainwater is allowed to fall into the holding broodstock tank.

Feed equivalent to 5 per cent of the bodyweight of the stocked prawns should be supplied twice every day, in the morning and evening. It is preferable to supply raw food in the night and prepared food during daytime. Chopped fish and mussel, small shrimp, chopped fish, and adult Artemia are good for broodstock. Dry pellets with good food value can be provided, if available.

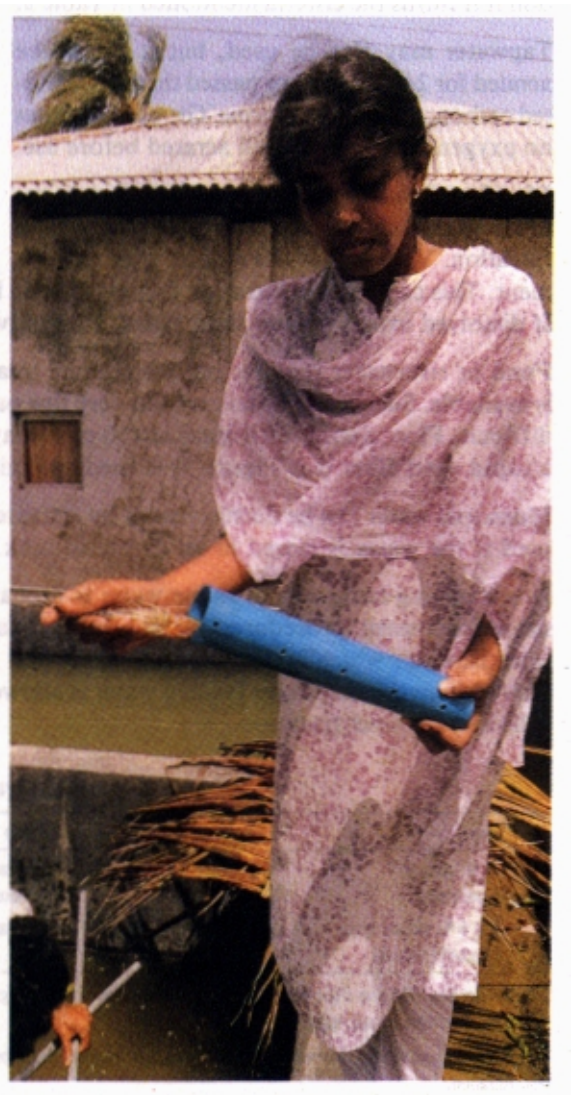
Before the food is given everyday, the bottom of the tank should be cleaned by siphoning. This removes uneaten food, leftover moults and faecal waste. The tank bottom and wall should be brushed and cleaned every two days.

The tank and broodstock can be disinfected by introducing 20 ppm formalin solution in the tank, followed by a 100 per cent change of water after 24 hrs. This must be done very carefully.

From time to time, the water level should be decreased and injured prawns removed,

Berried females should be transferred to the spawning tank as they become available.

Fig. 15 Plastic tube used for long distance transport of berried females.



Broodstock collection and maintenance

The female prawn carries the fertilized eggs under her abdomen in a brood chamber. Prawn that are carrying eggs are referred to as 'berried'. They are generally found in rivers and their tributaries, canals, ponds and deep depressions. Berried prawns can also be produced in the holding tanks in a hatchery. During cool periods, broodstock can be produced in a hatchery under controlled temperature conditions.

Berried female prawns are available throughout the year in the lowlands of Malaysia, Thailand, Indonesia, southern Vietnam and similar locations where water temperatures remain high through the year. In Bangladesh and West Bengal, natural reproduction occurs only during the warm months – from late March through September.

Transport of berried prawns is similar to that of adult prawn, but because of the eggs in the abdomen, they need to be handled with more care than immature prawns. Berried female prawns should be held in individual perforated plastic tubes if they are to be transported long distances (Figure *15).* The ends of the tube are closed with gauze held in place by rubber

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bands. The tubes are transported in plastic bags containing oxygenated water. After broodstock are collected, they should be disinfected with formalin. Weak, wounded and diseased animals should be discarded and only healthy and disease-free berried prawns stocked or transferred to the hatching tank.

In the hatching tank, it is advisable to stock four prawns/sq.m in water of depth 30-40 cm. The tank bottom should be cleaned every morning and evening and 50 per cent of the water exchanged. The salinity should gradually be increased to 12 ppt.

The feed ratio should be 5 per cent of the total bodyweight of broodstock. Bivalve meat, snails and worms can be fed.

Aeration should be continuous and the temperature maintained at 28-29°C. If temperature is not controlled, the growth of embryo will be delayed and the newly-hatched larvae will be weak and undersized. The main purpose of this tank is to provide proper conditions for the embryo to grow.

Full development of the eggs in the abdomen of the female prawn takes about 19 days. The female prawn remains busy during these 19 days by brooding the eggs. As the eggs develop, their bright orange colour changes to a greyish colour.

Ag. 16a Newly **spawned** eggs.

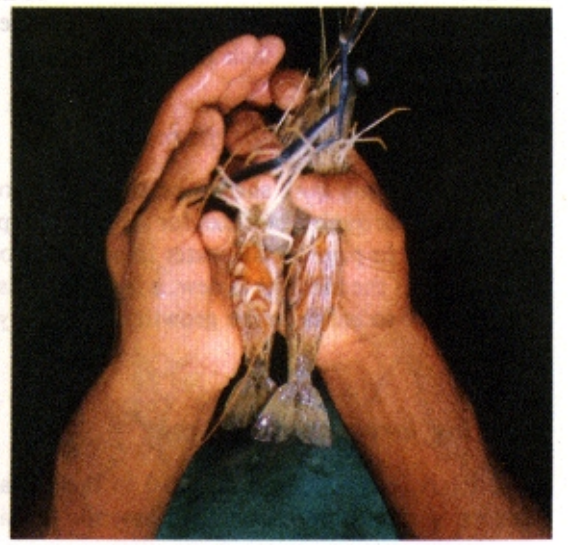

Fig. 16b **Berried female** ready **for transfer to hatching tank**

Selection and disinfection

Egg development should be observed every alternate day: a 'conical scoopnet is used to take out each prawn and its yellowish ventral egg sac examined. When the colour of the eggs in the sac becomes dark grey, the prawn should be transferred to hatching tanks after disinfection (see Figure 16). Disinfection is done by keeping them in aerated water with 25 ppm formalin for 1½hour. When the elliptical eggs are ready to hatch, fully developed larvae may be seen inside the egg with the aid of a microscope.

Berried females should be selected carefully, applying the following criteria. They should be:

- Healthy and disease free;
- Strong and active;
- Bright-coloured;
- Laden with a large number of eggs;
- As large as possible.

The chances of larvae surviving from prawns having these qualities is good. Their growth will also be fast.

Hatching tank management

Hatching can be done in the larvae rearing tanks. However, the use of a separate hatching tank helps to prevent the spread of disease to larvae rearing tanks. Larvae rearing density is also more easily controlled. In case separate tanks are used, for every 100 g prawn weight a tank or aquarium of at least 500 l capacity is required.

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Before stocking prawns in a hatching tank, it should be filled to a depth of 30-40 cm with treated 12 ppt brackishwater. The sodium salt of ethylene diamine tetracetic acid (EDTA) appears to have very beneficial effects on the hatching rate and larvae survival, so 5-10 ppm EDTA should be added to the water in the hatching tank. Aeration should be continuous and temperature maintained at 30°C. After hatching of larvae, spent broodstock should be returned to the holding tank or broodstock pond.

No **feed** need be supplied to berried prawn in the hatching tank. During the time they spend in this tank, they are busy taking care of their eggs and **do not feed. Larvae generally hatch during** the first half of the night, although sometimes hatching may take place during late evening. In some cases, partial hatching also occurs.

Movement of larvae starts five minutes after hatching. At this time, larvae swim in a head-down position.

Larvae are made to concentrate in one corner of the hatching tank by covering all but a small portion of the surface with an opaque material. Larvae are attracted to the light and will gather in the exposed corner, from which they can be easily removed with a siphon or small bucket.

Larvae rearing tank preparation

The larvae rearing tank should be filled with treated 12 ppt water. The water is conditioned by circulating it through a biofilter for several days prior to the tank being stocked with newly-hatched larvae (Refer to page 19 for water treatment procedures). Add 10 ppm of EDTA before stocking with larvae. If the biofilter is new, a few handfuls of urea, or another ammonia salt, can be thrown into the tank to enhance the growth of denitrifying bacteria in the filter during the conditioning period.

Stocking larvae rearing tank

Stage I larvae are stocked at 100/1 in the larvae rearing tank. The remaining larvae should be transferred elsewhere. After ten days, the density of larvae can be adjusted to 60-80l.

By keeping all these parameters favourable and by controlling management accordingly, the end result can give up to 30-40 PL/l. In some cases, 60-100 PL/l have been produced. Fifteen to 20 PL/l seems to be about average.

Tank management

Maintaining stable water conditions in the tank is what ensures successful larvae rearing. This is much more critical in a recirculation system than in an open system hatchery. Salinity, temperature, ammonia and pH must all be kept within the limits necessary for the good health of the larvae.

Salinity control

The salinity of water in the larvae rearing tank should be maintained at 12 ppt up to the PL stage. However, ±2ppt does not affect growth of the larvae. Sudden fluctuations during water changes are to be avoided. Salinity can be checked and controlled by a hand refractometer.

In a recirculation system, salinity may increase a little due to evaporation. Freshwater of the same temperature as the tank water should be added as needed to maintain the salinity at 12 ppt. Salinity should be checked every four or five days and freshwater added as required. If turbidity increases dramatically, an immediate water change is required. For this reason, **treated 12 ppt water should always be available in the mixing tank.**

Temperature regulation

The ideal temperature for rearing is 28-30°C.Temperature can be controlled by using immersion heaters. It has to be borne in mind that temperature below 24°Cand above 33°Care lethal to larvae. Fluctuations of temperature by more than 1°Care stressful and cause mortality.

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To heat the water in the tank, different types of heaters are available on the world market. About 400 watts per 1000 l of water is required. Water temperature should be checked five or six times daily if a thermostat is not being used. Covering the tank at night will help to reduce diurnal fluctuations.

Larvae at all stages are attracted by light, but **direct sunlight is harmful.** It has been shown that growth and survival of larvae are improved in lighted tanks compared to dark tanks. Low, even illumination is preferable. Uneven illumination will cause 'clumping' of larvae in the brightest areas of the tank. Strong aeration counteracts the clumping tendency. Covering individual tanks is recommended to maintain temperature and to inhibit the spread of disease between tanks. Fluorescent tubes can be used to evenly disperse light if tanks are inside a closed building.

Ammonia, Nitrite and pH control

Chemical changes take place in the water of the larvae rearing tank. Such changes take place because of the waste products of larvae and *Artemia,* dissolved fractions of feed supplied to the larvae, unused feed and spoilage of dead larvae. Some of these changes are very harmful.

Un-ionized ammonia is the result of one such change. High pH increases the amount of un-ionized ammonia. Both nitrates and nitrites are harmful. Excessive nitrates increase mortality and retard growth, while 1.8 ppm of nitrite is lethal. The presence of un-ionized ammonia in very small quantities also induces mortality.

The concentration of nitrites and nitrates in the water of the larvae tank should not exceed 1 ppm and 20 ppm respectively. The concentration of ammonia nitrite and nitrate is reduced and controlled by recirculating the water through the biofilter. Oyster or clamshells are good media for the biofilter, as the calcium carbonate content acts as a buffer against sudden changes in pH. The best pH range is 8.0 - 8.2.

Maintaining water quality

Cleanliness must be strictly maintained to ensure best results. Constant vigilance is required to prevent or control outbreaks of disease. Instruments and glassware should be kept separately for each tank, to prevent the transfer of disease between tanks. All tools and glassware should be disinfected by soaking them in a solution of potassium permanganate or formalin. After every larvae cycle, the tank should be washed and disinfected to prevent the growth of *Zoothumnium, Epistylis,* hydroids and other disease organisms.

The following precautions should be taken:

- Larvae should not be given feed in excess of their requirements.
- The walls of the tank should be cleaned with a soft brush every third day.
- After the first feed every day, aeration should be stopped and solid waste and dead larvae allowed to settle at the bottom. These should then be removed by siphoning and the aeration turned on immediately thereafter.
- If the hatchery is located on a sea beach, only 50 per cent of the water needs to be exchanged daily. In a recirculation system, an even smaller proportion of tank volume needs be changed. A 20 per cent replacement on Day 10 and on Day 20 is beneficial.
- If for any reason the condition of the water deteriorates, or the movements of the larvae become weak, then 100 per cent of the rearing water should be changed.
- EDTA (usually 5-10 ppm) added to both hatching and larvae rearing tanks improves production.
- After completing a larvae rearing cycle, the side walls of the tank should be brushed well and kept moist for 24 hours with a strong solution of commercial bleaching powder. Formalin at 250 ppm may be used in place of bleaching powder. The disinfectant rinse should be followed by washing with clean water and drying for at least one day. Before starting work again, the tank should be rinsed with tapwater.

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Counting larvae

Larvae are strongly phototactic (light sensitive) and tend to group in even a well-aerated tank. It is almost impossible to get an accurate count under these conditions. Taking several samples with a 250 ml beaker and counting the larvae caught in it will give a rough estimate. It is worthwhile estimating the Stage I larvae so that the stocking rate could be kept within reasonable limits.

Counting dead larvae during daily tank cleanings will give a clear indication if something is amiss and enable remedial measures to be taken. If there is a large increase in dead larvae, behavioural **and colour changes will usually be seen in the live** larvae in the tank.

Feeds and feeding

Prawn larvae feed by filtering particulate matter. Food particles must be small enough to enter their mouths, yet large enough to be retained by their setae (filters).

Larvae in the first stages do not actively search for food. *Artemia* density must be high enough so that the larvae will frequently encounter their feed. Late stage larvae and post-larvae are more active in searching for food.

In preparing food for the larvae, the following should be remembered:

- The feed has to contain components which attract larvae.
- Feed quality is of paramount importance; cost is secondary.
- Feed should be hygienically prepared and stored.
- Prepared feed should be immediately used in the tank and any additional feed should be kept under refrigeration.
- Prepared feed should remain in suspension in the water.
- The particle size of the feed should suit the requirements of each stage of the larvae.

Live food

Macrobrachium larvae cannot collect food directly by themselves. Live Artemia salina nauplii (Brine Shrimp Nauplii, BSN), a small crustacean rich in protein and essential fatty acids, is given as feed to prawn larvae. No commercially effective substitute has yet been found for Artemia nauplii.

Where to find Artemia?

Artemia, popularly known as fairy shrimp, brine shrimp or 'sea monkey, is a primitive crustacean inhabiting very saline water beyond the tolerance of finfish. It is found in natural and man-made salterns, lakes and flats, from the temperate to subarctic regions.

When conditions of salinity and oxygen content are right, the brine shrimp bears its young alive, in the form of nauplii. But if the salinity rises above 120 to 180 ppt, each embryo becomes encased in a highly resistant cyst. The development of the embryo then ceases, but it remains viable for many years, even centuries! The resistant cysts are transported by birds and wind. When conditions return to normal, development of the embryo resumes and within 24 hours the nauplii hatches.

There are many strains of brine shrimp, each with differing nutritional qualities and hatching rates. Cysts with low hatching rates are sold cheaper, but the poorest quality are actually quite expensive in terms of the number of nauplii produced per gram of cyst. The best quality cysts come from southern San Francisco Bay and the Great Salt Lake, Utah, in the USA. Other sources are China, Brazil and Australia. Attempts are underway in several tropical cQuntries to commercially produce *Artemia* cysts. Locally produced cysts are marketed in India and Thailand, but their quality is not consistent.

Artemia nauplii, or brine shrimp nauplii (BSN), are the major operating expense of a freshwater prawn hatchery. Its proper use should therefore be considered in some detail.

Calculating the weight of cysts required

The number of cysts/gram and the hatching rate are given on each can of *Artemia* cysts. For example, one popular brand contains 250,000 cysts/gram and their hatching rate is 80 per cent. Therefore, to get 25 million nauplii, you need:

$$\frac{25{,}000{,}000 \text{ nauplii}}{250{,}000 \text{ cysts/g} \times 0.8 \text{ nauplii/cyst}} = 125 \text{ g of cysts}$$

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Decapsulation

The outer shell of the *Artemia* cyst is removed by decapsulation. This has several advantages:

- It disinfects the *Artemia* cysts.
- Disinfection reduces the chance of introducing disease from the cysts.
- There is no need to separate empty cyst shells from the newly hatched nauplii.
- No empty cysts get introduced into the larvae rearing tank, thus helping to keep the water clean and preventing the clogging of the siphon intake screens.
- Unhatched decapsulated cysts are more nutritious than newly hatched BSN.

Several buckets and a fine meshed cloth, or a Nitex screen bag, are required for the decapsulation procedure. Sufficient ice must be kept on hand to control the temperature of the decapsulation solution. The following steps should be followed to remove the capsule:

1. Put 200 g of cyst in three litres of freshwater and hydrate them for about an hour with strong aeration.
2. After an hour, drain the cysts on a screen cloth of 120 micron mesh. Then wash them thoroughly in running water.
3. Prepare the decapsulation solution as follows:

   Dissolve 160 g of bleaching powder and 120 g of sodium carbonate together to make a solution of 4-5 l. Mix well, allow the solution to settle undisturbed for 30-45 minutes, then decant the clear liquid. This should be done while the cysts are hydrating.
4. Add the cysts to the decapsulation solution and strongly aerate for about 20-25 minutes. Simultaneously add ice to keep the temperature below 40°C. If the bleaching powder is weak, two solutions prepared according to the instructions for Step 3 may be necessary. In this case, decapsulation should be limited to ten minutes in each solution (see Figure 17a facing page). When the cysts become orange, decapsulation is complete. If the cysts have not become orange, then this stage has to be repeated.
5. When decapsulation is complete, stop aeration, filter the decapsulated eggs through a net of 120 micron mesh and wash them under tapwater until no chlorine odour is detectable.
6. Dissolve 10-20 g sodium thiosulphate in two litres of water and add this solution to the washed cysts (as in step 5). Aerate now for 5-10 minutes to neutralize any residual chlorine.
7. Rinse thoroughly in tapwater.
8. Place the decapsulated cysts in a small quantity of freshwater (2-3 l) for a few minutes. The decapsulated cysts will sink to the bottom of the container, while the small quantity that are undecapsulated will float. Remove these by siphoning and store in brine; they could be used in the next decapsulation.
9. The cysts at the bottom should be filtered as before and washed for the last time, then taken for hatching or for preservation in saturated brine for use later. For storage, 50 ml brine should be mixed with every 100 g of cysts (see Figure 17b).

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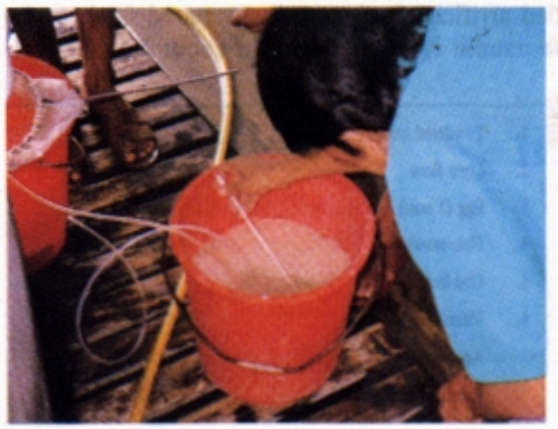
Fig. 17a Controlling the temperature in decapsulation solution.

Fig. 17b Decapsulated cysts stored in brine



Hatching

Artemia cysts can be hatched immediately after decapsulation. The necessary conditions required for this are:

The cysts should be stocked in the incubator at 1-2 g/l (dry weight) or according to the supplier's recommendation. Therefore, the volume of the incubator depends on the hatchery's daily requirement of ***Artemia*** cysts.

Whatever the volume, the salinity should be 28-31 ppt or should follow the supplier's recommendation. The pH should be between 8 and 9. Sodium bicarbonate ($NaHCO_3$) can be added to bring the pH of the hatching water to 8.5-9 before adding the cysts. Hatching begins within 18 hours and may continue for a further 12-18 hours. The procedure thereafter should be as follows:

1. About 30 minutes before collection, add ***50*** ml of ***50*** ppm formalin to disinfect the nauplii.
2. When hatching is complete, stop aeration and cover the tank with opaque cloth or plastic so that the nauplii can settle to the lighted bottom of the tank. *Artemia* nauplii should be fed to larvae as soon as possible after hatching. Since hatching begins after 18 hours of incubation and extends to 36 hours, it may be advisable to partially harvest a tank before hatching has been completed. ***Artemia*** nauplii are most nutritious while they contain the yolk sac, which is why they should be fed as soon as possible after hatching.
3. Collect the nauplii in a phytoplankton net of ***250*** micron by siphoning or draining. The water used for hatching of ***Artemia*** nauplii should be thrown away. Collect the cysts that have not yet hatched. These may hatch in the larvae rearing tank or the larvae may consume them. If the cysts have not been entirely decapsulated, stop siphoning, or close the plug of the drain pipe, just before completion of drainage, so that the shells of the eggs can be separated. This will allow the shells to float and will help in their collection for removal.
4. Before supplying the nauplii to the tank, they should be rinsed in clean, 12 ppt saline water.
5. Transfer the nauplii from the phytoplankton net to a bucket and put them in the larvae rearing tank in the quantities necessary.

A new batch should be hatched every day, following this procedure, and fresh nauplii should be supplied to the larvae.

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Prepared food

Along with the live feed, larvae should be supplied artificial feed as well. Prepared feed can be given from the tenth day onwards, when *Arternia* nauplii can be reduced to about 2.5 BSN/ml.

Feed preparation

Taking into consideration the protein needs of the larvae, the ingredients listed alongside may be used for preparation of an artificial feed.

1.	Powdered milk	60g
2.	Cornflour	20g
3.	Egg (2 nos.)	70g
4.	Fish/prawn	BOg
5.	Cod liver oil	3.5 ml
6.	Vitaminmix	2g
7.	Agarpowder	4g
8.	Tetracycline	0.50g

Mix these ingredients in a blender, then steam to prepare a 'custard'. After cooling, grind in the blender. Particles both too large and too fine should be removed by wet screening (see Figure 18 a). Custard particles should be fed manually and feeding behaviour carefully observed (see Figure 18 b).

Fig. 18a Wet sieving **prepared food**

Ag. 18b Monitoring behaviour during hand feeding

Feeding

It is generally not necessary to supply Artemia naupili on the day of hatching. From the 2nd to the 10th day, the density of the Artemia nauplii should be maintained at 5 BSN/ml by adding newly hatched nauplii in the morning and evening. Subsequently, the density of the naupffi may be halved, as prepared feed is given.

The quantity of *Artemia* naupii to be added will depend on the volume of water in the tank and not on the number of larvae. At the rate mentioned above, a 5-*t* tank will require 4.3 kg of *Artemia* cysts for a 50-day rearing cycle. The density of the nauplii should be determined before feeding so that the number given can be adjusted to maintain the desired level.

In the case of prepared feed, the following should be considered:

- The size of the particles should correspond to the size of the fry.
- Overfeeding will pollute the rearing water and may cause mortality of the fry. Underfeeding causes malnutrition and cannibalism, and may effect normal growth.
- Quality and cleanliness of the feed should be checked before feeding.

The best way to give prepared feed is as follows:

- Turn off aeration;
- Hand feed until all larvae are actively feeding;
- Resume aeration.

Careful observation during hand-feeding prevents overfeeding and detection of health problems (see Figure 18 B).

As the age of the larvae increases, the amount of feed should be increased. When prepared feed is first given, at Day 10, every 5-t tank should be. given 15-30 g/tank. Subsequently, the rate may be increased to 100 g/tank/feeding. Completion of the larvae cycle may require 6-8 kg of feed.

From Day 10, particle size may be gradually increased up to 1 mm as the larvae grow.

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Potential problems

Fry mortality may increase due to improper or careless management as well as from disease. Some causes of mortality are:

- Inadequate cleaning of rearing tanks, insufficient change of water, low recirculation rate or carelessness of the operator during siphoning.
- Spoiled food, under or overfeeding.
- Lack of proper observation of the condition of the larvae.
- Extended power cuts, leading to stoppage of water circulation and aeration and disruption of the temperature control system.
- Just before metamorphosis, the larvae tend to jump and die due to striking the tank walls.

Characteristics of healthy larvae

After feeding, observe the behaviour of the larvae regularly. Healthy larvae show the following characteristics:

- They move about in the surface water (particularly during the first ten days).
- They start taking food immediately.
- They look reddish-brown.
- They are not cannibalistic.
- They swim with their heads down, 'jumping' when contacting any surface. Healthy larvae swim actively and do not settle at the bottom of the beaker or tank.

On the other hand, unhealthy larvae

- look bluish;
- often exhibit black spots or irregularities in or on their bodies;
- do not take food;
- settle at the bottom of the tank or beaker; and
- swim in a downward spiral path.

Disease and its prevention

The known diseases of *M. rosenbergii* larvae are caused by bacteria, protozoa and nutritional deficiencies. All the disease-causing organisms are probably present in the rearing water and only affect larvae when they are stressed due to inadequate feeding, overcrowding and poor water quality. These types of infections are termed 'opportunistic'. That is why good **tank management and proper** feeding have been emphasized throughout these pages. "An ounce of prevention is worth a pound of cure!"

The major known diseases and their possible treatment are discussed below. It should be kept in mind that as the causes of some of these diseases are not known, no cure can be specified. Some treatments, however, do appear to work.

Mid-cycle Larvae Disease (MCD)

This disease is the most serious threat to production. It was first reported from Hawaii, but has since been experienced by hatchery operators in many countries. Typically, mortality begins around the end of the first third of the larvae rearing cycle, say Day 10. Mortality increases quickly for 3-5 days, then stops, or dramatically decreases. PL production may be reduced to 1 or 2 PL/l.

The cause of the disease is not known, but it is infectious and has an incubation period of about five days. After mortality has ceased, survival of the remaining larvae is good.

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The symptoms of the disease are spiral swimming behaviour and poor feed consumption. The larvae assume a bluish-grey coloration.

MCD does not respond to any tested antibiotic. The best procedure is to discard the infected larvae and disinfect the affected tank.

If the infection spreads through the hatchery, general disinfectation may be required. Disinfectation requires thorough washing of all tanks, filters and equipment with formalin and/or chlorinated water, followed by drying for at least one week.

Bacterial Necrosis (BN)

Larvae affected by this disease turn bluish and stop feeding. The intestinal tract will be found to be empty. Small black spots and lesions can also be seen on the exoskeleton. Stage 4 or 5 larvae may suffer 100 per cent mortality, but older larvae and PL seem to be resistant.

The disease can be treated with the following antibiotics: bipenicillin-streptomycin @ 2 ppm, Furanace @ 0.1 ppm, or erythromycin phosphate at *0.65-1* ppm. Chloramphenicol may also be effective. Prophylactic treatment consists of giving the above dosages every three days, while daily treatments should be given if there is an outbreak of the disease. However, the prophylactic use of antibiotics is not recommended, as it usually leads to severe, untreatable disease problems later.

Exuvia Entrapment Disease (EED)

EED affects Stage XI larvae and early post-larvae. Infected larvae are unable to extricate themselves from their exuvia during moulting. The larvae generally have malformed appendages. Mortality usually ranges from 20-30 per cent.

It is believed that nutritional deficiencies are the principal cause of EED. For example, larvae of other shrimp species (Paleomon) experienced EED when fed *Artemia* nauplii from the Great Salt Lake, Utah, USA, whereas nauplii from the San Francisco Bay did not produce EED. Adding lecithin to the prepared feed may help to prevent, or reduce, EED.

Microscopic Epibiont Diseases (MED)

A variety of protozoa may be found attached to the exoskeleton of larvae. Some of these are illustrated in Figure 19 (see facing page). Some species may attack the eggs of broodstock. Others interfere with the feeding and moulting of larvae. They can be controlled by formalin treatment. One reason for giving spawners a formalin bath is to prevent the introduction of these ectoparasites into the larvae rearing system.

Filamentous and non-filamentous bacteria may also foul the external surfaces of broodstock, eggs and larvae. Antibiotic treatment may be effective in controlling outbreaks of bacterial fouling.

The use of antibiotic treatments in a recirculation system must be **done with care as many of the** commonly available antibiotics can 'kill' the biofilter so that it is no longer effective in eliminating ammonia and nitrite. Table 4 shows the effect of antibiotics on the nitrification in freshwater aquaria. Care should **be taken in applying these figures to brackishwater,** but they may serve as a guide. Some substances, such as malachite green, are clearly very toxic. Of course, their use in an open system could be freely determined by required therapeutic levels.

Table 4 Effects of antibiotics on nitrification in freshwater aquaria*.

Compound	*Concentration ppm*	*Inhibition (%)*
Chloramphenicol	50	0-84
Oxytetracychne	50	0
Sulfamerazine	50	0
Salfanilamide	25	65
Erythromycin	50	100
Nifurpirinol	1	0
Chlortetracycline	10	76
Formalin	25	0
		27
Malachite green	0.1	0
Methylene blue	5	100
Copper sulphate	1	92
	1	0
	5	0
Potassium permanganate	4	0
	1	86

* From Spotte 1979.

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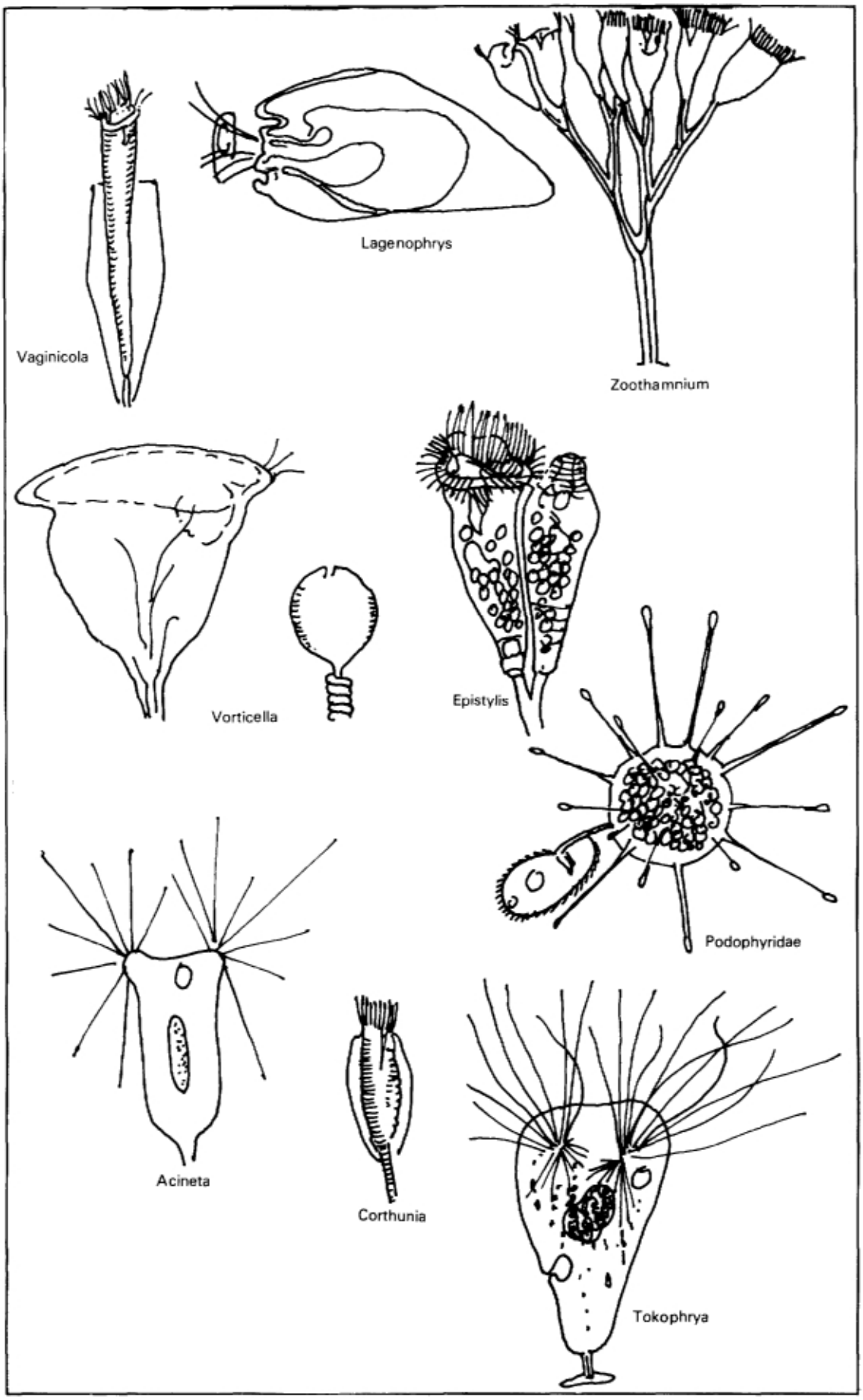
Fig. 19 Illustrations of some epibiont protozoan genera reported from M. rosenbergii.



Harvesting post-larvae

The time taken to complete metamorphosis depends mainly on water temperature and salinity. Given proper management of both, as well as of the supply of feed, it takes 35-40 days for metamorphosis into post-larvae to be completed. Post-larvae look like small prawn and move forward. Instead of swimming in the water, they crawl along the wall or bottom of the tank.

Post-larvae harvesting can begin when about half the larvae have metamorphosed. The following procedure may be used:

1. Turn off aeration.
2. Cover half the rearing tank. Larvae will be attracted to the light; PL will go to the dark side. PL's will settle to the bottom and on the sides of the tank, while larvae remain swimming.
3. After 10-15 minutes, lift up the cover and scoop net the PLs. Transfer the harvested PLs to a 30-litre basin. Since some larvae will also be caught, they should be separated from the PL and returned to the rearing tank. Gently stir the water to create a circular current in the basin. Larvae will concentrate in the centre of the basin and can be easily netted and returned to the rearing tank.
4. After the larvae have been returned to the rearing tanks, the PLs, in the buckets or basins can be counted. The PLs are mixed with a rapid up and down motion of the hand or a plunger, followed by quickly sampling the container with a 250 ml glass beaker. At least three repeat counts should be made; there should not be wide discrepancies between each count.

Acclimatization of post-larvae to freshwater

Post-larvae in 12 ppt saline water may suffer high mortality if stocked immediately in freshwater. Therefore, they should be acclimatized in freshwater before they are transferred to post-larvae holding tanks or sold.

After the post-larvae have been transferred, the basin water should be reduced by about 50 per cent and freshwater gradually mixed with it through a porous hose. In this way, the water level of the basin can be brought up to full in about three hours and the salinity brought down to 6 ppt.

Six hours later, 50 per cent of the water should again be removed and freshwater mixed to raise the water level as before, now bringing down the salinity to 3 ppt. After 2-3 hours more, two-thirds of the water should be siphoned from the basin and refilled with freshwater. The salinity will now be about 1 ppt. After keeping the post-larvae in this water for 2-3 hours, they should be transferred to post-larvae holding tanks. This acclimatization should be done over at least 12 hours.

Another way to reduce salinity is by continuous flushing through the screened stand pipe. The freshwater inflow can be adjusted to bring the salinity down to 0 ppt in 12 hours. **But BEWARE** : Deep well water may be toxic and should be tested by bioassay before the flushing method is used.

Nursing post-larvae

Tanks of 10- 50 m^2 and 1.2 m depth are suitable for post-larvae rearing. However, PLs should be sold as quickly as possible. Mortality increases rapidly after only a few days in crowded holding tanks.

Halfthe water in post-larvae holding tanks should be changed daily. Thesetanks require continuous aeration and should be shaded, either with individual covers or a roof over thetanks. The bottom of the tank should be cleaned of excreta and leftover feed every morning by siphoning. Five thousand PL/m2 may be held for one week. PL density should be reduced to 2000/m2 if they are being held for longer periods. The holding capacity of the PL tanks can be increased by placing additional substrate. Palm fronds and branches may be used. Vertical panels of plastic mosquito mesh are easy to clean and long lasting.

Artemia nauplii are not necessary for post-larvae. Good quality pellets, ground fish and blended bivalve meat may be fed.

PL may also ben ursed in *happas* (mesh bags) suspended in ponds whose water-quality is good. Floating net cages in lakes or slow-moving streams may also be used. The *happas* have to be frequently cleaned. Care should be exercised in controlling the stocking rates. If the PL are held for any length of time, thinning may be required.

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Packing and transportation

Packing density is determined on the basis of the distance to be covered. If travel time is less than an hour, 30,000 fry can be transported in 40 l of aerated water. Five hundred PL of 1 cm may be transported per litre of water in aluminium or earthen pots for 1 ½ hour-journeys. If transport takes more than 16 hours by land, oxygenated plastic bags should be used.

Transport in plastic bags

- Take a 45 x 80 cm bag, put some water in it and examine it to see if there is any leakage. (If there is, discard the bag.)
- The corners of the bag should be tied off with rubber bands to prevent animals getting trapped in them.
- Fill the bag with eight litres of water in which the PL were acclimatized.
- Put in 1000-2000 PL (at a rate of 125-250 PL/l of water).
- Fill two-thirds of the bag with oxygen so that it is fully inflated.
- The top of the bag should now be twisted, bent over, and sealed tightly with rubber bands.
- The inflated bag is then put in a carton after lining the bottom and sides of the box with styrofoam and ice mixed with rice husks. The carton should then be closed and sealed with tape. In this way, fry can be transported for 16-24 hours by land or air. In place of rice husks, wood shavings may also be used (1-1 ½kg/kg of ice). A mixture of salt and ice (50 g/kg of ice) can also be used.

If transportation is by land, battery-operated fans along with ice may be used to keep the temperature low. If the post-larvae are to be transported by air, the carton must be leak-proof. An inner lining of plastic sheet or heavy-duty plastic bag will have to be inserted first.

Causes of transport mortality

- Lack of precautions while packing for transportation.
- Toxic levels of ammonia in warm packing water.
- Careless acclimatization by the pond operator.
- Excessive PL density.
- Faulty packaging.
- Transportation during moulting.

The hatchery operator should take pains to ensure that only healthy PL are sold. Direct sales to farmers are preferable to dealing with middlemen, because it enables direct feedback from the customer. As with any other business, it is the responsibility of the hatchery operator to ensure that the product is of the highest possible quality.

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NOTE:

Apart from these publications, the BOBP has brought out several folders, leaflets, posters etc., as part of its extension activities. These include Post-Harvest Fisheries folders in English and in some South Indian languages on anchovy drying, insulated fish boxes, fish containers, ice boxes the use of ice etc. Several unpublished reports connected with BOBP's activities over the years are also available in its Library.

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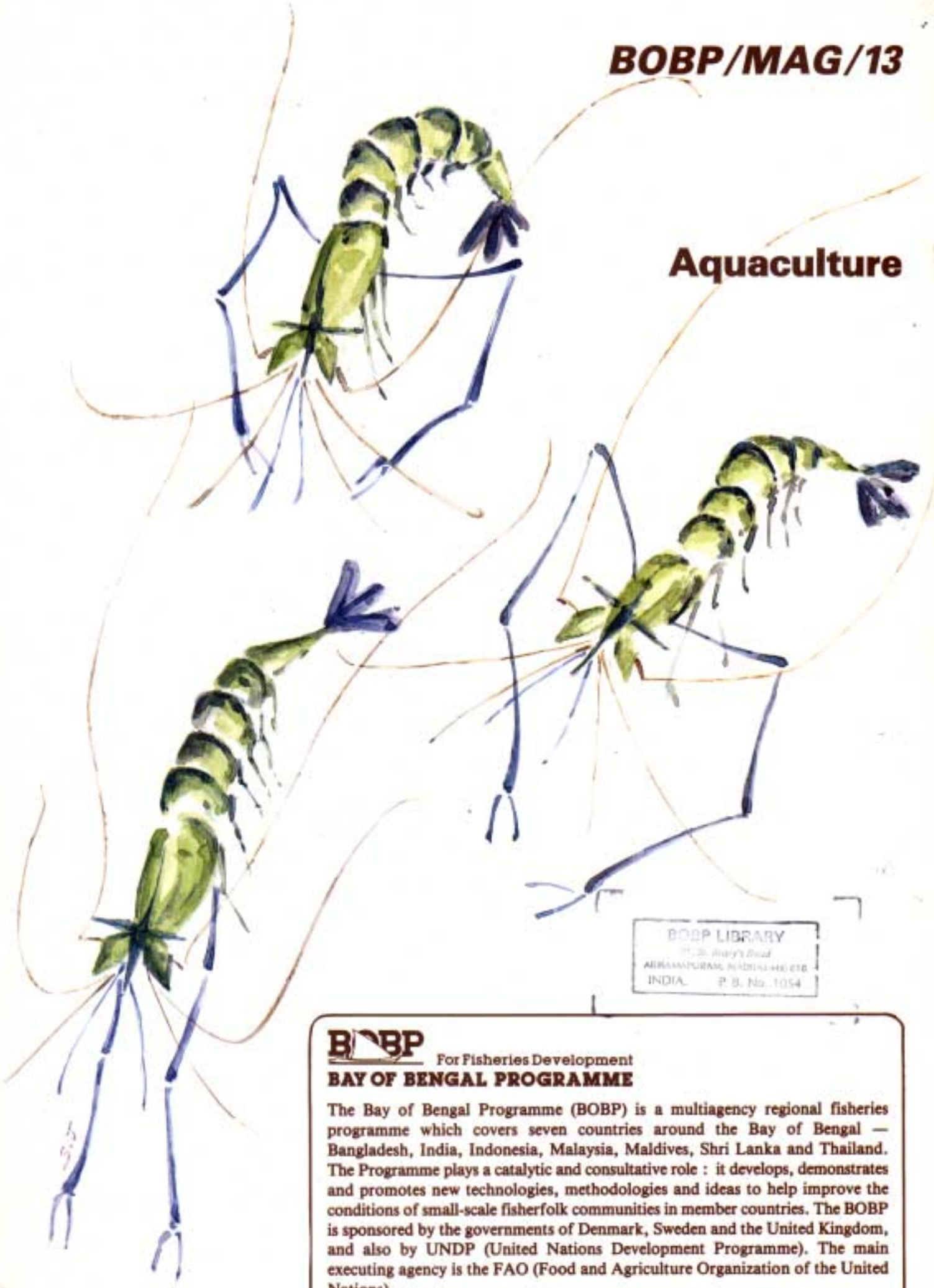

BOBP For Fisheries Development

BAY OF BENGAL PROGRAMME

The Bay of Bengal Programme (BOBP) is a multiagency regional fisheries programme which covers seven countries around the Bay of Bengal — Bangladesh, India, Indonesia, Malaysia, Maldives, Shri Lanka and Thailand. The Programme plays a catalytic and consultative role : it develops, demonstrates and promotes new technologies, methodologies and ideas to help improve the conditions of small-scale fisherfolk communities in member countries. The BOBP is sponsored by the governments of Denmark, Sweden and the United Kingdom, and also by UNDP (United Nations Development Programme). The main executing agency is the FAO (Food and Agriculture Organization of the United Nations).

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