

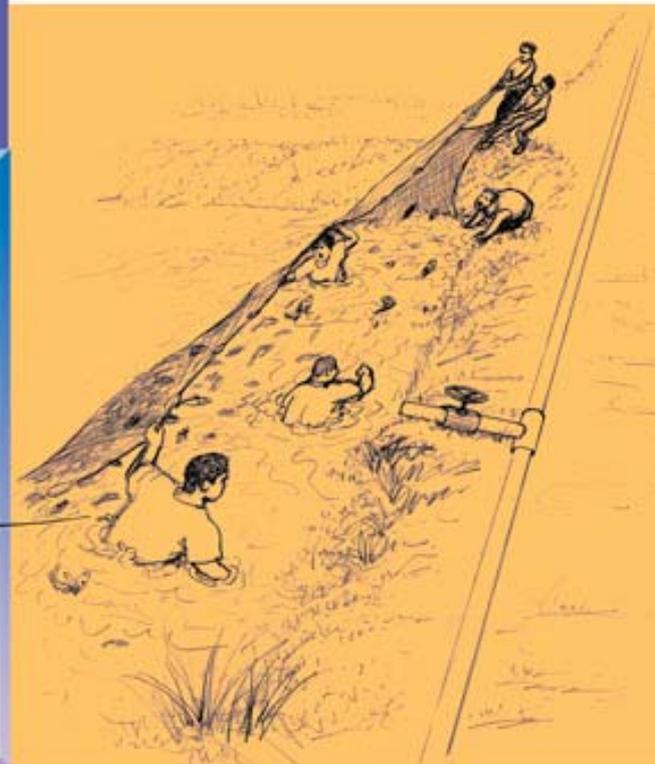
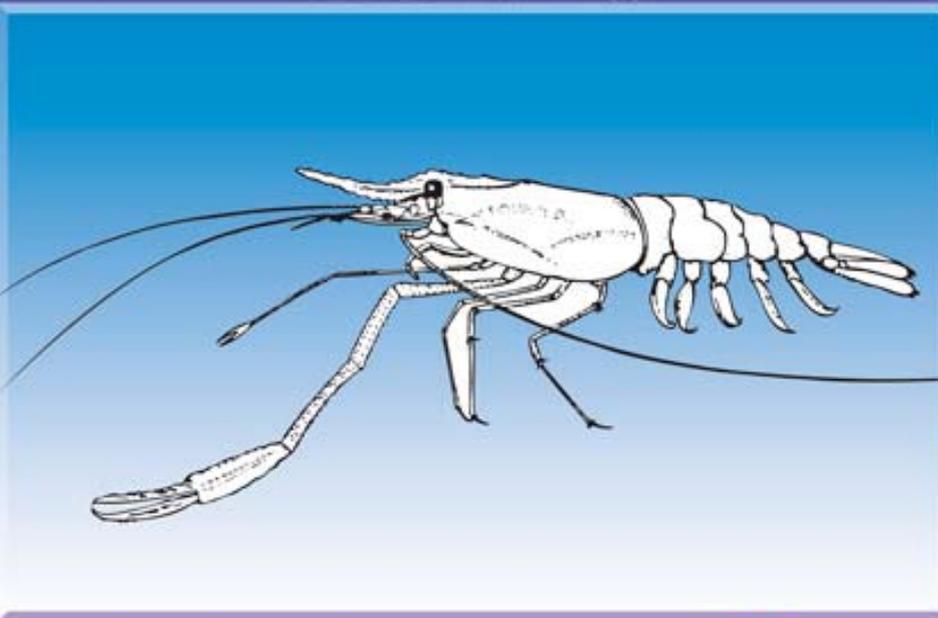
Freshwater prawn *Macrobrachium rosenbergii*  
farming in Pacific Island countries

# Hatchery Operation

Volume: 1

By  
Satya Nandlal

and  
Timothy Pickering





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***Hatchery Operation***

**by**

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Secretariat of the Pacific Community**

**and**

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## Preface

The farming of freshwater prawn is expanding in Fiji and in the Pacific. The grow-out sector of the industry depends entirely on hatchery-produced postlarvae, and the primary problem facing this 'infant' industry is the unavailability of postlarvae. Due to expansion in production and the trend towards intensification of prawn farming, there will be a need for development of more hatcheries for production of postlarvae to meet the demands in the Pacific Island countries.

The book is intended for use by fisheries department aquaculture staff, other NGO officers, and staff of large commercial freshwater prawn farms which have established their own prawn hatcheries. It provides a practical, hands-on guide to the clearwater larvae rearing method to produce *Macrobrachium rosenbergii* postlarvae for stocking into grow-out ponds which is in the Pacific Island countries.

Techniques for grow-out of freshwater prawns are covered in a companion SPC publication, Freshwater prawn *Macrobrachium rosenbergii* farming in Pacific Island countries: Volume two: Grow-out in ponds.

It is assumed that the people using this book already have education to at least to secondary-school biology/or some technical work experience in aquaculture.

This manual was developed from training workshops run in Fiji from 2002 to 2004 jointly by the Institute of Marine Resources of the University of the South Pacific (USP), the Aquaculture Programme of the Secretariat for the Pacific Community (SPC) and Fiji Fisheries Department, with funding provided by the Government of Canada under the Canada-South Pacific Ocean Development Program Phase II (C-SPODP-II). The book's production costs were met by funds provided by AusAID to SPC's Aquaculture Programme.

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## Contents

<b>Introduction</b>	<b>5</b>
<b>Biology and life cycle</b>	<b>5</b>
<b>Distribution</b>	<b>6</b>
<b>Identity and morphology</b>	<b>7</b>
<b>Life cycle</b>	<b>9</b>
<b>Growth and survival</b>	<b>13</b>
<b>Food and feeding habits</b>	<b>13</b>
<b>Prawn hatchery</b>	<b>14</b>
<b>Site for hatchery</b>	<b>14</b>
<b>Water quality</b>	<b>15</b>
<b>Hatchery components</b>	<b>15</b>
<b>Hatchery operation</b>	<b>18</b>
<b>Broodstock</b>	<b>18</b>
<b>Hatch tank management</b>	<b>21</b>
<b>Hatching of larvae</b>	<b>22</b>
<b>Larval culture environment</b>	<b>22</b>
<b>Larval culture cycle</b>	<b>24</b>
<b>Larval diets</b>	<b>25</b>
<b>Larval water quality</b>	<b>30</b>
<b>Larval health</b>	<b>31</b>
<b>Larval rearing: routine tasks</b>	<b>32</b>
<b>The postlarval stage</b>	<b>35</b>
<b>Harvest of postlarvae</b>	<b>36</b>
<b>Packing and transportation of postlarvae</b>	<b>37</b>
<b>Record keeping</b>	<b>38</b>
<b>Further reading</b>	<b>Error! Bookmark not defined.</b>



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## Introduction

Aquaculture of the giant river prawn *Macrobrachium rosenbergii* is an emerging industry in the Pacific Island region. Increasing demand and rising prices for seafood are raising the profile of freshwater prawn as an important aquaculture commodity in Pacific Island countries and territories (PICTs).

Attempts during the last three decades to culture freshwater prawns in Hawaii, French Polynesia and New Caledonia have not had a lasting impact owing to high costs of production in these countries. Fiji has had some success in prawn farming, and Vanuatu and Papua New Guinea are moving in this direction. Other PICTs have been constrained by lack of technical capacity, particularly in hatchery operations.

Freshwater prawn farming is not as highly technical or capital-intensive as aquaculture of sea prawns (penaeid shrimp) and therefore is a more accessible industry for small-scale operators. Freshwater prawns can be raised in farming systems similar to those used for tilapia with and it can be more profitable than tilapia farming (depending upon the farm site and the skill of the farmer, as well as local prices and demand).

The major constraint to expansion of prawn farming in PICTs is difficulty in obtaining reliable supplies of postlarvae (PL), usually referred as 'prawn seed', for stocking into grow-out ponds. This book seeks to address this constraint and is based on experience of prawn hatchery techniques developed at Naduruloulou Aquaculture Station and at Marine Studies Laboratory, the University of the South Pacific, both in Fiji.

## Biology and life cycle

For successful hatchery operation, the technician or operator needs to reproduce the environmental conditions experienced by the prawn in its natural environment. For the proper management of the hatchery systems, the operator needs some knowledge of the biology and life cycle of *Macrobrachium rosenbergii* in order to understand its larval rearing requirements in captivity.

A lack of knowledge on the part of the operator not only is likely to cause difficulties for the smooth operation of the business, affecting the success or profit of the business, but may also damage the reputation of the aquaculture industry in the region.

This section of the manual contains a brief description of the biology and life cycle of *M. rosenbergii*. More detailed information is available in the book: *Freshwater Prawn Culture: The farming of Macrobrachium rosenbergii*, by M.B. New and W.C. Valenti.

## Distribution

The freshwater prawns belong to the genus *Macrobrachium*. There are about 200 species of this genus described in books and various journals. Almost all of these prawns live in freshwater and some spend part of their life in brackish water or seawater in their larval stages of development.

Various *Macrobrachium* species have been introduced to places outside their natural range for use in aquaculture, for example to North and South America and to countries in the Pacific like Fiji, American Samoa, Hawaii, Tahiti, New Caledonia, and even New Zealand. *M. rosenbergii* remains the most common species for farming and thus has been introduced to more countries compared to other species.



## Box 1

### 'Family tree' of giant river prawn *Macrobrachium rosenbergii*

Kingdom	Animalia - animals
Phylum	Arthropoda - insects, spiders, crustaceans etc.
Subphylum	Crustacea - crabs, lobsters, shrimp, etc.
Class	Malacostraca
Order	Decapoda
Sub-order	Pleocyemata
Infraorder	Caridea, sometimes called Natantia
Superfamily	Palaemonoidea
Family	Palaemonidae
Subfamily	Palaemoninae
Genus	<i>Macrobrachium</i>
Species	<i>Macrobrachium rosenbergii</i> – giant river prawn

The freshwater prawn *M. rosenbergii* (Fig. 1) was the first species to be studied extensively and farmed commercially. It is indigenous to South and Southeast Asia, and the northern oceanic and western Pacific islands. The adults of the species are usually found in freshwater bodies such as the lower reaches of rivers and lakes, ditches, canals and pools connected to the sea. It has been transferred extensively within its natural range and has been introduced into many countries where its farming has been established.

*M. rosenbergii* has become the main freshwater prawn species for small-scale and large-scale farming because of its fast growth, large size, better meat quality, omnivorous feeding habit, and established domestic and export markets in Asia. Its farming is well developed in China, India, Thailand, Viet Nam, Bangladesh, Malaysia and Taiwan, as well as Ecuador in South America. There are also capture fisheries for *M. rosenbergii* in India, Viet Nam, Bangladesh, Papua New Guinea and several other countries in Asia.

Another freshwater prawn, *Macrobrachium lar* (monkey river prawn), is also native to many countries in the Pacific Islands region, and also grows to large size. Its natural geographical range is from East Africa to the Marquesas Islands. Techniques to rear larvae of *M. lar* in hatcheries have not yet been developed. However, in some PICTs (for example, Vanuatu and Futuna) growing of wild juveniles in taro swamps is a traditional practice.

## Identity and morphology

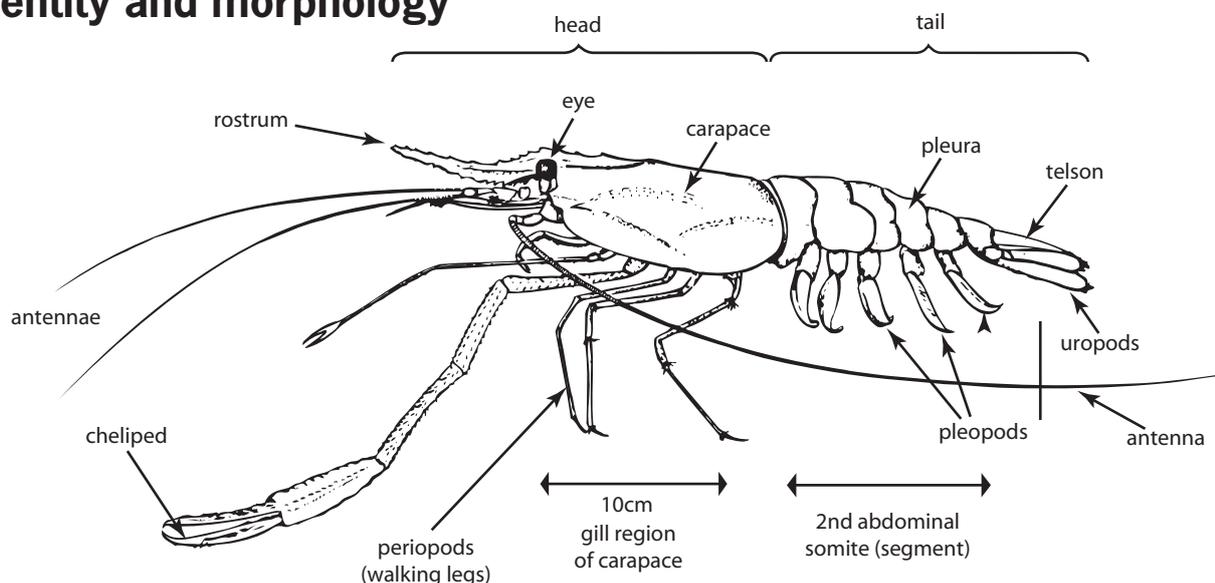


Fig. 1. External anatomy of freshwater prawn *Macrobrachium rosenbergii* (male)



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***M. rosenbergii*** (Fig. 1) is the largest natantian (swimming) prawn in the world and belongs to the family Palaemonidae (refer to Box 1). The adult prawn can easily be identified from other species in the genus by the following characteristics:

- Adult male has a pair of very long legs (chelipeds)
- The rostrum is long and bent in the middle with 11–13 dorsal teeth and 8–10 ventral teeth
- The movable finger of the leg of the adult male is covered by a dense mat of spongy fur
- Distinct black bands on the dorsal side at the junctions of the abdominal segments

The body consists of the head (cephalothorax) and tail (abdomen) and is divided into 20 segments. Of these segments, 14 are in the head and covered by a shield known as the carapace.

The front portion of the head has 6 segments, and features:

- stalked eyes
- first antennae
- second antennae
- mandible, used to grind food
- first maxillae, which transfer food into the mouth
- second maxillae

The rear portion of the head (thorax) has 8 segments, each of which has a pair of appendages:

- 3 sets of maxillipeds (function as mouthparts)
- 5 sets of legs (pereiopods)

The first and second pairs of legs end in claws and are used for capturing and holding food and the others, the third, fourth and fifth, are used for walking.

The tail (abdomen) consists of 6 segments. The first five have a pair of pleopods each, used for swimming. The sixth segment has a pair of pleopods called uropods, and a telson. The prawn moves or jerks backwards using the telson and the uropods.

Male prawns are larger than females of the same age. The male has a head (cephalothorax) proportionally larger than the abdomen, which is narrow, and the chelipeds are long, massive and large. There are three major types of males and a number of intermediate forms:

- blue claw males (BC) have large blue claws. This type are sexually most active.
- small males (SM) have small claws, and are sometimes referred to as runts.
- orange claw males (OC) have light-orange claws, shorter than the claws of BC males.

The female prawn, which is smaller in size than males of the same age, has a smaller head and slender claws. There are three main types of females:

- virgin females (V or VF)
- berried females (BF), which are egg carrying females
- open brood chamber (spent) females

The first three abdominal pleura of the female are elongated and broad, and form a brood chamber for incubating eggs. The genital pores are located at the base of the third walking legs. The reproductive setae (bristles) appear on the thorax and pleopods of mature females. These bristles help to guide and propel the eggs during spawning and to anchor the eggs to the pleopods for incubation.

In males, the internal reproductive structure consists of a testis (actually two testes fused together), the coiled vasa deferentia (sperm ducts) extending as a tube and ending in an ampulla (Fig. 2). The testis is situated dorsally in the carapace and gives rise to the coiled vasa deferentia



which are located anterior to the heart. These extend laterally and open at the base of the fifth pereopods, in an enlarged and partially enclosed space like a box (ampulla). During ejaculation, muscles surrounding the ampulla contract, extruding the sperm into a spermatophore.

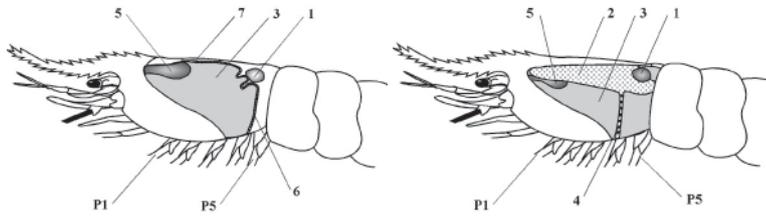


Fig. 2. Internal reproductive structures of *Macrobrachium rosenbergii*. A: male. B: female. P1 to P5 are pereopods

In females, the ovaries are located dorsal to the stomach and hepatopancreas in the carapace cavity (Fig. 2). When the female is in ripe condition the orange coloured ovaries are visible through the carapace, extending from just behind the eyes to the first abdominal segment. An oviduct extends from each ovary (anterior to the heart) backwards to the gonopore of the third pereopod.

## Life cycle

Like all crustaceans, freshwater prawns regularly cast off their exoskeleton in order to grow, a process known as moulting. There are four distinct phases in the prawn life cycle: egg, larva (zoea), postlarva (PL) and adult. The time spent in each phase and its growth rate is affected by the environment, especially water temperature and food. The male and females reach first maturity at about 15–35 g within 4 to 6 months.

## Mating

Ripe females undergo a pre-mating moult and are soft-shelled. Males do not undergo any change before mating and remain hard-shelled. Fertilization is external and takes place soon after the eggs are extruded. During mating, the male deposits the sperm as a gelatinous mass on the ventral thoracic region between the pereopods (walking legs) of the female. The female starts to lay eggs about 5–6 hours after mating. As the eggs extrude, they are fertilized by the sperm attached to the exterior of the female body. The fertilized eggs adhere to the setae of the first four pairs of pleopods.

## Embryonic development

The egg development begins with the successful mating between ripe females and mature males. Incubation of the fertilized eggs takes 18–21 days, depending on the temperature (best results are obtained when the water temperature is 28°–30°C). During this time the berried female aerates the eggs by movement of the pleopods. A 'berried' female is an adult female carrying eggs under its tail. The eggs are slightly elliptical in shape and initially yellowish to bright orange in colour, then gradually change to greyish a few days before hatching. This colour change occurs as the eyes get larger and embryos utilize their food reserves and grow in size.

The number of eggs carried by a female depends on her size, and varies from 3000 to 80,000.

## Larval development

Though the prawn inhabits rivers and estuaries, the adults normally prefer freshwater environments. Thus in the wild, mating and incubation take place in freshwater. The berried females may migrate from freshwater to brackish water regions, where the eggs hatch; or, alternatively, the hatched



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larvae flow down the river to the coastal zone. The larvae complete their development in the estuarine environment. The larvae swim in the water column of estuaries and coastal lagoons as part of the zooplankton community.

The newly hatched larvae require brackish water within 1–2 days, or they will die. They swim actively, upside-down and tail-first with their eyes looking upwards towards the surface. They are attracted to light, and will aggregate together in dense groups at the brightest spots of a tank if the water is still. The larvae eat continuously. In nature their diet is primarily zooplankton and larvae of other aquatic invertebrates.

As the larvae moult, they not only increase in size but also increase in complexity, with new body features appearing at each stage. There are 11 distinct larval stages and it takes about 22–35 days for a larva to complete these 11 stages, to become a postlarva (PL). The change from the larva form to the PL form is called metamorphosis.

In captivity, all the larvae develop at the same pace up to stage IV (synchronous larval development), then the timing of moulting and appearance of the developmental stages differs between individuals until the PL stage.

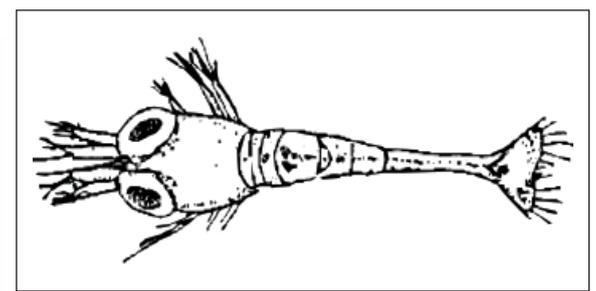
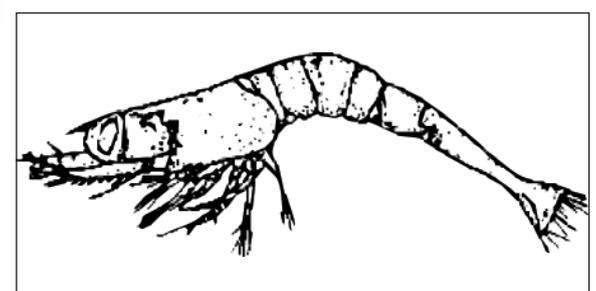
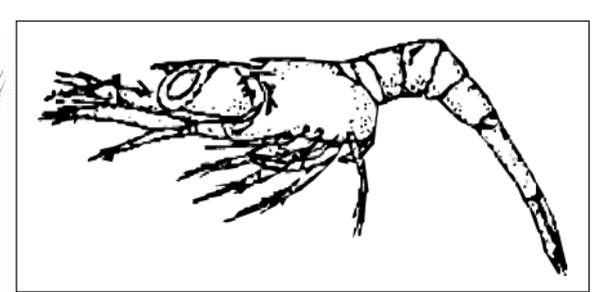
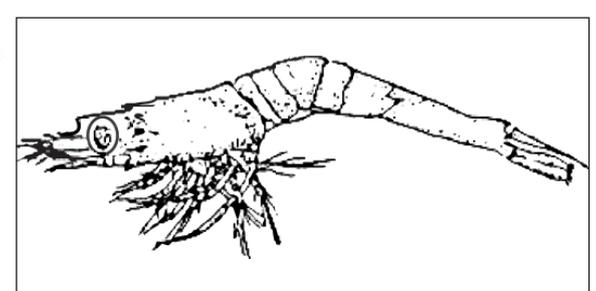
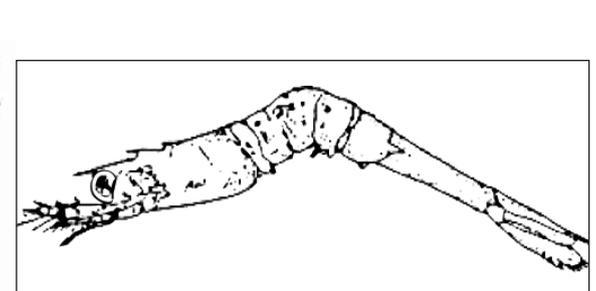
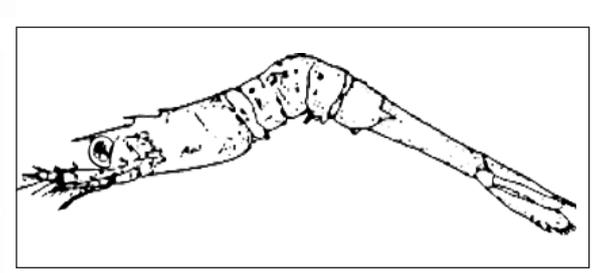
A guide to identification of the larval stages is given in Fig. 3. A typical timeline for these stages, with their recognised characters, is shown in Table 1.

**Table 1. Key for identification of larval stages of *Macrobrachium rosenbergii*.**

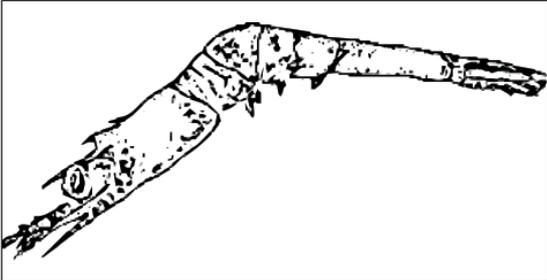
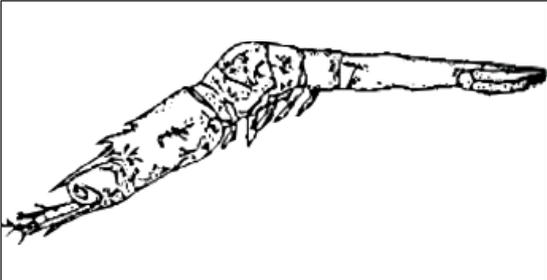
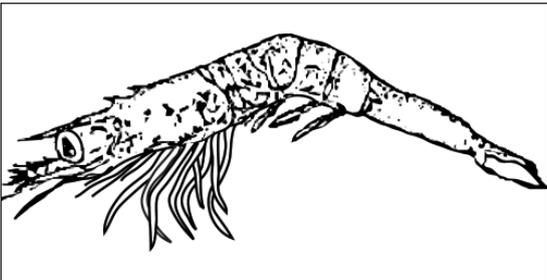
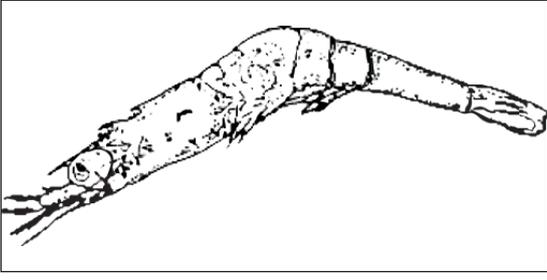
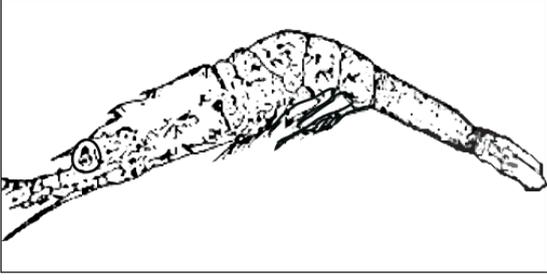
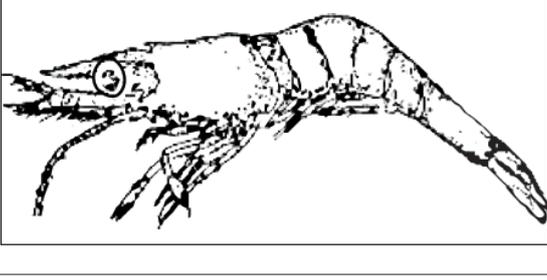
Larval stage	Age (days)	Recognized characters
I	1	Sessile eyes
II	2	Stalked eyes
III	3-4	Uropods present
IV	4-6	2 dorsal teeth
V	5-8	Telson narrows and elongated
VI	7-10	Pleopod buds present
VII	11-17	Pleopods biramous
VIII	13-20	Pleopods with setae
IX	15-22	Endopods of pleopods with appendices internae
X	17-23	3-4 dorsal teeth on rostrum
XI	23-35	Teeth on half of upper dorsal margin
PL	23-35	Adult behaviour



**Fig. 3. A guide to identification of the larval stages of *M. rosenbergii***

Larvae	Stage Age (days)	Characteristics	Pictures
I	1	Sessile eyes	
II	2	Stalked eyes	
III	3-4	Uropods appear	
IV	4-6	Two dorsal teeth on rostrum	
V	5-8	Telson narrower and elongated	
VI	7-10	Pleopod buds appear	



Larvae Stage	Age (days)	Characteristics	Pictures
VII	11-17	Pleopods biramous and bare	
VIII	14-19	Pleopods with setae	
IX	15-22	Endopods of pleopods with appendices internae	
X	17-24	3 - 4 dorsal, teeth on rostrum	
XI	19-26	Teeth on half of upper dorsal margin	
Post Larvae	23-27	Now benthic, swims forwards with dorsal side uppermost. Teeth on upper and lower margin of rostrum (also behavioural changes, mainly in swimming)	



## Postlarva to adult

After metamorphosis, the PL settle to the bottom to become crawlers and like to cling to submerged objects like fine-mesh netting. PL now change to bottom-feeders, and will pick up and eat pieces of clam, snail or squid meat, shrimps and fish flesh, and a variety of formulated pellet feeds.

The PL in the larval rearing tanks need to be slowly acclimated from brackish water (12ppt) back to freshwater, because this is the stage when in nature they would migrate from coastal waters back to rivers and grow into adults (as depicted in Fig. 4).

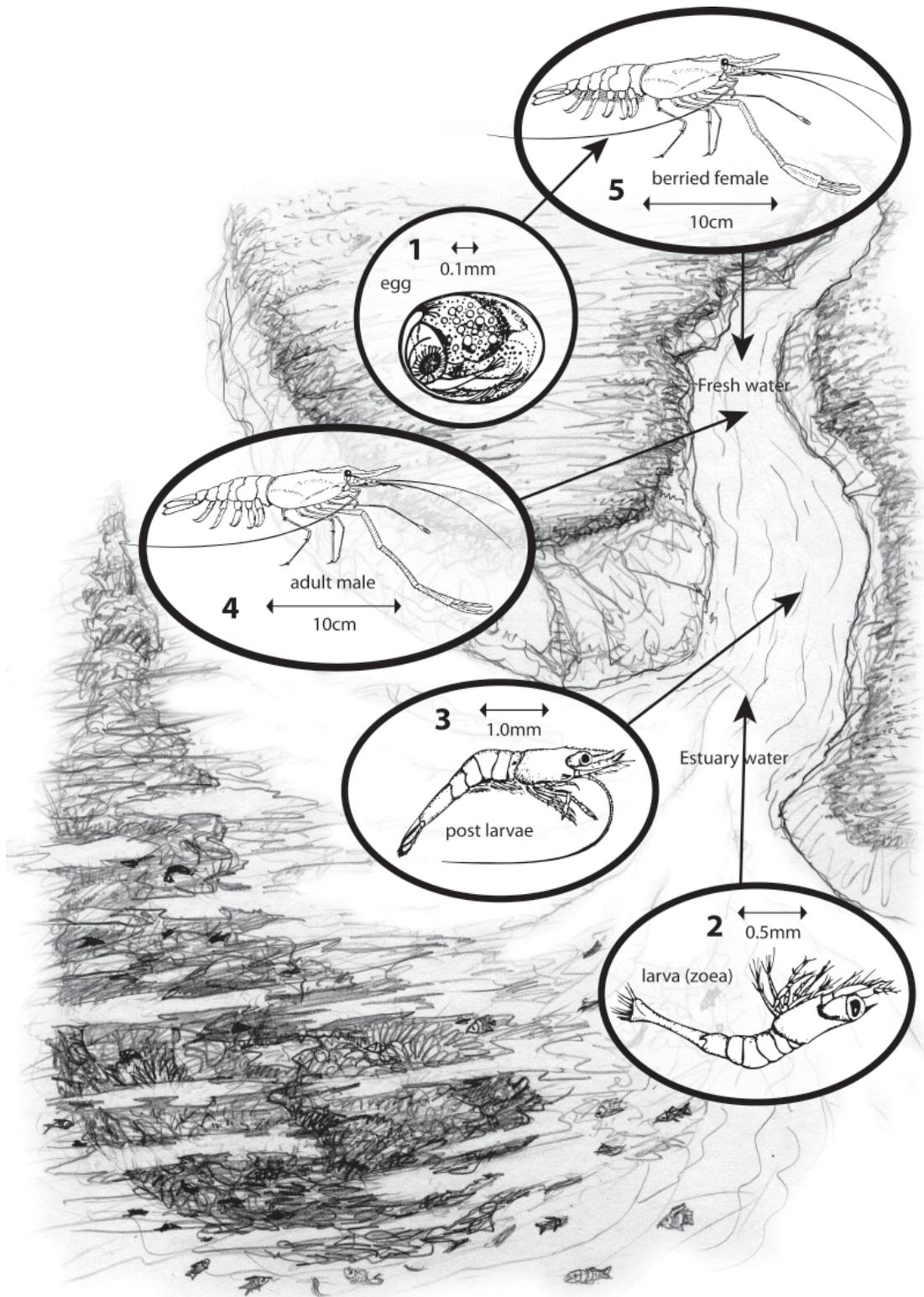


Fig. 4. Life cycle of freshwater prawn



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PL are whitish, grey and brownish in colour and gradually change to light brown and bluish as they grow into juveniles and to adults.

The PL, known as juveniles as they grow older, have five horizontal lines on the carapace, which are characteristic of the species. These lines disappear when they attain the length of 7–9 mm, and a dark belt-like patch develops at each of the junctions of the abdominal segments, which persists in adults. In juveniles, the rostrum does not have a crest and the teeth are arranged compactly without much space in between.

The juveniles and adults are slow moving and hide in shade and under shelter in the shallow areas of rivers, canals and ponds during daytime, but are very active during night-time. Adult males have strong territorial behaviour, and individuals will maintain a clear area around themselves within the radius of the sweep of their antennae into which no other members are allowed.

### **Growth and survival**

In prawns, growth does not appear continuous, because of the hard exoskeleton (shell) covering the body and appendages. From time to time the prawn casts off the old shell leaving a soft skin which allows the animal to swell up, then a new, larger shell hardens to take the place of the old one. Growth is only visible at this time of moulting. The frequency (of moulting) is an indicator of growth rate, and depends on the age of the animal, quality and quantity of food consumed and the environmental conditions.

In captivity in Fiji hatcheries, larvae take 22–25 days after hatching to reach the PL stage. The time varies depending upon water temperature, food quality and availability, water quality, the genetic stock used for breeding and, most importantly, skills of the hatchery operator. Since in nature larval development takes place in brackish water, so in the hatchery the newly hatched larvae must be acclimated into brackish water, failing which they will die, as explained earlier.

Survival from hatching to PL should be 80% or higher but can be as low as 10% if any of the above factors are not suitable.

Growth of PLs is relatively rapid, but is highly variable. Both the males and females grow at similar rates until the onset of sexual maturity (15–35 g), when females begin to divert energy more into egg production and less into growth, so males end up a lot larger than females. Some males will become dominant in a social hierarchy, which leads to reduced growth of other males, the result being wide size variations.

### **Food and feeding habits**

The larvae are carnivorous and in culture they are fed on live, newly hatched brine shrimp (*Artemia salina*). This is supplemented by other high protein, locally obtainable food. In Fiji, for example, the larvae are fed with blended fresh ox liver obtained from butchers or abattoirs, or minced tuna meat. Smaller island countries with no regular beef slaughter may substitute fresh tuna flesh for ox liver. Recipes for other food supplements like egg custard are available but so far these have not been tried much in PICTs.

Juvenile and adult prawns are omnivorous, and feed on a wide variety of food items such as aquatic worms, insects and their larvae, small molluscs and crustaceans, flesh and offal of fish and other animals, grains, nuts, seeds, fruits, algae, tender leaves and stems of aquatic plants. They prefer animal sources of food, and sometimes may even be cannibalistic. They also consume their shells which have been shed off as a result of moulting.

Prawns locate their food mostly by touch with their antennae. When prawns are farmed, food is often not completely eaten immediately because of the prawn's territorial nature, so feeds which last well in the water and maintain an attractive odour are needed.



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## **Prawn hatchery**

A freshwater prawn hatchery produces PL for growing out in ponds and for sale to other prawn grow-out enterprises. The hatchery consists of a building, where prawn larvae are hatched and reared in tanks of water which are set up to imitate the environment of the prawn larvae in the wild, together with outdoor ponds or tanks where prawns for breeding are grown out and maintained.

The hatchery is usually designed to suit the specific site and the techniques which will be employed by the operators, and design will also depend on availability of freshwater and seawater, financial input, climate and PL production requirements.

The hatchery building is usually associated with the nursery tanks and grow-out ponds in terms of water supply and other requirements.

The following account provides basic information on the design, required facilities and operational aspects of a hatchery.

### **Site for hatchery**

Water supply is an important factor in hatchery operations and therefore special attention should be given to water availability at the site chosen. The larval stages of freshwater prawn require brackish water and therefore hatcheries should be sited where both freshwater and seawater are available at reasonable costs.

Ideally a hatchery should be located where both types of water are close by. The alternative is to choose a site with a good freshwater source, because freshwater is needed in greater quantity. In this case, saltwater can be piped from the sea or brought to the hatchery by water-tanker truck. The hatchery site should, where possible, be based away from sources which may pollute the water supply, for example industrial areas, harbours, towns, cities and rubbish dumps.

A feasibility study should be conducted to determine the suitability of the proposed site. An important requirement during this study is a water survey and water analysis. Criteria for site selection must be strictly followed as it is very expensive to relocate hatcheries.

### **Facilities**

Road access, power supply, communication facilities and emergency generator are all essential components to run the equipment and operating systems in the hatchery.

### **Water quality**

Water quality and quantity are critical to the success of a prawn hatchery operation. If water quality is good, then good results can be achieved easily. Freshwater from a river, stream or lake, rainwater, or groundwater can be used. The USP prawn hatchery in Fiji uses treated drinking water from the Suva city supply, after this water has been aerated vigorously for a day or so to drive off dissolved chlorine. Use of treated drinking water is not recommended, however, and pure water from a natural source is much preferred. Hardness (as CaCO<sub>3</sub>) should be in the range 50–100 ppm.

Seawater is needed to mix with the freshwater to produce brackish water for the larvae. Seawater should be clean and free from pollution, collected from open sea or from a well on the sea shore, and preferably pumped through a bed filter. The seawater is disinfected with 10 ppm of calcium hypochlorite and stored with vigorous air bubbling for at least a week before use.



The pH of the water should be in the range 7.0–8.5. Ammonia concentration in the water should not exceed 1.5 ppm of ammonia ion ( $\text{NH}_4^+$ ) and 0.1 ppm of un-ionized ammonia ( $\text{NH}_3$ ).

## Hatchery components

Every hatchery requires site-specific adaptations for optimum production and cost-effectiveness. The layout of a typical small hatchery is given in Fig. 5. Some of the basic hatchery components and equipment are:

- Building to house the larval rearing space
- Hatch tanks 1000 L
- Larval rearing tanks (LRTs)
- Holding tanks 1000 L for PL, also used for broodstock holding
- Nursery tank 5000 L for PL (optional)
- Brine shrimp hatch tanks
- Water (bio-filters)
- Freshwater storage tank
- Saltwater storage tank
- Mixed water storage tank
- Water mixing tank
- Air blower (1 HP), air tubes and air stones
- Immersion heaters
- Generator (2 kVA) with auto-changeover switch
- Water pump
- Domestic refrigerator and freezer
- Plastic buckets, basins, containers
- Siphons and hoses
- Microscope, refractometer, pH meter, dissolved oxygen meter
- Equipment for packing and transport of PL
- Feed and chemicals like formalin and chlorine, with storage facilities for these
- Ponds (200–400 m<sup>2</sup>) for rearing and maintaining adult prawns for breeding
- Equipment for pond culture as described in manual *Freshwater prawn Macrobrachium rosenbergii farming in Pacific Island countries: Volume two: Grow-out in ponds*

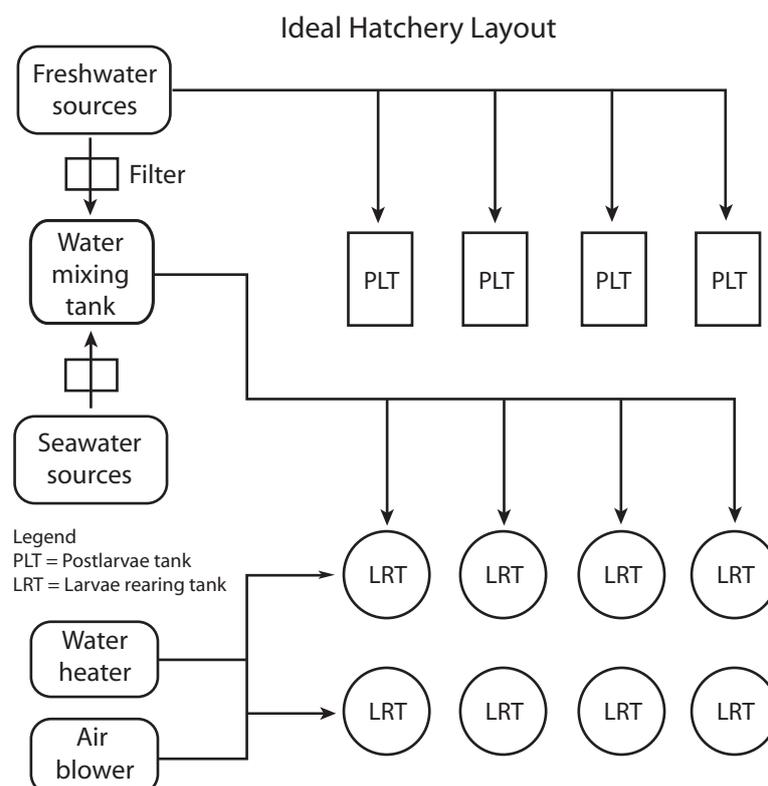


Fig. 5. Ideal layout of a semi-commercial/back-yard freshwater prawn hatchery



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## Hatchery building

The size of the building depends on the intended production capacity of the hatchery. The roof should be covered with corrugated iron sheets with some translucent sheets to allow for enough sunlight. The LRTs are placed such that sunlight falls directly on them.

## Water storage tanks

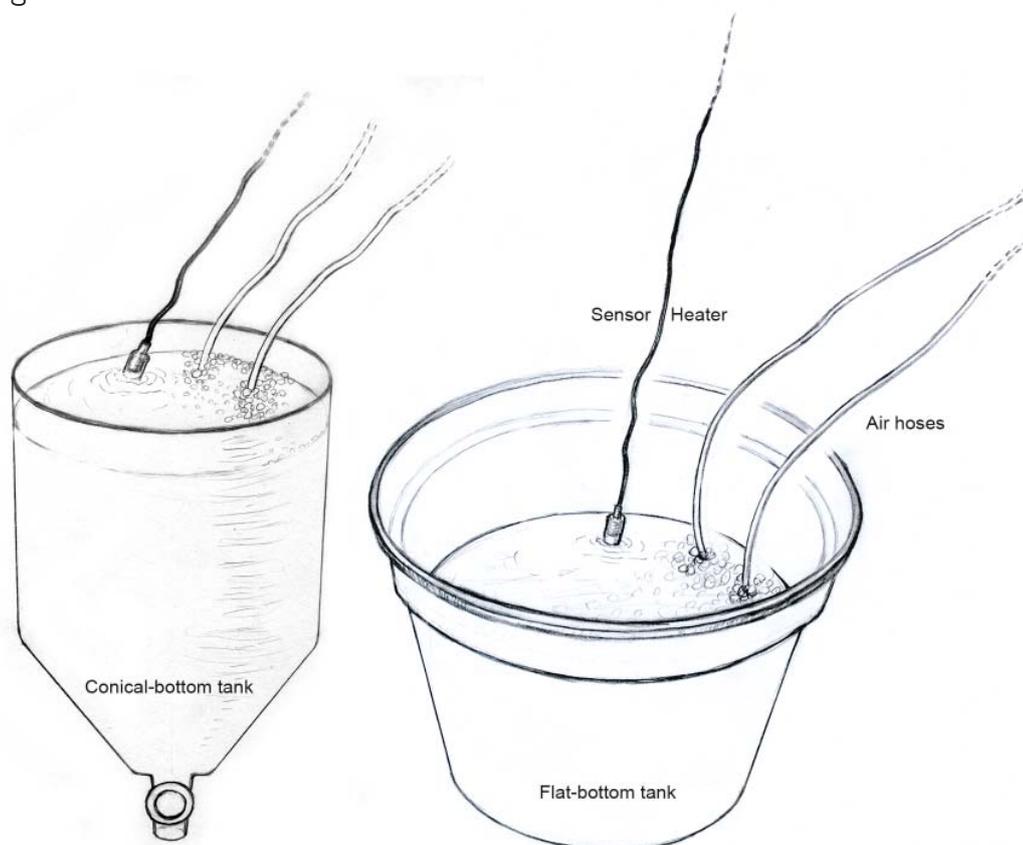
Three ferro-cement or FRP (fibreglass reinforced plastic) tanks each with 5–10 tonne capacity are required for storing freshwater, seawater and mixed water. These tanks should be constructed or placed at a higher level than the LRTs to allow gravity flow of water into the hatchery. It is strongly recommended that both the freshwater and seawater are filtered to at least 5 micron. If possible, the temperature of the stored water should be maintained in the range of 28°–30°C.

## Larval rearing tanks (LRTs)

A range of tank types are suitable for use as LRTs. Some hatcheries use the same type of LRT throughout the larval culture cycle. Others use a two-phase larval rearing system, using conical-bottom tanks for early larval stages followed by larger tanks with flat or U-shaped bottom for later stages.

Conical-bottom tanks are more efficient at the beginning of the culture cycle for maintaining food circulation thus minimizing the need for water exchange. The best are purpose-built fibreglass or plastic cylindrical tanks of 500–1000 L with conical bottoms and a bottom drain valve (Fig. 6, left). This type is the easiest to keep clean, but is more expensive.

Later-stage larvae (stages IV or V) are moved from the conical tanks to 1000 L, flat-bottom tanks. These tanks have greater bottom surface area than the conical tanks and are thus more suitable for the settling PL.



*Fig. 6. Left: Conical bottom larval rearing tank. Right: Flat-bottom larval rearing tank (showing aeration lines and two immersion heater with sensor).*



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Alternatively, flat-bottom or U-shaped bottom, round or even square tanks in fibreglass or PVC plastic may be used (Fig. 6, right) for the whole larval rearing period. Cement tanks of suitable design can be used but must be aged sufficiently to reduce poison in the cement.

### **PL holding tanks, hatch tanks**

FRP or ferro-cement tanks of 1000 L are required for holding PL. These tanks are also used for holding berried females when they are brought into the hatchery, and for hatch tanks.

All the tanks should be cleaned properly and are stored in a dry place when not in use. They should be disinfected with chlorine or hypochlorite bleach and rinsed and dried in the sun before use.

### **Nursery tank**

A large nursery tank with large bottom surface is desirable for holding PL to further increase in size and hardness before transfer to grow-out ponds. A rectangular flat-bottom 2000–5000 L tank is suitable.

### **Generators and other equipment**

Power is required for lighting, heating water, aerators and water pumps. A back-up generator is needed to keep this equipment running in case of failure of mains power. A number of other miscellaneous items like freezer, plastic ware, seine nets, chemicals, refractometer, pH meter, dissolved oxygen meter and siphon are also required.

### **Broodstock tanks or ponds**

Normally a hatchery is associated with a commercial prawn grow-out operation. The hatchery broodstock are grown and maintained in ponds, as described in *Freshwater prawn *Macrobrachium rosenbergii* farming in Pacific Island countries: Volume two: Grow-out in ponds*. Some hatcheries rear their broodstock in nursery tanks, although pond culture normally gives better results.

### **Hatchery operation**

There are two main hatchery systems: flow-through and recirculation. There are two types of flow-through system: clearwater and greenwater. This manual describes a simplified version of the flow-through system with clearwater management, which was developed at Naduruloulou Aquaculture Station and at USP Marine Studies Laboratory.

### **Broodstock**

The adult male and female prawns chosen for breeding are called broodstock. Some people use the term broodstock only for berried females netted from the broodstock ponds.

In our tropical and sub-tropical climates the prawns will breed year-round. The availability of berried females at all times is an important factor in hatchery management. To ensure that sufficient numbers of berried females are available at any one time with eggs which are ready to hatch at about the same time (synchronous hatching), a large number of broodstock females need to be maintained. On average, 500–1000 prawns (male and female) need to be kept as broodstock to ensure that at any one time there are 15–20 females with eggs ready to hatch.

The number of larvae needed will depend upon the PL requirement, with allowance for typical larval mortality in culture. As a rough guide, 1 g weight of berried female produces 1,000 larvae. Berried females 10–12 cm long usually carry about 10,000–30,000 eggs each.



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In Fiji, broodstock are usually reared in ponds, starting with an initial stocking density of 4–5 PL/m<sup>2</sup> of pond area and reducing to 2/m<sup>2</sup> at adult size.

When required berried females are caught and brought to the hatchery. Broodstock maintained in ponds, rather than in tanks, usually give better hatching rate (at low stocking density with appropriate management) and good healthy larvae.

### **Growth to maturity**

The PL grow to maturity within 4–7 months in freshwater ponds, depending on temperature, food and environmental conditions. The maturity stages of females can be determined by external examination of the ovary, as follows:

- Stage 1 *Immature/resting* (neuter period). Ovary is tiny, transparent, confined to the posterior-most region of the carapace cavity
- Stage 2 *Early maturing*. Ovary is yellow and occupies about a quarter to half of the length of the carapace cavity
- Stage 3 *Maturing*. Ovary occupies more than three fourths of the length of the carapace cavity and is light orange in colour
- Stage 4 *Ripe*. Ovary occupies entire carapace cavity and is dark orange in colour

### **Selection of broodstock**

If broodstock are stocked into a pond as adults, they should be stocked at a density of 2/m<sup>2</sup> of pond area, in the ratio of one male to four females.

When selecting prawns to use as broodstock, the most active animals should be chosen. For males, the majority selected should be BC, although some OC and SM should be selected to ensure that there are some less developed males (OC and SM) which are able to develop to replace losses of reproductively active BC males. The largest (that is, fastest growing) females should be selected.

### **Feeding broodstock**

The stocking density in the broodstock pond is kept low (2 prawns/m<sup>2</sup>). This reduces stress from crowding, and increases a prawn's chances of getting the natural food in the pond, thus enhancing the quality of its nutrition.

It is essential to feed broodstock with a nutritionally complete diet, preferably with 40% crude protein, as this will promote proper egg development leading to good egg yolk quality and hence good embryo development and healthy larvae. In Fiji, broodstock are fed with pelleted diets formulated for tilapia (containing 29% crude protein) or commercial penaeid shrimp feeds. The broodstock should be fed once a day, usually in the evening.

An example of a pellet feed formulated for broodstock feeding should roughly consist of:

Protein	40%
Fat	10%
Carbohydrate	33%
Ash	9%
Fibre	8%
Gross energy	4.3 kcal/g (18 kJ/g)

The ingredients of the pellet feed should consist of copra meal, fish meal, meat–bone meal, rice pollard or wheat bran, vitamins and mineral mix.

If pelletised feed is not available, a variety of feeds of plant and animal origin can be used to feed the prawns.

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## **Selection of berried females**

The prawns are netted when required from the broodstock pond by seine or cast-net and berried females are selected. As eggs get near to hatching, the colour changes from bright orange to brownish and greyish. Always choose females with greyish coloured eggs as these will hatch within 1–3 days time. (Females with orange eggs should be maintained at 0 ppt until the eggs start turning grey.)

Care should be taken to select large females which are healthy and active, with ripe egg clutches (without damage) firmly attached to the underside. Berried females with eggs which are damaged or appear like loose gelatinous mass should be discarded.

Large females carry more eggs. One large female can provide enough larvae for one LRT and, when hatched, the larval batch will be of same age. A larval batch of different ages in an LRT will pose problems in properly maintaining them. For example, it is cumbersome to adjust feed sizes to suit larvae hatched over a period of 4–6 days (different ages).

As an alternative to broodstock mating in ponds, unberried females can be put with males in tanks (2000–5000 L size) in the hatchery for mating. The largest blue claw males should be selected and held in the tanks at 1:4–1:6 ratio with healthy and active females. Broodstock held in a tank are fed a clean and nutritious food like squid, *Anadara* marine clams (kaikoso) or *Batissa violacea* freshwater clams (kai). The water must be kept clean through regular changes.

## **Transport of broodstock to hatchery**

If the broodstock pond is close to the hatchery, berried females can be transported in buckets of water to the hatchery. For journeys of 2–3 hours, broodstock can be transported in open containers of pond water, with air bubbled through the water by an aerator powered by a car battery or torch batteries.

Long-distance transportation involving 12–15 hours or more is usually done in double polyethylene (plastic) bags with oxygen added, at 2–5 prawns per bag. The bags should be placed inside insulated containers to avoid temperature fluctuations and movement. The temperature should be maintained at 27°–30°C. The rostrum of each prawn should be blunted with a scissors or inserted into pieces of styrofoam to prevent the bags being punctured.

## **Handling berried broodstock**

Berried prawns should be handled with care to prevent egg damage which would lead to shedding of the eggs. Do not use scoop nets as females struggle in the net. The eggs will get damaged when in contact with the mesh, resulting in loss of eggs. When transferring berried females to treatment tanks or LRT, catch the prawns underwater using a 2 L glass beaker or a bucket, and keep them immersed in water as they are being carried about.

## **Disinfecting berried females**

The berried females should be treated (or disinfected) before they are put into the hatch tanks. They are held in a container of aerated freshwater with 0.2–0.3 ppm copper sulphate or 20–30 ppm formalin solution for 30–60 minutes, to inhibit fungus like *Lagenidium* or fouling zooplankton like *Zoothamnion* or *Epistylis* from growing on the egg mass. If not controlled by chemical dips like formalin or copper sulphate, these organisms can infect the larvae once hatched and cause heavy larval mortality. (Mixing the disinfectant solution is described later, in Box 6.)



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## Hatch tank management

Whether the berried females were obtained from a tank or from a pond, it is best to hold them in hatch tanks. Berried females ready for spawning should not be disturbed and should be kept secluded in the hatch tanks. They should be covered by black plastic sheeting to reduce light and provide a sense of security to the prawns.

Start with 500 L freshwater in a 1000 L hatch tank, and stock a maximum of 3–4 berried females. Keep the temperature at 25°–30°C and pH 7.0–7.3 until the eggs hatch. Temperatures below 25°C promote fungal growth on the egg mass, and above 30°C may lead to protozoa infection.

Tank water should be kept clean and free of dirt and debris through regular water changes and bottom-siphoning with a hose of 2 cm diameter ( $\frac{3}{4}$  inch). Gentle aeration should be provided. Small amounts of food like pieces of squid or clam should be fed to females once a day. If feed is not given, in some instances females pick at their own egg mass. However, the top priority for management of hatch tanks is water quality and not food availability.

## Hatching of larvae

When hatching has occurred, thousands of tiny black dots will be seen at the surface of the water. These are the newly hatched larvae. Once the eggs have hatched, remove the females from the tank with a coarse dip-net and transfer them back to the broodstock holding tank. The spent females are returned to the broodstock pond or in some cases discarded.

Once the eggs have hatched and the mother prawns have been removed, increase the salinity of the water in the hatch tank by 3 ppt per day until 12 ppt is reached. The water is topped up daily with mixed water to achieve the desired salinity (see fig.11).

In Fiji, LRTs are used for the hatching and the larvae are then reared in the same tank. The grey-coloured egg-bearing females are disinfected before being put into LRTs with water at a salinity 0 ppt. Immediately after the disinfection treatment they are acclimated from 0 ppt to 3 ppt in the LRT. Once the eggs are completely hatched, the female is removed from the LRT, leaving the larvae in the LRT. The salinity is increased gradually over the next 1–2 days to 12 ppt, in 3 ppt steps which are at least 2–3 hours apart.

## Larval culture environment

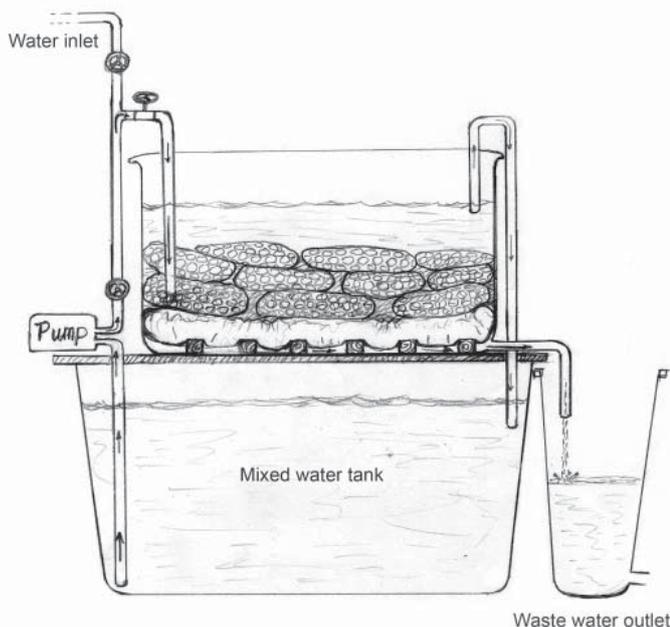
In the wild, prawn larvae live at very low density in open waters of high water quality. In the hatchery, they are held at high density into an enclosed space (the LRT) with hard sides and bottom, and fed on foods which quickly rot and break down in the water. It is therefore important to provide water of good quality at the optimum temperature and nutritious food of the right size, and to remove uneaten food daily so it does not foul the water. Every day the LRTs must be cleaned and some of the water drained off and replaced.

### **Preparation of rearing water**

The water for the larval rearing tanks is prepared by mixing freshwater and seawater together in the mixing tank (Fig. 7) to achieve a salinity of 12 ppt (measured with a refractometer). Since the salinity of seawater is normally about 30–35 ppt and freshwater is 0 ppt, the mixture is roughly 1 part seawater to 2 parts freshwater. Final adjustments can be made using a salinity meter to check for the correct salinity value.

Water used in the mixing tank should be filtered to at least 5 micron. This can be done using a filter-tank (Fig. 7) or an in-line cartridge filter (Fig. 8).





*Fig. 7. Biological filter tank*



*Fig. 8. Bio-filter (with bio-filter cartridge shown separately)*

The water in the mixing tank water should be warmed to 28°–30°C, using an immersion heater (glass or titanium type electric heater with thermostat).

The following factors need to be monitored and controlled to obtain good results in the hatchery.

### **Water temperature**

Temperature regulates growth, and determines the rate of larval development and thus the length of the larval production cycle. The optimum temperature for freshwater prawn larvae is 29°–30°C. Larval growth and survival will be poor below 25°C. A temperature above 33°C is lethal to prawn larvae.

Even when temperature is in the optimum range, any sudden change of temperature by more than 0.5°C will have an adverse effect on prawn larvae.

### **Salinity**

Though larvae can withstand a wide range of salinity (8–18 ppt) best results have been obtained at 12 ppt. Sudden salinity variations of no more than 2 ppt are not detrimental to prawn larvae. However, there should not be any sudden wide variations in salinity while changing water in the LRTs. This can be avoided by adding water from the mixing tank which is ready-mixed at 12 ppt.

### **Dissolved oxygen**

The dissolved oxygen (DO) level should be maintained at close to saturation level throughout the larval rearing cycle. Continuous aeration of the water will maintain sufficient oxygen concentration and help to expel ammonia. Aeration also keeps larvae evenly dispersed and suspended in the water column. As a precaution against air failure, always use two air stones in each LRT.

### **pH and ammonia concentration**

Ammonia (NH<sub>3</sub>) is toxic to prawn larvae. Ammonia concentration in water should not exceed 1.5 ppm of ammonia ion (NH<sub>4</sub><sup>+</sup>) and 0.1 ppm of un-ionized ammonia (NH<sub>3</sub>). The amount of ammonia in the larval rearing water will rise when the pH is above 8.

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Provided the pH of the water source was checked when the hatchery was first built, it is not usually necessary to measure pH or ammonia concentration in the LRTs on a daily basis, as clean water is added each day.

## Larval culture cycle

### **Stocking LRTs with larvae**

After the larvae hatch, they are transferred from the hatch tanks to LRTs by ladling them across in buckets or beakers. If they hatched in freshwater they need to be acclimated by adding mixed water, as already described.

Larvae should be stocked in the LRT at 75–80/L with a maximum of 90/L. If you have more than this, thin them out by moving some to another LRT. If you have less, consider combining two LRTs of larvae into one.

When conical-bottom LRTs are used, at first the larvae are reared at high densities of 90–100/L, and when the larvae reach stages IV or V they are moved to larger tanks at 50–80/L.

To estimate the number of larvae in the tank, count larvae in samples of water from the tank and calculate the total number as described in Box 2.

It is important to know the number of larvae stocked (initially and also during rearing) to ensure:

- LRT is stocked at the recommended larval stocking density
- There are sufficient larvae in the hatchery to meet the PL production targets
- You can control the feeding, knowing the number of larvae in the tank
- The survival rate can be calculated

### **Box 2**

#### **How to estimate the number of larvae in a 500 L LRT**

You cannot count the number of larvae in a 500 L tank — there are simply too many. But the number of larvae can be estimated by counting the number of larvae in a sample of the water.

Make sure there is vigorous aeration of the LRT water so that larvae are all evenly dispersed. Scoop a sample of water of known volume using a beaker. Experiment with different sizes of beakers until you are collecting an easily countable number of larvae (10–30 larvae/50 ml beaker gives good results).

Slowly pour the water out of the beaker back into the LRT, counting the larvae as they pass over the spout. Do this 10 times, and take an average by adding the 10 counts together then dividing by 10.

Then work out the total number in the tank as in the following example.

For example, if the volume of your sample is 50 ml, the volume of the LRT is 500 L, and the average sample count is 4 larvae/50 ml, then:

$$500 \text{ L} \times 1000 \text{ ml}/50 \text{ ml} \times 4 \text{ larvae} = 500 \times 20 \times 4 = 40,000 \text{ larvae}$$

In this example, there are approximately 40,000 larvae in the 500 L LRT, giving a density per litre of 40,000/500, or about 80 larvae/L.



## Larval diets

Newly hatched, live *Artemia* brine shrimp are the main food given to prawn larvae. Brine shrimp are fed to the larvae throughout the larval culture cycle. Brine shrimp are aquaculture's 'fast food', because they do not need to be cultured until you are ready to use them. This is very convenient for a hatchery operator. The method to hatch and prepare live brine shrimp is provided in Box 3.

Brine shrimp eggs are expensive and labour-intensive to prepare. Much work has been done over the years to develop cheaper substitute feeds. Recipes for feeds like egg custard, ox liver, etc. are available in the scientific literature. Zooplankton (e.g. *Moina*, *Acetes*), *Tubifex* worms, mussel (*Lamellidens* spp. or *Batissa violacea*), ox liver, and prepared feeds have all been successfully used. Currently, Fiji prawn hatcheries rely on live brine shrimp as the main feed and use ox liver as a supplementary feed.

The larvae begin grabbing and eating ox liver at stage III and, even though their guts are poorly developed at this age for digesting ox liver, there are nutritional benefits in introducing it as a dietary supplement as early as stage III.

### Box 3a

#### Production of live brine shrimp

Brine shrimp (*Artemia salina*) are small shrimps which in nature live in very salty lakes. When these lakes dry up, the shrimp lay eggs which form cysts with a hard outer shell. These cysts are collected, and can be bought from mail-order aquarium suppliers. The cysts can last up to 2 years if kept cool (in the refrigerator), dark and dry. On exposure to water and to light, they will begin to hatch out.

#### Brine shrimp hatch tank

You will need three or four suitable tanks for hatching brine shrimp cysts. For large-scale production these may be conical fibreglass tanks, usually 200–500 L, with a transparent cone at the bottom. For smaller-scale production, hatch tanks may be made of glass in a four-sided pyramid shape (Fig. 9).

Brine shrimp can also be hatched in 25 L polythene bags. Bags can be cut from 460 mm wide clear polythene film tube (wall thickness 125 µm), and heat-sealed at the bottom at a 30° angle. The bag can be suspended from a stand by an aluminium clip. A 5 cm slit cut vertically at the top front of the bag allows water and cysts to be added to the bag.

You will also need an airline and air stone to aerate each hatch tank, a fluorescent tube light, and an immersion water heater.

Brine shrimp egg cysts have a hard outer shell called the chorion. Although cysts can be hatched complete with the shell ('undecapsulated'), the chorion harbours bacteria so unhatched cysts and empty shells can foul the brine shrimp cultures. A better way is to remove the shell first, by a step called decapsulation.

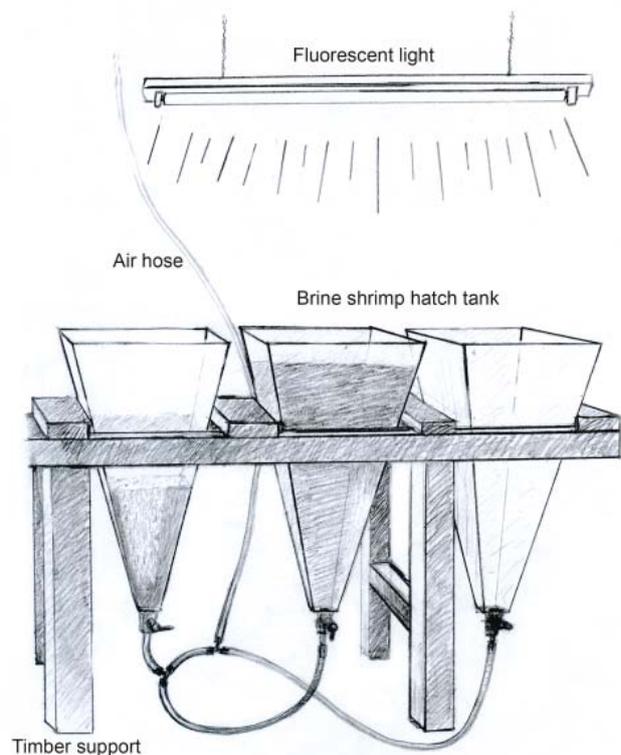


Fig. 9. *Artemia* brine shrimp hatch tank



### **Making the decapsulating solution**

Dissolve 15 g (dry weight) of sodium hydroxide in 900 ml of freshwater, and add 500 ml of sodium hypochlorite solution with 5% available chlorine (commercial bleach like Chlorodu or Janola). This makes enough decapsulating solution for 100 g of cysts (dry weight).

### **Decapsulation**

First, soak the cysts in freshwater at 25°C for 2 hours, keeping the water stirred continuously by vigorous aeration.

Drain the rehydrated cysts onto a 120 µm mesh screen, then place them in freshly made decapsulation solution in a glass beaker, and stir for 5–15 minutes. The cysts change colour from dark brown through whitish-grey to bright orange as the chorion dissolves away. The treatment is completed when all cysts are bright orange. Check the temperature of the suspension repeatedly with a thermometer, as the chemical reaction will heat the water. Temperature should not be allowed to exceed 30°C. If necessary the beaker may be placed in a tray of cold water with ice to cool it down.

When decapsulation is complete collect the cysts on a 120 µm screen and wash repeatedly with freshwater. A few drops of ammonium hydroxide solution can be added to the wash water to neutralize any remaining chlorine.

The cysts can then be hatched immediately, or stored in a beaker of seawater in a refrigerator for up to one week. For convenience 100–150 g of cysts can be decapsulated and stored and about 25 g can be hatched every 2–3 days for feeding.

### **Box 3b**

### **Hatching**

Follow the instructions on the package label (if there are any). The usual procedure is as follows.

Fill the hatch tank halfway with freshwater. The water in the hatch tank is aerated to maintain oxygen levels above 2 mg/L and illuminated by a fluorescent light. Add the cysts and allow to soak for 30 minutes. Then add an equal amount of seawater.

The water temperature should be 25°–30°C. Water temperatures below 25°C reduce the hatch rate and increase the hatching time. Temperatures above 33°C can be lethal to developing brine shrimp.

The cysts hatch within 24–48 hours depending on temperature. Typical hatch rates should be about 75%, depending upon the grade of cysts and on how good your hatching technique is (see Box 4).

### **Collection**

Stop the aeration or remove the air stone. Allow the brine shrimp to settle (5 minutes). Because the brine shrimp tend to swim towards light, they can be encouraged to swim towards the transparent bottom portion of the tank by putting a dark lid on the top of the tank. The empty egg cases and undecapsulated cysts tend to float, so by opening the bottom drain valve or by siphoning from the bottom of the tank the hatched brine shrimp only can be taken. Collect the brine shrimp by filtering the water through a 120 µm mesh screen.



Wash the brine shrimp on the collection screen under tap water, then add them to a bucket of LRT water from the mixing tank. Keep the hatched brine shrimp in the refrigerator until they are used for feeding.

#### Box 4

##### **Measuring the brine shrimp hatch rate**

Before hatching, take four 1 ml samples of the water containing the cysts, and count the cysts in each sample under the microscope using a counting tray. After hatching, take another four 1 ml samples and count the hatched brine shrimp. Calculate the hatch rate, as follows:

$$\% \text{ hatch rate} = \frac{\text{Sum of 4 brine shrimp counts} \times 100}{\text{Sum of 4 cysts counts}}$$

Typical hatch rates should be about 75%, depending upon the grade of cysts and on how good your hatching technique is.

##### **Method and frequency of feeding**

The prawn larvae are fed with brine shrimp from the first or second day after hatching. For the first few days (until stage III) only live brine shrimp are fed. Once larvae reach stage III, prepared feed like ox liver can also be provided.

The stocking density of brine shrimp in the water should be 3/ml. Although prawn larvae can hunt, early-stage larvae catch their food mainly as a result of chance collisions with prey. Maintaining brine shrimp numbers at the recommended stocking density will increase the probability that larvae will catch their prey.

Daily counts of brine shrimp in each LRT should be done. Count the brine shrimp in a sample under the microscope and calculate the number in the tank using the formula in Box 2, and add more brine shrimp to the LRT if necessary. After a while you will learn to tell, just by looking, whether there are enough brine shrimp in an LRT or not.

The larvae normally take 1–4 days to complete each stage of their development. The progress of each stage can be checked daily by examining some larvae under the microscope and comparing them with Fig. 3.

In the early stages (stage I to IV or V), brine shrimp are fed to the prawn larvae two times a day (morning, and evening). From about stage V the larvae are fed brine shrimp in combination with prepared feed, once per day in the evening.

Brine shrimp should preferably be given in the evening to ensure the presence of food in the LRT throughout the night. Prepared feed like ox liver can be given as a light feeding 2–3 times per day during the daytime. The method to prepare ox liver is given in Box 4.

In practice, the exact quantity of prepared feed to be given each time is not something a set rule can be given for. The quantity of feed depends on the utilization of the feed by the larvae, and must be decided by visual observation by the hatchery operator.

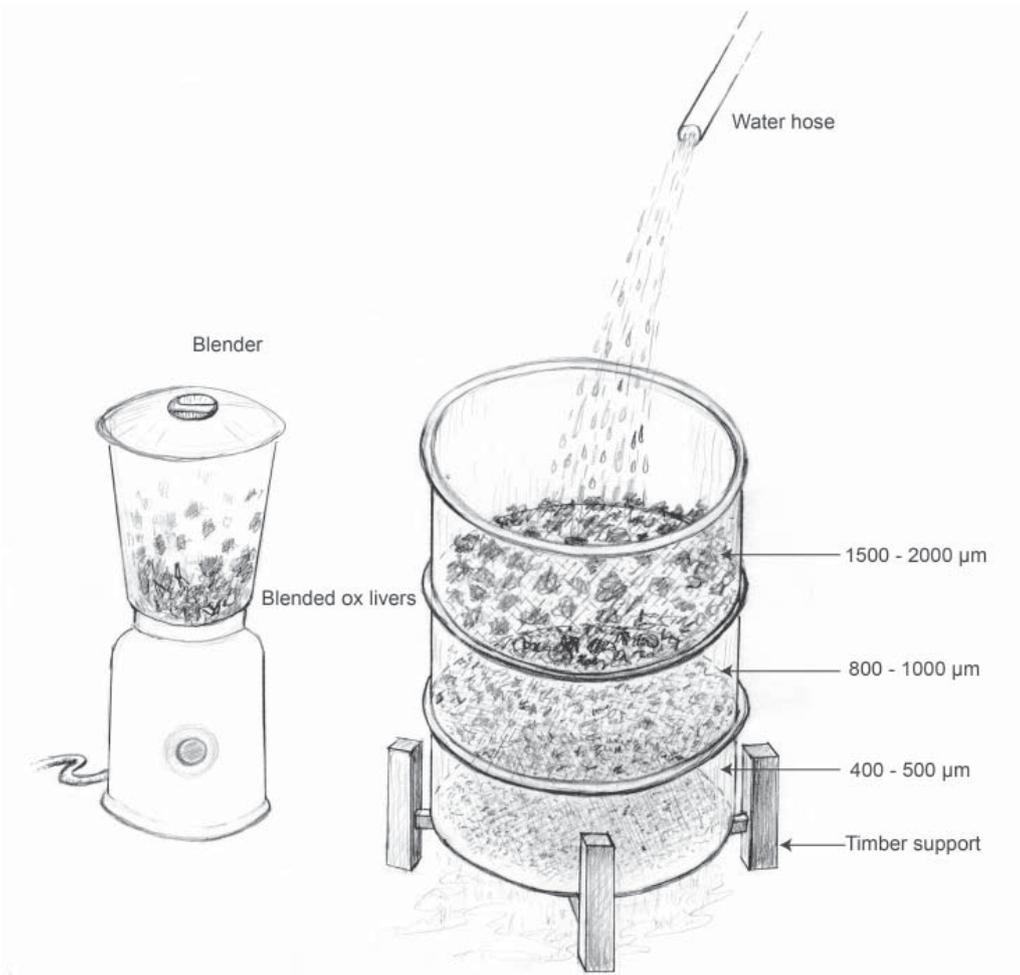


## Box 5

### **Preparation of ox liver feed**

If possible, only fresh ox liver from cattle slaughtered in the last one or two days should be used. This may be kept wrapped in plastic and stored in the refrigerator for use over a 3–4 day period. Fresh liver should be bought at least twice a week.

Remove any fat or tough connective tissue (the white parts), and cut the liver into small pieces about 2.5 cm (1 inch) square. Put the pieces into a kitchen blender with just enough water to cover all the liver. Blend the liver in short bursts of a few seconds by pressing the pulse button on the blender, about 4–5 times. Don't overdo it, or the liver will turn to fine sludge. Pour the contents of the blender through a stack of three sieves with stainless-steel mesh in the size range of 1500–2000  $\mu\text{m}$ , 800–1000  $\mu\text{m}$  and 400–500  $\mu\text{m}$ , from top to bottom (see Fig. 10).



*Fig. 10. Preparation of ox liver*

Wash away blood and fine particles under the tap or hose the sieves. Let the water drain out, and transfer the finely chopped ox liver from the two small-mesh sieves to a beaker of tap water. Pour away the excess water.

The large chunks of ox liver from the top sieve can be blended again and the above steps repeated several times until only connective tissue is left. Larger pieces of ox liver collected on the top sieve can be given to the batches of bigger larvae and the finer pieces from the two bottom sieves to the early stage larvae.

The liver particles can be used immediately, or stored in the fridge for use later the same day. Any liver not used the same day it is prepared should be thrown out.



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Remove air stones prior to feeding ox liver. When the larvae have gathered in swarms at the surface, administer feed from the beaker using a dropper. Drop the feed amongst the dense swarms of larvae in small amounts with the dropper, and wait for larvae grab on to all the liver pieces before you add more. Do this several times until most larvae have seized on to a liver particle. This technique reduces the amount of liver which gets wasted by settling to the bottom of the tank. Replace air stones after feeding.

Feed sparingly, based upon your observations of how much the larvae can eat without there being large amounts left uneaten next day. Prepared feeds like ox liver are 'dirty' feeds because, unlike living brine shrimp, these foods are dead tissues which begin rotting and fouling the water as soon as they are added to the LRT. They need to be used sparingly in order to maintain water quality.

### **Checking feeding rate**

Overfeeding can be recognized by the presence of left-over feed on the bottom of the LRT at the next scheduled feeding time, or by the presence of foam on the water surface or scum on the side of the tank. If overfeeding is noticed, it is best to exchange 70–90% of the tank water, and reduce the amount of feed being given to a level where only a few particles still remain at the next scheduled feeding.

When checking larvae under the microscope each day to identify their larval stage, at the same time check for signs of fungus or pest zooplankton like *Epistylis* on their bodies and for food in their gut. Food in the gut can be seen as brown or golden colouration to the gut passage.

### **Larval water quality**

If the gut is transparent (no colour) then it is empty and larvae are either being underfed, or a water-quality or other health problem has made them stop eating. If water quality is okay, and there is no visible health problem like fungus or *Epistylis*, increase the amount of food in the LRT.

Inadequate feeding leads to starvation, cannibalism, and delayed larval development. If larval development falls behind the timeline shown in Fig. 3 while water temperature is in the optimum range, you should suspect that the larvae are being underfed.

The main challenge during larval culture is maintenance of good water quality. The two functions of (1) feeding prawns and (2) maintaining good larval environment are in opposition to each other, because adding food spoils water quality.

Water quality in the LRT will deteriorate due to decomposition of uneaten food, larval faeces, and build-up of toxic chemicals like ammonia. Food left in the water will also encourage bacterial growth in the water and on the sides of the tank. These bacteria use up oxygen and release toxic substances.

If daily cleaning and water exchange are done, then LRT water quality should not become a problem. You will know that water quality is deteriorating if there is a slimy, slippery feel to the tank surface underwater when you run your fingertip across it. The tank sides should have a 'squeaky-clean' feel if they are sufficiently free of bacterial build-up. You should also be on the look-out for any cloudiness or colour in the water as you look down into it. Good tank water will appear 'crystal-clear'.

This manual describes the clearwater method of larval culture. If good-quality water is likely to be scarce, then other hatchery methods can be tried (see References).



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## Larval health

The most common health problems in prawn hatcheries are surface infections of fungus such as *Lagenidium*, or settlement of protozoans like *Epistylis* or *Zoothamnion* on the larva shell. These will affect the larva's feeding ability by slowing it down and preventing its appendages from moving properly, and ultimately it will die. Fungus will be seen as a mass of fuzzy threads (hyphae) on the larva. Protozoans appear like little buds on stalks (like tulip flowers) attached to the larva shell. Some will be seen to be moving, in characteristic retractions and extensions of the bud by the stalk.

These health problems are not usually a primary cause of trouble, but rather are usually a secondary problem caused by something wrong in the culture system. For example, dirty water with high bacterial levels will encourage the growth of filter-feeding protozoans which feed on these bacteria. Similarly, dirt in the water encourages the growth of fungus. Any other environmental factor which causes larvae to become stressed will reduce their resistance to pathogens.

Even well run hatcheries will see these infections make an appearance, however. The answer is to carry out a regular chemical dosing to reduce the growth of these species on the larval prawns. Chemical dips which are useful for regular dosing include copper sulfate solution, or formalin solution. Most commonly used in Fiji freshwater prawn hatcheries is a formalin treatment, described in Box 6.

In Asian freshwater prawn hatcheries a wider range of prawn larval pathogens can occur. Pacific hatchery operators need to know something about these in order to be vigilant in case of their possible appearance in our region. Information about these can be found in the References section.

### Box 6

#### **Treating larvae with formalin**

The prawn larvae in LRTs should be treated every second day with 30 ppm formalin for up to 1 hour, to reduce the incidence of fungus and protozoan infestations.

Formalin may be sold almost pure (100%) or as a 40% solution. Read the label on the bottle to determine the concentration.

100% formalin represents 1,000,000 ppm  
40% represents 400,000 ppm.

To calculate how much formalin to add to an LRT to provide a concentration of 30 ppm, use the formula:

where:  $C_1 V_1 = C_2 V_2$   
 $C_1$  is the concentration of formalin in the bottle  
 $C_2$  is the concentration of formalin needed in the LRT (30 ppm)  
 $V_1$  is the volume of formalin needed from the bottle  
 $V_2$  is the volume of water in the LRT

#### **Worked example:**

You have 40% formalin in a bottle, and the LRT contains 500 L of water. Calculate the volume of formalin required to treat the larvae at 30 ppm:



$$\begin{aligned}
C_1 &= 400,000 \text{ ppm} \\
C_2 &= 30 \text{ ppm} \\
V_2 &= 500 \text{ L} = 500,000 \text{ ml} \\
C_1 V_1 &= C_2 V_2 \\
400,000 \times V_1 &= 30 \times 500,000 \\
V_1 &= 15,000,000 / 400,000 \\
&= 37.5 \text{ ml}
\end{aligned}$$

Accurately measure the formalin in a measuring cylinder and transfer it into a large beaker or bucket. Top up the beaker or bucket with pre-mixed brackish water from the mixing tank to dilute the formalin. Add the diluted formalin to the LRT, and wait for one hour. Then drain out at least 80% of the LRT water and replace it with clean water from the mixing tank.

### Larval rearing: routine tasks

The routine tasks of larval rearing are critical in the hatchery management. The management of water quality, larval food, temperature, salinity and cleanliness are important factors for the success of PL production. Best results can be obtained by starting with proper stocking density, following suitable feeding regime, keeping tanks clean and managing water quality carefully. A diagrammatic representation of the different steps and daily activities of the hatchery are depicted in Fig. 11.

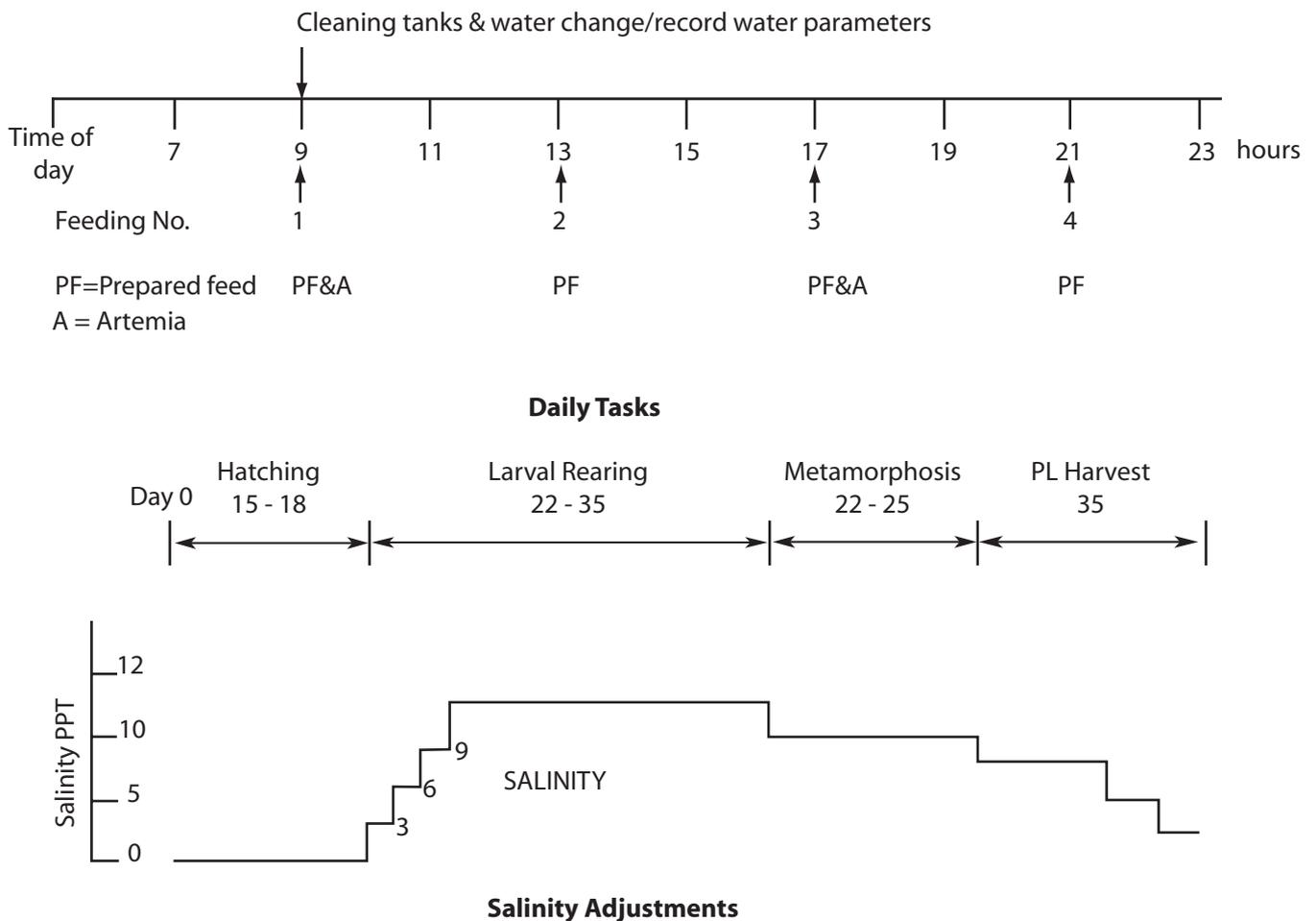


Fig. 11. Timeline of LRT operation over a full larval cycle



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## Morning duties

1. Measure temperature and salinity of each LRT and record in the logbook. Record any other observations, such as tank cleanliness or larval activity.
2. Examine a few larvae from each LRT under the microscope to check (1) stage of larval development, (2) food in the gut: a clear or transparent gut passage is a sign of under-feeding, and (3) health of the larvae, in particular fungus or protozoans like *Epistylis*. Record these observations in the logbook.

### Clean and disinfect LRTs

3. Switch off heaters in the LRTs.
4. Remove air stones from each LRT.
5. Wipe tank sides below the waterline and bottom of the tank using a sponge bound to the end of a PVC pipe. Use a hand-held sponge to clean the rim of the tank at the water line.
6. Gently swirl the water in the LRT so all dislodged dirt collects in the middle.
7. Siphon out debris from the bottom of tank into a white plastic bucket, using a ½ inch or ¾ inch hose connected to PVC pipe (Fig. 9).
8. Once the dirt is all removed and before the bucket overflows, swirl the water in the bucket and leave it to stand for a few minutes until the dirt has collected in the middle. Healthy larvae will be seen swimming mostly near the sides at the top of the bucket. Check if there are large numbers of dead larvae amongst the dirt: this is a sign of problems in the LRT.
9. Using a smaller hose (e.g. 4 mm), siphon out the living larvae into another bucket of clean water and return them to the LRT. Alternatively, if there are more larvae than dirt, it can be easier to siphon out the dirt instead of the larvae.
10. Replace air stones.
11. Every second day, dose each LRT with 30 ppm formalin or other chemical disinfection treatment for one hour (see Box 6).

### Water exchange

12. Drain down LRT water, using a sieve-covered siphon hose (see Fig. 11) until about 30% (or 80%, if formalin-dosed) of water has been removed.
13. While water level is low, wipe the tank sides above the waterline with a hand-held sponge.
14. Replace the water with water at correct temperature and salinity from the mixing tank.
15. Switch heaters on.

### Feeding

16. Estimate the number of brine shrimp left in the LRT water, record in logbook, and calculate number of brine shrimp to be added. Feed early-stage larvae with brine shrimp (otherwise leave feeding until the afternoon).
17. Prepare brine shrimp cysts for hatching, if needed (see Box 3a).

## Evening duties

1. Feed larvae with more brine shrimp if necessary.
2. Remove air stones and feed later stage larvae with ox liver (Box 4). Replace air stones.
3. Set up rehydrated brine shrimp cysts to hatch out over next 1–2 days (see Box 3b).
4. Mix a new batch of 12 ppt water in the mixing tank, and leave it overnight with an immersion heater and strong aeration to come up to temperature and lose any chlorine.
5. Clean up all buckets, hoses and other equipment, wash them and leave them to dry.
6. Inspect all LRTs to ensure aeration is running and heaters are on, and there are no other problems. Close up the hatchery.



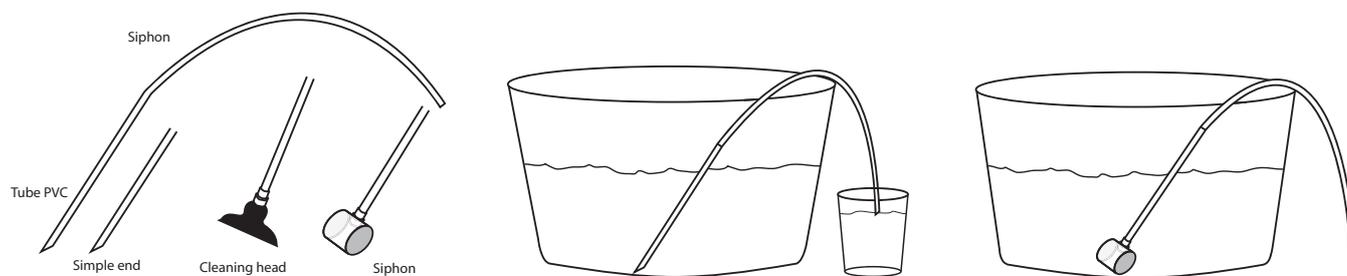


Fig. 12. Using a siphon to remove dirt and draining of LRT

## The postlarval stage

The first PL are expected at about Day 23 of the larval culture cycle. Usually 90% of larvae metamorphose within the next 10 days from appearance of the first PL, but there may be late-stage larvae which still have not undergone metamorphosis into PL, through till Day 35.

*Note.* The practice of designating the development stage of PL by the days in culture since metamorphosis (PL1, PL25 etc) as in the case of penaeid prawns cannot be followed for *Macrobrachrium rosenbergii* due to the non-synchronous metamorphosis.

PL are radically different from larvae in behaviour and appearance. For the first time they resemble miniature adult prawns. While they swim freely (now frontwards, and dorsal-side uppermost), they also crawl or cling to the tank surface.

### Acclimation to freshwater

When about 90% of larvae have metamorphosed into PL, they should be acclimated to freshwater ready for pond stocking or for a nursery phase of culture. The salinity can be decreased by 3–5 ppt every time a water exchange is done, until 0 ppt is reached.

### Nursery system

Overcrowding of PL causes a lot of mortality due to cannibalism, so PL should be moved out of LRTs and into a nursery tank or grow-out pond without delay. Losses of about 10–15% per day can occur if PL are left overcrowded in LRTs.

Ideally PL should be transferred into a large nursery tank with large bottom surface area from the time that 90% became PL, to further increase in size and hardiness before transfer to grow-out ponds. A rectangular, flat-bottom 5000 L tank is suitable. PL can be stocked in nursery tanks at a density of 25/m<sup>3</sup> of water and reared for 2–5 weeks before stocking into grow-out ponds. In Fiji they are normally kept in the nursery tank until they are 15–20 mm long (½ to ¾ inch), or 0.015–0.02 g average body weight. Keeping the juveniles in nursery tanks or a nursery pond at the hatchery longer, until they reach a size of 3–5 g, gives better growth and survival when they are moved to the grow-out ponds.

A nursery system helps the farmer to monitor the productivity of seed, and improves predictability of yield and efficiency of utilization of the grow-out facilities with improved prawn yields. Most farmers in Fiji bypass the nursery phase, however, and stock PL directly into ponds.

PL are primarily bottom-dwelling organisms. Whether in a nursery tank or in a pond, they should be provided with an artificial substrate or structures like plastic shade cloth or mosquito netting hanging from polystyrene floats in the water. They will cling to this and spread themselves out, thus reducing stress from crowding and cannibalism.



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## **Feeding postlarvae**

The PL in a nursery tank can be gradually weaned off ox liver and brine shrimp, and onto a formulated pellet diet. Commercially available pellets for penaeid shrimp are suitable. A variety of other feeds such as small shrimp, worms, or mollusc meat can also be given, subject to local availability.

Detailed information about rearing PL in ponds is given in *Freshwater prawn Macrobrachium rosenbergii farming in Pacific Island countries: Volume two: Grow-out in ponds*.

## **Harvest of postlarvae**

PL can be harvested from the LRT or nursery tank by reducing the water level, collecting them with fine-mesh hand nets, and putting them into clean water in a well aerated container.

The number of PL harvested should then be counted or estimated.

### **Counting individually**

Scoop PL, a few at a time, into clean water in a small container and then count them while pouring them into a larger container. This provides an exact count, but is time-consuming.

### **Estimating by sampling**

1. Transfer the harvested PL into a known volume of aerated water in a container.
2. Aerate or agitate the water thoroughly to disperse the PL evenly in the water.
3. Take four equal-volume water samples (e.g. 100 ml) and count the PL in each as they are poured slowly back into the container.
4. Use the calculation in Box 2 to estimate the number in the container.

## **Packing and transportation of postlarvae**

During transportation a large number of fragile PL are going to be crowded together into a small volume of water. Many could die before they reach their destination, unless they are packed properly.

There are two basic systems for transporting PL. The closed system is a sealed container partly filled with oxygen. The open system consists of a container in which the oxygen requirements are supplied from the air, either with an aerator stone for long distance delivery of PL or without a aerator stone for short distance delivery (10–15 minutes). Three methods usually practised in the Pacific are described in Box 6.

If the hatchery is a long distance by road from the grow-out ponds, or located on another island and PL need to be sent by airfreight, then the PL will need to be carefully prepared and packaged for transportation in plastic bags provided with oxygen and fitted into boxes, as follows.

1. Harvest and count PL.
2. Provide sufficient aeration while holding them in containers ready for packing.
3. Use clean water.
4. Use two bags, one inside the other, as insurance against damage to the plastic. Water is poured into the bag and PL are transferred into it. Pack 50/L for long journeys (up to 18 hours).
5. Press the bag above the water to remove atmospheric air. Insert a hose from an oxygen cylinder in to the bag, hold the neck closed around the hose, and fill the bag up with oxygen (Fig. 13).



6. Withdraw the hose, then twist and double back the neck of the bag, using strong rubber bands to hold it closed and gas-tight.
7. Fit the plastic bags into Styrofoam boxes or any suitable box.
8. Add an ice-pack to each box before taping up its lid. Cooler temperature will reduce the PL demand for oxygen. Avoid direct sunlight or conditions where bag water temperature could rise above 30°C.

*Fig. 13. Packing PL for transport*



## Box 7

### **Three methods of transporting PL**

#### **Household container**

A household bucket or other container is ideal for carrying PL from a hatchery on the farm or very close by. The container should be covered with a lid to keep sunlight from reaching the water. Density of PL should be below about 25/L.

#### **Plastic bags with oxygen**

Two large, strong, clear plastic bags are used, one bag inside the other as insurance against damage to the plastic. They are filled about one-third with clean water, the PL are put into the water and oxygen is added. With oxygenated bags, the PL density can be twice as much as when buckets are used.

#### **Tanks on trucks**

Fibreglass or plastic containers, or frames or boxes lined with plastic, can be used for transport by truck. The containers have an open top. Any size can be used, but a recommended size is 1 m<sup>3</sup>. The open top is covered with plastic or a sack and tied around with a rope, to keep the water from splashing out and also to protect from exposure to the sun. The container should be filled with water to 70% of its volume and where possible fitted with an aerator. If an aerator is used, higher densities of PL can be transported this way than in unaerated buckets.

Forward and backward movement of water when the vehicle is in motion should be minimized, as this affects PL and may even kill them.

On arrival at the pond site, care should be taken to acclimate the PL to the temperature of the pond water by floating the transportation bags in the pond for 15–20 minutes before opening them. Sudden changes in temperature of more than 5°C can harm the PL.

When you open the bag, allow in some pond water to mix 50:50 with the transport water. Sudden changes in water quality can also be harmful, so the PL need time to adjust to their new water conditions. After another 2–3 minutes, tip the bag on its side and allow the PL to swim out (Fig. 18). Do not tip them out all at once, but rather let them swim out by themselves. This will leave any dead ones remaining in the bag, so they can be counted and the mortality of PL during transportation can be ascertained. A survival rate of 90% should be possible if correct procedure is followed.



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## Record keeping

Hatchery production records are important for good management. They provide detailed information about the inputs and outputs of the operation, and can help to identify any problems which occur. It is recommended that records be maintained in a logbook and, where possible, in a computerized database.

If good hatchery records are made available to researchers, they can compare the efficiency and profitability of different hatchery techniques and also compare performance of broodstock of different origins. This information helps the hatchery operators to choose techniques and broodstock which are most appropriate and profitable.

Hatchery records provide the information necessary to:

- Compare the efficiency of various production techniques
- Compare the productivity of different broodstock
- Compare the cost-effectiveness of alternative inputs
- Improve the efficiency of the hatchery operation

### What should be recorded?

Records should be made in a logbook should be kept, with daily entries for each LRT:

- Initial stocking density and estimated numbers of larvae stocked into LRT at hatching
- LRT temperature
- LRT salinity
- Observations of tank cleanliness or larval activity
- Stage of larval development
- Whether there is food in the guts of larvae
- Health of the larvae, in particular fungus or protozoans like *Epistylis*
- Whether formalin was dosed or not that day
- Brine shrimp stocking density left uneaten, and brine shrimp density re-stocked
- Every 3 or 4 days, estimate of numbers and % survival of larvae in LRT

At the completion of each larval culture cycle, a Larval Culture Cycle Record Sheet can be compiled from the logbook data. This is the record of progress and profits from each culture cycle.



# Larvae Culture Cycle Record Sheet

Name of farm: \_\_\_\_\_

Broodstock source: \_\_\_\_\_ Date sourced: \_\_\_\_\_

No. of broodstock: \_\_\_\_\_ Average weight: \_\_\_\_\_

Hatch dates: from \_\_\_\_\_ to \_\_\_\_\_ Total larvae hatched: \_\_\_\_\_

LRT No.	Date stocked	No. larvae stocked	Average water temp.	Date of 1st PL	Date all PL	No. PL	% survival	No. PL packed & delivered	PL price per 1000

## References

Profiles of High Interest Aquaculture Commodities for Pacific Island Countries

Economic Models for Aquaculture and Agriculture Commodities

Freshwater Prawn Culture: The Farming of *Macrobrachium rosenbergii*. Edited by M.B. New and W.C. Valenti. Published by Blackwell Publishing. 2000.

There is a reference to a recipe for egg custard feed for larvae.

There is a reference to other hatchery methods

<http://www.freshwaterprawn.com/html/diseases.htm>





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