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FOREWORD

SEAFDEC Aquaculture Department's research on the breeding, larval production, and grow-out of the freshwater prawn since the 1980's has continuously been evolving. Studies conducted at its Binangonan Freshwater Station have made significant contributions to the implementation of effective and practical farming systems for *Macrobrachium rosenbergii*, an aquaculture commodity with economic importance and high export potential.

Proper understanding of the giant freshwater prawn, from its biology to its culture system, is necessary for the successful and optimized farming of this species. Accordingly, relevant science-based techniques on its breeding and seed production are presented in this manual.

Included in this publication are essential information such as collection, transport, holding, and management of broodstock; hatchery facilities; stocking density and larval rearing techniques; feeding management; post-larvae harvesting and nursery; and health management. May this manual benefit the various stakeholders of the aquaculture industry, especially the freshwater prawn farmers, and consequently guide them in effecting sustainable aquaculture practices.

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INTRODUCTION

All farmed freshwater prawns belong to the genus *Macrobrachium*. The largest species in the genus is *Macrobrachium rosenbergii* (Fig. 1), hence the name giant freshwater prawn. The species thrive in many tropical and subtropical areas of the world. It is native to the Philippines, Thailand, Malaysia, Indonesia, Vietnam, Myanmar, India, Bangladesh, and Australia. It has been introduced for culture in other areas beyond its natural distribution such as in China, USA, and Latin American countries. The species live in freshwater environments with links to saline waters since the larvae of the species require brackishwater for complete development.

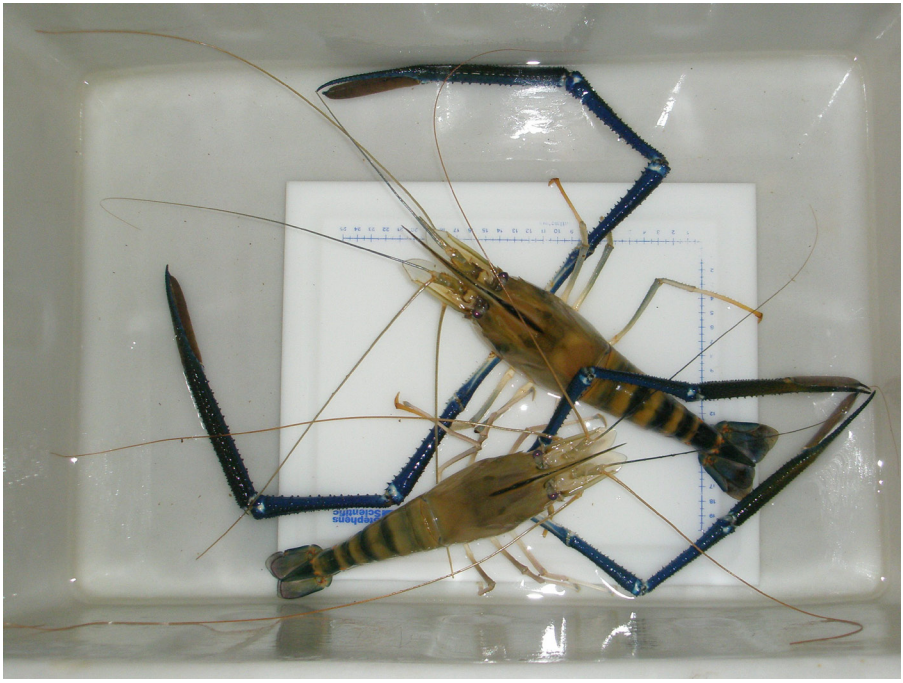

Fig. 1. *Macrobrachium rosenbergii*

BIOLOGY

The external features of the species are shown in Fig. 2. The body is divided into two parts, the "head" or cephalothorax and the "tail" or the abdomen. The cephalothorax ends in a long pointed rostrum. On both sides of the rostrum are two pairs of antennae used for sensory reception. The eyes are stalked with a dark round cornea. Prawns have ten pairs of appendages or legs. The first five pairs are called the "walking legs" or pereiopods. The first two pairs of walking legs, called the chelipeds are provided with chelae or pincers. The first pair of cheliped is slender and is used primarily for capturing food. The second pair of cheliped is well developed and has strong claws. This cheliped is used for food capture, aggression/defense, and for mating. The remaining walking legs are similar to each other and terminate in a pointed dactyl.

The abdomen is divided into six segments. The first five segments are provided with swimming legs or the pleopods. The shell covering the second segment overlaps the first and the third as opposed to penaeid shrimps (e.g. *Penaeus monodon*) where the first abdominal segment overlaps the second, and the rest of the succeeding segments are overlapped by the preceding ones. The abdomen ends in a pointed telson flanked by a pair of uropods.

Like other crustaceans, the species undergo moulting, or shedding of the exoskeleton or outer shell for growth to take place. The frequency of moulting depends on the growth rate of the individual. The newly moulted prawns (Fig. 3) are vulnerable to predation and cannibalism since the new shell is still soft. After about 24 hours, the shell is completely hardened. The species exhibit aggressive behavior and are territorial which may preclude culture in very high stocking densities.

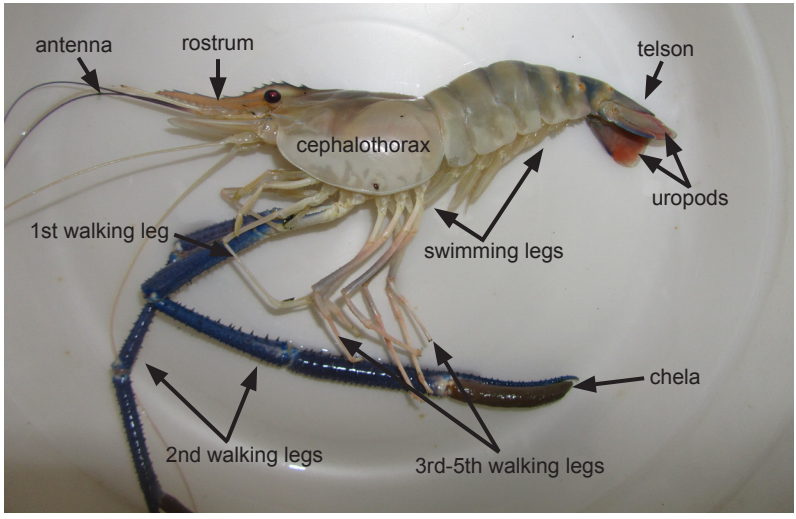

Fig. 2. External features of the giant freshwater prawn.

Another characteristic of the species is their "heterogeneous individual growth" (HIG) particularly the males. The females exhibit the normal size distribution common to other aquaculture species, but the males show large size variation and distinct physical characters or morphotypes. The male morphotypes are: blue-claw (BC), orange claw (OC), and small males (Fig 4). The males exhibit a strong social structure which should be considered in the culture of the species.

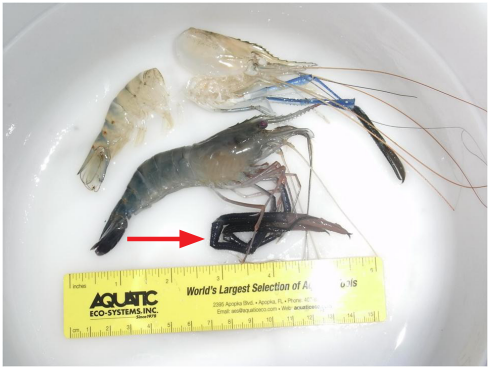

Fig. 3. Newly moulted adult prawn showing recently shed exoskeleton. The chelipeds are weak and soft (red arrow).

This species thrives in freshwater environments in the tropics. Although other *Macrobrachium* species can complete their life cycle entirely in freshwater, *M. rosenbergii* require brackishwater during larval development. For this reason, its natural habitats are bodies of water with direct link to brackishwater or the sea. Berried (egg-carrying) females migrate downstream to brackishwater where the eggs hatch into larvae. The free swimming larvae pass through a series of stages (zoea I to XI) until they metamorphose into post-larvae (PL). It takes an average of 28 days for the larvae to metamorphose into PL. In nature, PLs migrate upstream to low salinity and freshwater environments. The PLs grow into juveniles and eventually into adults. The summary of the life cycle of *Macrobrachium rosenbergii* is illustrated in Fig. 5.

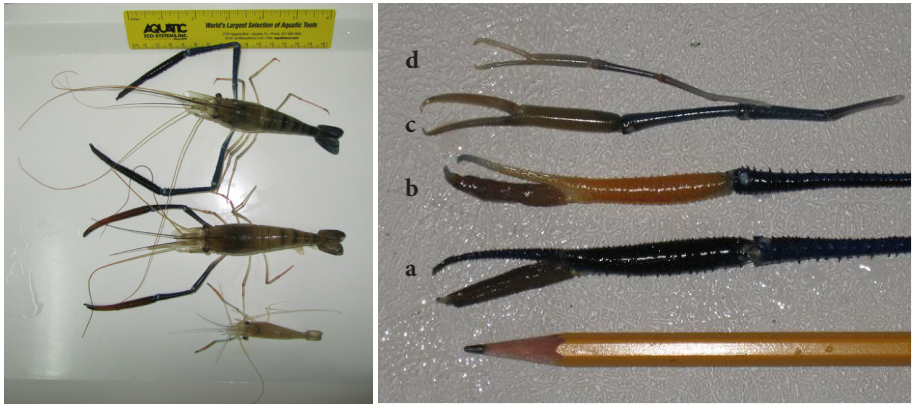

Fig. 4. Left photo shows blue claw (BC, top), orange claw (OC, middle), and small males (SM, bottom). Right photo shows detail of claws of different male morphotypes: (a) cheliped of BC; (b) transitional OC to BC with mixed characteristics of the chela; (c) OC male; (d) SM chela with white to transluscent claws.

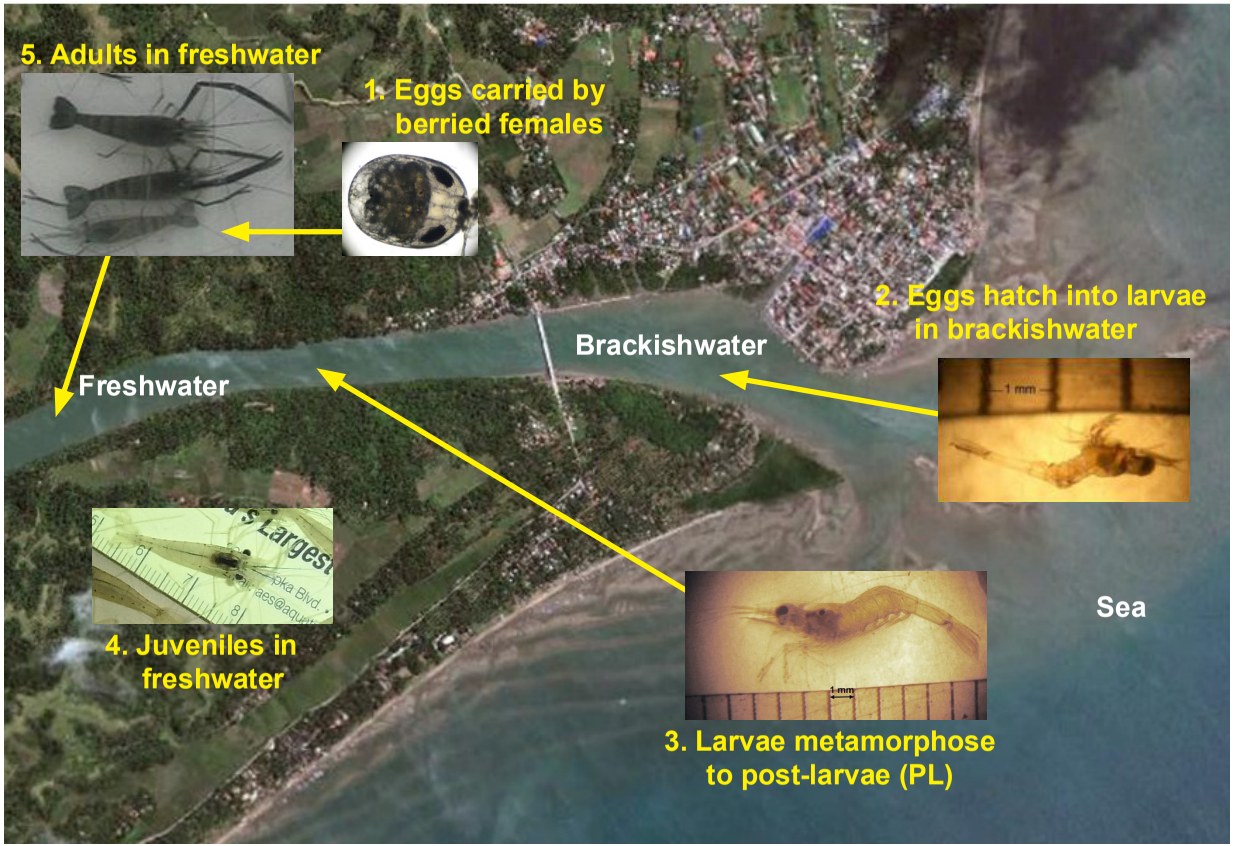

Fig. 5. Life history of the giant freshwater prawn.

BROODSTOCK COLLECTION

Prawn broodstock may either be sourced from the wild or culled out from grow-out operations. Since the giant freshwater prawn inhabits natural water bodies in the Philippines and other tropical countries, broodstock may be collected from the wild. Rivers are the most common collection sites for this species. Grow-out operations are also a good source of broodstock. Collection from the wild or from grow-out operations, usually involves selection of egg-carrying or berried females (Fig. 6). Males may also be collected if the farmer plans to extend holding the broodstock for succeeding spawnings.

Characteristics of a good broodstock are: (1) healthy and active; (2) well pigmented; (3) no missing appendages or other damage; (4) and carrying large egg masses in the case of berried females.

If the female breeders are being culled from grow-out operations, early maturing females are recommended which may be observed within 3-4 months of the grow-out operations, depending on the age at initial stocking. Selection of broodstock in the early part of the production cycle results in a beneficial effect on the growth potential of broods in the succeeding generation. If collecting from the wild, another alternative is the selection of the biggest females since number of eggs produced is proportional to female size.

Fig. 6. Egg-carrying or berried females of prawns.

TRANSPORT OF BROODSTOCK

Care should be taken during the transport of broodstock from the grow-out farm or the collection site to the hatchery facility. Berried females tend to loose eggs totally or partially due to transport stress (Fig. 7).

To avoid damage to the rostrum and at the same time prevent the rostrum from puncturing holes in the transport bags, it may be protected by inserting packing styropore material or plastic tubing (Fig. 8a&b). For berried females, placing them individually in smaller double layer extra strength plastic bags with water and oxygen

is recommended because it reduces egg loss. Another method is to keep the broodstock inside a perforated PVC pipe cut to appropriate lengths according to the size of the broodstock. The open ends of the pipes are then closed with nets (Fig. 8c&d). The pipes are then placed in the transport containers. The last method has been proven by the authors to be effective for transport by both air and land with travel times exceeding 8h.

Fig. 7. Female carrying a whole (bottom) and partially aborted (top) egg mass.

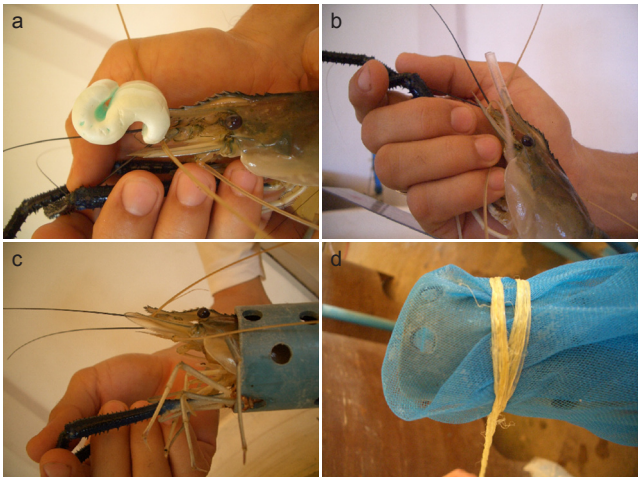

Fig. 8. Packing of prawn broodstock for transport. The rostrum may be blunted with styropore packing material (a) or with short plastic tubing (b). The prawns may also be kept inside perforated PVC pipes (c) and the open ends covered with netting material (d).

HOLDING OF BROODSTOCK

If berried females are not readily available, mature females can be collected and kept in holding tanks with males (Fig. 9). Stocking four females to one male is sufficient. Blue-claw (BC) and orange claw (OC) males are preferred over small males. Males have a tendency to develop a "harem" and defend the members of its harem and its territory against other males.

The stocking density of brooders in holding tanks affects the egg carrying capacity of the females. The number of eggs that will eventually hatch is reduced at higher stocking densities. A stocking density of 20 to 60 L per individual is recommended for broodstock. It is advisable to keep the temperature of the broodstock holding tank between 27 to 30°C.

Fig. 9. Broodstock in holding tanks. The branches (left) and cut-off PVC pipes (right) serve as shelter.

BROODSTOCK MANAGEMENT

The prawn hatchery operator has the option to discard the female broodstock as soon as the eggs hatch or keep the broodstock for future use. The former has the advantage of eliminating inbreeding in the hatchery but has the disadvantage of the added expense of sourcing broodstock from the wild. The latter has the advantage of having readily available broodstock. However, if broodstock for hatchery operations is continuously culled from grow-out operations, after several cycles of production, the performance of the giant freshwater prawns tends to decline. This is manifested by poor growth and survival due to inbreeding or genetic degradation of the stocks, especially if the practice is done for several generations of prawns. It is advisable to replenish broodstock from other sources from time to time. Another advantage of holding the broodstock even after the berried females have released their eggs, is that it allows the females to increase their egg clutch size as they grow. The ovaries of prawns are able to remature even if they are still berried. Thus, shortly after the eggs hatch, the females may be ready to undergo another pre-mating moult in preparation for another reproductive cycle. Interval between rematuration can vary depending on the nutritional state and environmental factors, thus the number of rematuration in the female's lifetime is variable. The shortest observed interval for the ovaries to remature is 30 days.

In penaeid shrimps, cutting the eyestalk or ablation is practiced to induce maturation and spawning. In the giant freshwater prawn, unilateral eyestalk ablation has been shown to induce gonad maturation in spent females. However, this is not as widely practiced as in penaeid shrimps.

The broodstock may be fed commercially formulated feeds, preferably with high protein content (40%) to improve rematuration, fecundity and egg hatching rate.

SEX IDENTIFICATION

Giant freshwater prawns are sexually dimorphic, that is, males and females exhibit distinct physical traits. Male prawns have a pointed structure at the ventral side of the first abdominal segment while females do not have this protrusion (Fig. 10). Sexually mature females also have a wider distance between the flaps covering of the abdomen (abdominal pleura), in preparation for carrying a clutch of eggs when they become berried. The adult males also have a pronounced appendix masculina in the second pair of swimming legs (Fig. 11). Each swimming leg in the male and female have two parts, an exopod and an endopod. In the males, the appendix masculina is found projecting from the inner side of the endopod. The rest of the swimming legs do not have this structure. The appendix masculina is used during mating.

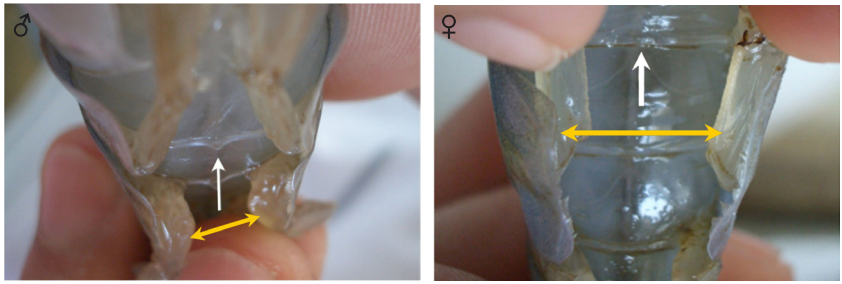

Fig. 10. Distinguishing features between a mature male (♂) and female (♀) giant freshwater prawn. White arrows show the presence (♂) or absence (♀) of the protrusion in the ventral part of the first abdominal segment. The wider distance between the covering of the abdomen in females compared to the males is another distinguishing trait (yellow arrows).

REPRODUCTIVE BEHAVIOR

Sexually mature female prawns undergo a pre-mating moult. During this time, it is vulnerable and prone to attacks by other females. The male protects the female undergoing pre-mating moult from the other females. Courtship follows characterized by the males stroking the females with their antennae and cheliped (Fig. 12). This is followed by the cleaning stage where the males, using its cheliped, clean the ventral region of the female in preparation for laying the eggs. The male flips the female so that their ventral side are aligned. In both males and females, the gonads are located in the cephalothorax. During copulation, the males release its spermatophore, a gelatinous mass containing sperm, through openings (gonophores) at the base of the fifth walking

legs. The gelatinous mass is deposited in the female. After copulation, the female will release the eggs through openings at the base of the third walking legs. The eggs are fertilized in the process by the previously deposited semen. The fertilized eggs are deposited in the brood chamber. The brood chamber is the expanded abdominal area of the female. The eggs are held together by a sticky substance produced by the female to keep the eggs together (like berries, hence berried females). The movement of the swimming legs of the female aerates the eggs as they develop.

The first few batches of eggs that a female carries at its first year of maturity is between 5,000 to 20,000. Fully mature females after subsequent rematuration can carry 80,000 to 100,000 eggs.

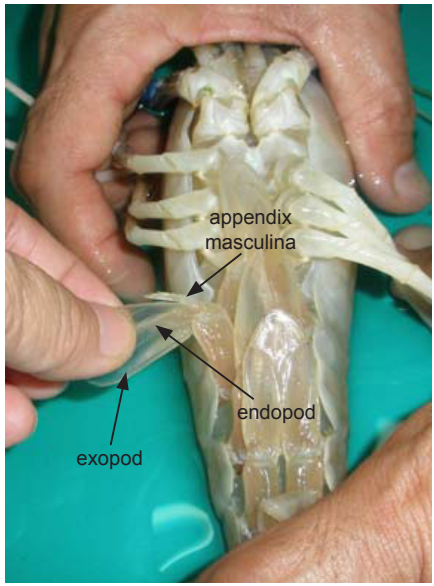

Fig. 11. Detail of the second swimming leg of the male giant freshwater prawn showing the appendix masculina.

Fig. 12. Courtship between a blue-claw male and a female giant freshwater prawn. The male is stroking the female with its cheliped and antennae.

EGG DEVELOPMENT

Once berried, the females carry the eggs until hatching. It takes an average of 20 days for the eggs to hatch at 28°C. Temperature affects egg development. Low temperature may prolong the process. The egg mass in the abdomen of berried females changes color from orange to gold to brown to brownish gray. The color of the egg mass depends on the stage of egg development (Fig. 13). The orange color is imparted by the yolk and the progression to darker colors indicates that the yolk is being used up as the embryo of the prawn develops (Fig. 14).

HATCHING OF LARVAE

The berried females may be transferred to a separate holding tank to await hatching of the eggs. Females with similar stage of egg development as indicated by the egg color are grouped together. Prior to transfer to the holding tanks, berried females are disinfected by immersion in 30 ppm formaldehyde solution for 30 minutes to 1 hour. The water in the holding tanks maybe freshwater or brackishwater. Studies show that egg hatchability is increased in brackishwater, with optimum at 6 ppt at 26 to 28°C. Higher temperatures, e.g. above 30°C, may be detrimental because it promotes growth of undesirable microorganisms. Hatching usually occurs at night. Covering the tanks

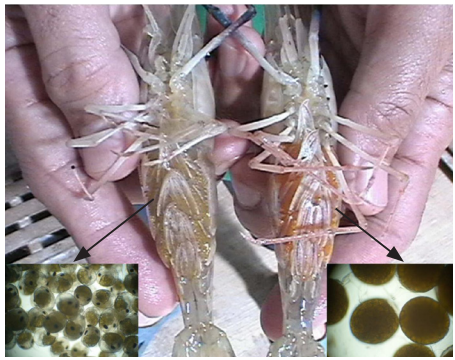

Fig. 13. The changes in the egg color in berried females is indicative of the development of the prawn embryo. Orange eggs are still predominantly yolk (right inset); the embryo of brownish gray eggs (left inset) has developed eyes and other body parts.

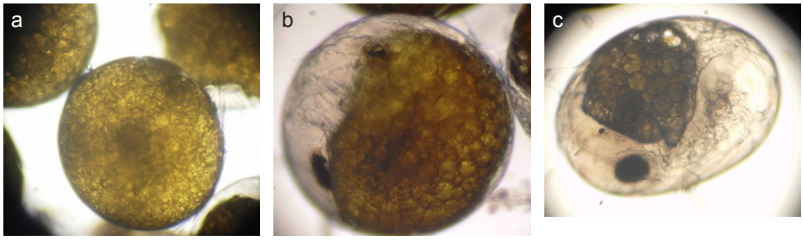

Fig. 14. Some stages of egg development of the giant freshwater prawn: (a) completion of segmentation 24h post fertilization; (b) eye pigment developed by day 9; (c) larva fully formed at day 14.

holding the berried females facilitate hatching during daytime. To minimize water quality deterioration for the newly hatched larvae, the berried females are not fed 2 days prior to hatching (indicated by the dark brownish gray eggs). The newly hatched larvae are collected by fine mesh nets and transferred to the larval rearing tanks. Alternatively, the water in the broodstock tanks are gently siphoned off through a fine mesh net to collect the larvae (Fig. 15). Care must be taken to ensure the larvae are not damaged by the transfer.

The larvae are transferred to a temporary holding tank where they can be gradually acclimated to the larval rearing salinity. Acclimation can be done in stages, by progressively increasing the salinity by 3 ppt until it reaches 12 ppt within 24 hours. The newly hatched larvae can survive in fresh water for two days, after which they start to die off unless acclimated to the appropriate salinity. Larval batches hatched within two days of each other can be pooled in rearing tanks. Pooling batches hatched beyond this time period is not recommended because younger and smaller larvae are prone to cannibalism by the larger, older larvae.

PREPARATION OF BRACKISHWATER

Salt water sourced from the sea can be diluted with freshwater to the desired salinity. Salt farms are also a good source of brine or extremely high salinity water which can also be diluted to the desired salinity. A refractometer (Fig. 16) can be used to measure the salinity of the water. Suspended particles in the seawater are allowed to settle or filtered out with fine mesh cloth or microfiber filter (Fig. 17). This is followed by chlorination using 5 ppm active chlorine, and the water is left to stand for 24 hours. Prior to use, the water is aerated vigorously (Fig. 18) for at least 6 hours to remove residual chlorine.

Fig. 15. Newly hatched larvae are removed from the broodstock tanks by siphon.

If the source of seawater is quite far from the hatchery and transport cost is prohibitive, formulated brackishwater can be used. Table 1 shows the chemical composition of artificial brackishwater for the larval rearing of the giant freshwater prawn. Food grade quality for each of these chemicals is recommended. Each of these reagents should be dissolved individually in filtered freshwater prior to mixing them together. The use of artificial brackishwater reduces the risk of introduction of predators, parasites and unwanted microorganisms in the hatchery. However, it may prolong the larval rearing cycle by about 10% compared to natural seawater or brine.

Regardless of the source of water, recommended pH for use in larval rearing is between 7 to 8.5 and dissolved oxygen of at least 5 mg/L.

Fig. 16. A refractometer is used to determine salinity of the rearing water.

Table 1. Composition of artificial brackishwater to make up 1,000 liters of 12 ppt for the larval rearing of giant freshwater prawn (Valenti and Daniels, 2000).

Salt	Quantity (g)
Sodium chloride (NaCl)	9,200
Magnesium sulfate ($MgSO_4.7H_2O$)	2,300
Magnesium chloride ($MgCl_2.6H_2O$)	1,800
Calcium chloride ($CaCl_2.2H_2O$)	467
Potassium chloride (KCl)	200
Sodium bicarbonate ($NaHCO_3$)	67
Potassium bromide (KBr)	9

Fig. 17. Seawater is filtered prior to use in the hatchery.

Fig. 18. Reservoir of chlorinated seawater is aerated vigorously to remove residual chlorine.

Fig. 19. Indoor (a&b) and outdoor (c&d) freshwater prawn hatchery facilities. The roof is provided with skylight for natural lighting in the indoor facility. The tanks may be covered with canvas or other materials to protect the outdoor tanks from intense sun and inclement weather.

HATCHERY FACILITIES

The prawn hatchery facilities may vary depending on the resources available to the hatchery owner. It may be housed indoor or in the open (Fig. 19). Basic requirements include:

- Sufficient supply of good quality water
- Stable power supply
- Aeration system
- Larval rearing tanks/green water culture tanks
- Reservoir for brackishwater
- Hatching jars for live food organisms
- Standard equipment for hatchery (stereomicroscope, refractometer, thermometer, pH and dissolved oxygen meter, buckets, hose, filters etc.)

Basic types of hatchery

- **Closed recirculating system**

  The water is reused after undergoing a series of filtration and treatment. This type is recommended for areas where the larval rearing water needs to be conserved due to distance from source. The waste water passes through a series of physical and biological filters to clean up the water before it is returned to the rearing tanks. The physical filter (e.g. Fig. 20) removes solid waste such as uneaten feed, dead larvae, exuvia and fecal material. The biological filters remove harmful substances, primarily nitrogenous wastes e.g. ammonia, which may build up in the larval rearing water (Fig. 21). In some cases, the water may also pass through an ultraviolet (UV) light filter to remove harmful microbes. The filters have to be cleaned out regularly to ensure efficient clean-up of the water before it is returned to a reservoir, ready to be recycled back to the rearing tanks. Ideally, the total water volume in the tanks should recirculate at least 10 times a day.

Fig. 20. Physical filtration. The tank (red arrow) is packed with sand, gravel and charcoal. Water is filtered and pumped to an overhead reservoir before recirculating to the rearing tanks.

Fig. 21. Biological filters may be packed with materials which will provide good surface area (bottom photos) for the growth of beneficial microbes to remove nitrogenous wastes.

- **Flow-through system**

  Water is continuously supplied to the tanks and overflows through a pipe (Fig. 22). This is ideal if the site of the hatchery is near the source of saline and freshwater. This type of hatchery ensures good water quality. It may be costly in terms of power consumption for cases where continuous pumping of water is necessary. Flow rates may be adjusted depending on the water replacement rate required in the tanks.

Fig. 22. Flow through system in concrete (left) and polyethylene (right) tanks. Red arrows show the inlet and white arrows the outlet for both systems.

- **Static-renewal system**

  Water in the tanks are partially or fully replaced as needed. The waste water may either be discarded or reused after undergoing treatment. In case water needs to be re-used, the waste water undergoes filtration processes similar to the closed recirculating system. The difference is that the water is not automatically recirculated to the rearing tanks after treatment but kept in a reservoir.

- **Green water system**

Fig. 23. Outdoor tanks used to produce "green" water.

The green water hatchery for freshwater prawn uses brackishwater seeded with green algae as the rearing medium for the larvae. Based on the results of the study at SEAFDEC/AQD's Binangonan Freshwater Station, this system improves survival and shortens time to metamorphosis by several days compared with the clear water system described above. Green water is cultured outdoors using at least 1 m^3 tanks (Fig. 23). The green water culture tanks are filled with filtered natural or artificial seawater with a salinity of 6 ppt. Commercial NPK (nitrogen, phosphorus, and potassium) fertilizer is added at the rate of 0.1 g per liter (100 g per m^3). Pure cultures of *Chlorella* (Fig. 24) is inoculated into the tanks. Male tilapia fingerlings are added to the tanks at a stocking rate of 5 per m^3 after 3 days of inoculation of the algae. The tilapia is for the control of filamentous algae and the feces may also serve as fertilizer. After several days, depending on density of the initial *Chlorella* sp. inoculum, the water will turn green and the salinity is increased to 12 ppt. The culture of green water should be done on a staggered basis to ensure the continuous supply during larval rearing cycle. The green water (without the tilapia) is transferred to the larval rearing tanks after passing through a filter bag made from fine mesh cloth. The prawn larvae are then introduced in the tanks. Another microalgae used for the green water system is *Nannochloropsis* sp. Cell densities between 12.5 to 25 x 10^5 cells per ml have given the best results in terms of larval survival and time to metamorphosis to PL.

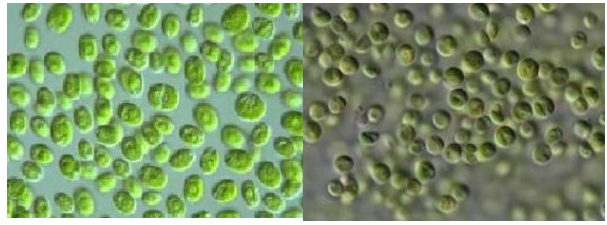

Fig. 24. *Chlorella* sp. (left) and *Nannochloropsis* sp. (right). Source: http://chlorella.co.nz/chlorellaimages/chlorella.jpg; http://rockncritters.co.uk/images/Nannochloropsis%20icon.jpg

The phytoplankton helps maintain good water quality by producing oxygen through photosynthesis. They also minimize ammonia build-up in the tanks since phytoplankton utilize ammonia as a growth nutrient. In addition, for outdoor hatcheries, the microalgae provide some shade against intense sunlight to the growing larvae. The disadvantage of this system is that it entails additional skill, labor and facilities to culture and maintain the algae. If the algal population in the larval rearing tanks crashes, it will contribute to water quality deterioration if not immediately checked. Hatcheries using the green water system have to provide either sunlight or artificial illumination to maintain the growth of the phytoplankton. This system precludes the use of recirculating systems since the mechanical and biological filters will eliminate the algae.

LARVAL REARING TANKS

Larval rearing tanks may be made of rigid plastic, wooden tanks lined with plastic, fiberglass, and concrete (Fig. 25). The size of the tanks vary depending on the volume of production planned for the hatchery. It may be rectangular, square, or circular. For rectangular or square tanks, the corners should be rounded out to prevent build-up and accumulation of waste at the corners and facilitate clean-up. Small volume (<100 liter capacity) tanks may be used for small-scale operations or to keep hatchlings from individual batches separate (Fig. 26). The tanks may be flat-bottomed or with bottoms sloping toward the drain (Fig. 27). Whatever the configuration, each tank should be provided with appropriate pipes for water supply and aeration line fittings. Aeration lines should preferably be above ground supplied by a blower system (Fig. 28).

Fig. 25. Larval rearing tanks may be made of rigid plastic (a), moulded fiberglass (b), and concrete (c).

Larval rearing tanks must be cleaned regularly to prevent build-up of organic pollutants from unconsumed feed, exuvia of moulting larvae, dead larvae and faeces. The leading end of the siphon may be provided with a brush to scrub clean the bottom of the tanks (Fig. 29).

Fig. 26. Small volume plastic tanks may be made from recycled plastic drums cut in half for small-scale operations.

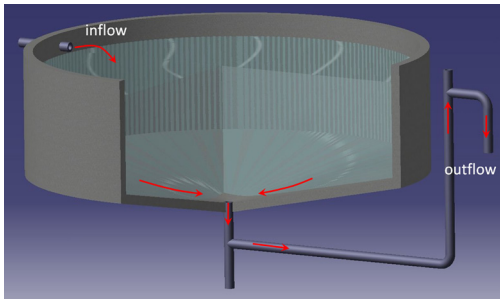

Fig. 27. Circular sloping bottom tank.

Fig. 28. Aeration fittings (black arrow) and aeration pump supplying oxygen to the tanks.

Fig. 29. Leading end of siphon may be fitted with a brush (a) to scrub the tanks during cleaning (b).

STOCKING DENSITY OF LARVAE

Larvae may be stocked at 50 to 100 individuals per liter if the larvae are to be reared from hatching to PL in the same tank.

Higher stocking densities of 500 larvae per liter can be used in the early stages of larval development. After about 10 days, the larvae, now zoea stage VI, are transferred to other tanks to reduce their stocking density to 50 individuals per liter. If larval transfer is not effected carefully, mortalities from stress and physical damage to the larvae may occur.

In the third method, progressive reduction in stocking density of the larvae by increasing the volume of the rearing water is employed. Larvae are initially stocked (100 per liter) in tanks with 40 cm of water. As the larval stages progress, more water is added in the tanks. This method reduces the stress of transferring larvae into different tanks, but requires larger tank volumes to accommodate the progressive dilution as the larvae grow.

To count larvae, water with the larvae is thoroughly but carefully mixed by hand and known volume of the water is scooped out and the larvae are counted. This will give the number of larvae per unit volume of water. This method is done several times to get a good estimate of the larvae in the batch. Counting is essential for estimating stocking density as well as for monitoring survival.

MONITORING OF LARVAL DEVELOPMENT

The larvae should be checked daily to determine the health status and progress in development. A good stereomicroscope may be used to observe the larvae. Healthy larvae are actively swimming and feeding. Prawn larvae (zoeae) tend to be planktonic and utilize the entire water column. They swim upside down and backward, with the tail end leading. The larvae undergo 11 stages of development (Fig. 30) before they finally metamorphose into PL (Fig. 31). The larvae undergo molting for each successive developmental stage. Stage I larvae is distinctive because the eyes have no stalk. In subsequent stages, the eyes become stalked, the rostrum develop "teeth", the tail becomes defined into its component parts of the uropod and telson, the swimming legs develop and the first two walking legs have fully developed chelae.

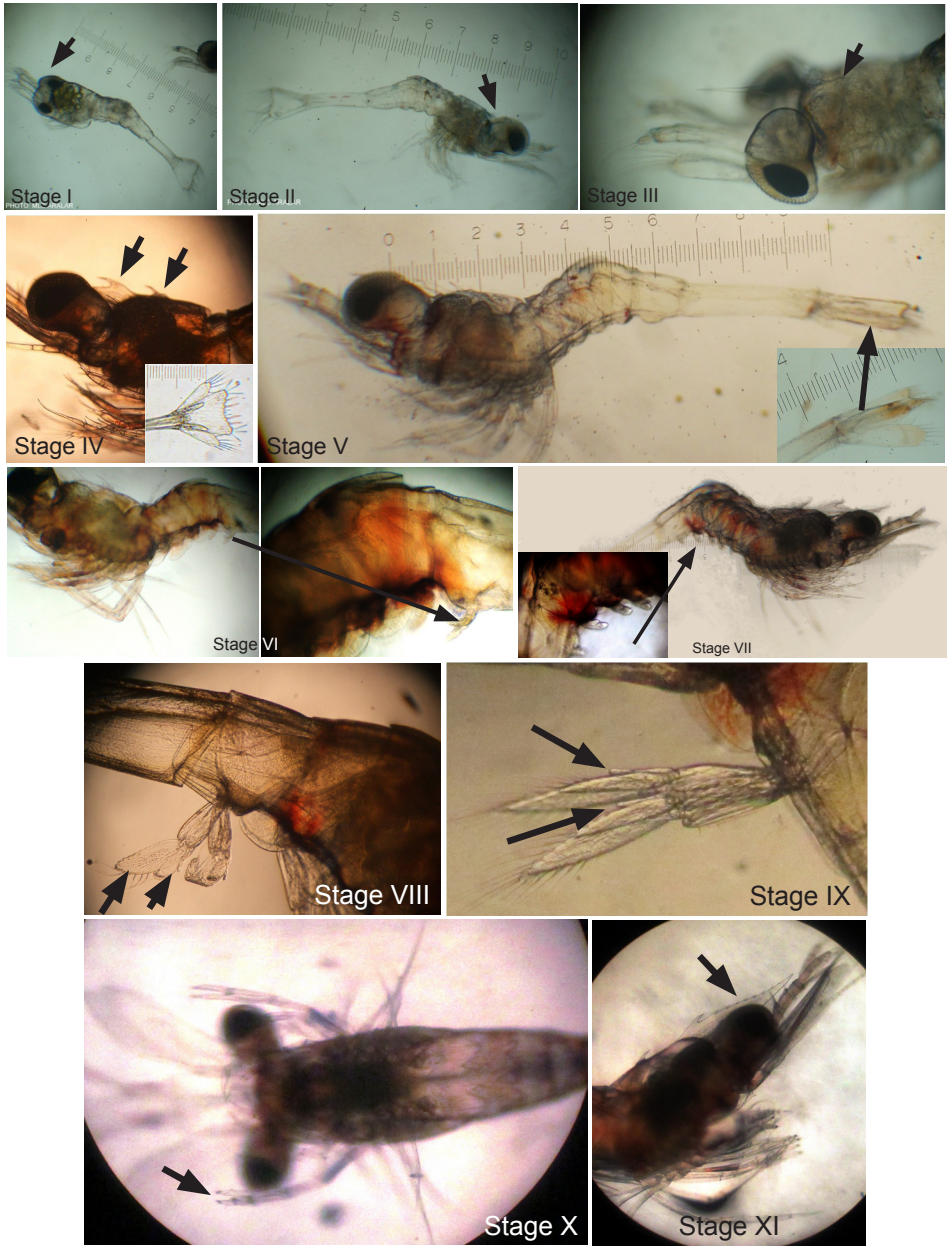

Fig. 30. Larval stages of the giant freshwater prawn. **Stage I:** eyes fused, no stalk; **Stage II:** eyes with stalk; **Stage III:** rostrum with a single dorsal tooth; **Stage IV:** rostrum with two teeth and uropods (inset) with setae; **Stage V:** telson elongated and narrower; **Stage VI:** first appearance of pleopod buds; **Stage VII:** pleopods or swimming legs biramous and bare (no setae or hairlike projections); **Stage VIII:** pleopods with setae; **Stage IX:** pleopods with developed appendices internae; **Stage X:** first two walking legs fully chelated; **Stage XI:** rostrum with many dorsal teeth.

Fig. 31. Newly metamorphosed post-larva, PL (left), of the giant freshwater prawn. The reddish color of the PLs (right) is imparted by the diet of *Artemia* nauplii.

It takes, on the average, 30 days for the Stage I larvae to fully metamorphose into PL. However, various factors such as temperature, nutritional status, and other environmental factors affect larval development.

WATER QUALITY FOR LARVAL REARING

The optimum temperature range for larval development is between 28 to 31°C. Growth and development slows down when temperature is below 26 to 24°C. Sudden changes in temperature, even 1°C difference, can be detrimental to the larvae. Dissolved oxygen should at least be 7mg/L, hence, an aeration system as described previously is an integral part of the hatchery. A pH of 7 to 8.5 should be maintained. Periodic additions of sodium carbonate (Na_2CO_3) and sodium bicarbonate ($NaHCO_3$) may be needed to maintain pH. Minimal fluctuations in the salinity of the larval rearing water is not so critical, however, drastic fluctuations can shock the larvae and cause mass mortality. Sudden changes in salinity may occur in uncovered tanks during sudden heavy rains or when there is an error in the mixing of the saline and freshwater.

FEEDING MANAGEMENT

Larvae maybe given feed on the first day of hatching. Although Stage I (newly hatched) larvae do not feed, they may progress to Stage II within the day, which will require feeding. The feeding management should be strictly monitored because this is an important aspect in larval rearing. There are several feeds and feed types that are used in larval rearing: brine shrimp nauplii *Artemia* sp. (Fig. 32), freshwater cladoceran *Moina* sp., egg custard, and commercial feed are used.

Artemia cysts are commercially available in aquaculture supply shops. They are widely used in the larval rearing of a variety of aquaculture species. When choosing the right brand, the hatching rate of the cysts should be considered. Unlike inert feeds, live food organisms such as *Artemia* nauplii (AN) have the advantage in their ability to survive

in the rearing water until they are eaten and thus will not pollute the water when unconsumed. Larvae fed solely on AN have estimated larval ingestion rates of 40 nauplii/day from Stages II to IV; 55 nauplii/day for Stages V-VI; and 80-100 nauplii/day for Stages VII-VIII. Prey size larger than newly hatched AN is advisable for the final larval stages. Feeding behavior of larval prawn is based more on chance encounter rather than active seeking of prey, hence, prey density per unit of larval rearing water is an important factor. The amount of feed given will depend on the volume of the rearing water as well as the stocking density of larvae. Five AN per ml of rearing water may be given from Day 1 to Day 6. This can be reduced to 3 AN per ml from Day 7 to metamorphosis to PL if supplemented with egg custard (EC) (see below).

Fig. 32. Brine shrimp, *Artemia* cysts packed in cans (top) hatched nauplii (bottom-left) and nauplii with prawn larva (bottom-right).

Since *Artemia* is quite expensive, the use of inert diets such as EC is used to supplement larval diets. A thick suspension of finely mashed EC is dispensed into larval rearing tanks. Although it has been demonstrated that prawn larvae ingestion rate for EC is independent of particle size, it has been the usual practice to finely mash and sieve with 33 micrometer mesh for Day 7 to 16. A larger mesh size of 45 microns is used for feeding from Day 16 until metamorphosis to post-larvae.

EC may be given when the larvae is at least Stage III or after four or five days after hatching. EC is given during daytime feeding and AN in the late afternoon feeding or the last feeding of the day.

The suggested feeding schedule in Table 2 may be used as a guide. The amount of feed may be adjusted depending on the feeding rate of the larvae. It is important to clean the tanks before the last feeding with AN to ensure that the unconsumed EC will not pollute the water overnight.

Table 2. Suggested feeding schedule for larval rearing of prawns.

Time	Feed type
8:00 A.M.	AN*
11:00 A.M.	EC**
2:00 P.M.	EC
5:00 P.M.	AN

* AN - *Artemia* nauplii
** EC - egg custard

LARVAL FEED PREPARATION

Artemia nauplii (AN)

The required amount of AN is prepared one day prior to feeding. Two grams of *Artemia* cyst is hatched for every liter of saline water. The salinity will depend on the strain of *Artemia*. The cysts are placed in an aerated conical hatching jar (Fig. 33) and allowed to incubate for 22 to 24 hours at a temperature of 25-30°C at a pH of at least 8. Provide continuous illumination (e.g. fluorescent lamp) over the AN hatching jars. Remove aeration and allow for the hatched *Artemia* to settle for five minutes. The nauplii can be siphoned off from the bottom of the hatching jar and concentrated using a fine mesh silk screen (100-150 micron mesh) (Fig. 34). Harvested *Artemia* nauplii are disinfected by immersion in 30 ppm formaldehyde solution for 30 minutes, and washed with brackishwater before feeding to the larvae. This practice is recommended to eliminate bacterial load which may have contaminated the nauplii during hatching.

The AN suspension is mixed well by hand and counting of the nauplii is done to determine the number of nauplii per ml of suspension and consequently, how much should be rationed to the larval rearing tanks.

Egg custard (EC)

This is prepared from a mixture of 10 g skimmed milk and one whole chicken egg. The ingredients are mixed well together with a pinch of red food color to make the EC visible to the operator and to monitor consumption. The mixture is placed in a container and steamed or cooked in a microwave. After cooling, the resulting egg custard is sieved through a fine mesh, to break down the particle size suitable for the prawn larvae (see foregoing section). Water is added to make a thick suspension of the EC before it is dispensed to the larval rearing tanks (Fig. 35). EC may be stored in the freezer for a couple of days but freshly prepared EC is more beneficial to the larvae.

Fig. 33. Sample of conical incubation jars for *Artemia*, made of fiberglass (left) and glass (right).

Fig. 34. *Artemia* nauplii is siphoned from the bottom of the incubation jars through a fine mesh screen (left) then washed thoroughly (right) (Photos: A Lazartigue).

HARVESTING OF POST-LARVAE

There are two methods of harvesting the newly metamorphosed PL. One method is by draining the larval rearing tank leaving about a third of the original volume. Freshwater is gradually added to the tanks until the salinity is zero in 12 h. Continuous aeration should be provided during the acclimation process. The PL remain in the original larval rearing tanks for a few days before transfer to nursery.

The other method involves partially draining the tank and the PL collected by a fine mesh net carefully to avoid damage or injury to the new PL. Although new PLs can withstand the shock of transfer from 12 ppt salinity to freshwater, it is still recommended to acclimate the PL to freshwater either by transferring them to progressively lower salinity water (e.g. 9 ppt to 6 ppt to 3 ppt to freshwater) in stages or gradual dilution of the brackishwater with freshwater. Once acclimated to full freshwater, the PLs can be transferred to the nursery.

Larvae metamorphosis to PLs do not occur at the same time. Some larvae develop faster or slower than the others. If the PLs are left together with the still developing larvae for several days, cannibalism may become a problem. If the development to PL is protracted, PL harvest may be done in batches, with the first batch timed when a third of the larval batch has metamorphosed to PLs. Two or three more subsequent harvests are done until all the PLs have been collected.

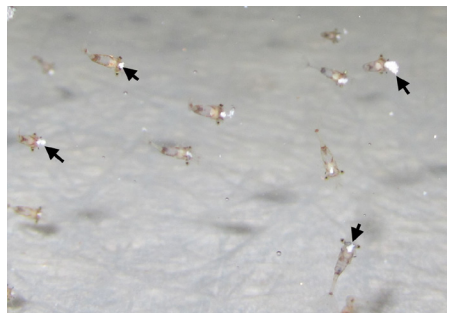

Fig. 35. Prawn larvae feeding on egg custard (black arrows).

NURSERY FOR POST-LARVAE

Nursing newly metamorphosed PLs before they are stocked in the grow-out systems significantly improves survival. The nursery's aim is to allow the PLs to grow bigger and stronger to enable better survival in the grow-out. PLs are categorized based on the number of days after they metamorphose, e.g. PL 15 for 15-day old PLs.

Primary nursery

Primary nursery facilities for newly hatched PLs may use similar ones for the larval rearing, concrete, plastic or fiberglass tanks. The PLs are reared for a few days to a month in the primary nursery, after which they may either be stocked directly in grow-out or in secondary nursery facilities for longer nursing. Tanks of various sizes can be used depending on the required capacity of the primary nursery. Water depth of 1 m is sufficient. PLs are substrate dwellers, thus stocking density is dependent on the unit area, instead of unit volume. Newly metamorphosed PLs may be stocked up to 5,000 pcs/m^2 provided additional substrates such as nets are added to the nursing tanks. The density should be progressively reduced to half after every week. The substrates provide additional surface area for the PLs (Fig. 36). If the larvae will be kept in the nursing tanks for a month without reduction in density, it is recommended to stock no more than 1,000 new PLs. Once or twice a day feeding is sufficient. Initially, egg custard used in the larval rearing may still be fed for a few days to the new PLs and gradually weaned to commercial dry diets, preferably with at least 35% crude protein content. Initial feeding rate may be as high as 50% of the total body weight equivalent of the PLs. Because of the high feeding rate and stocking density, tank hygiene should always be observed. Tanks are cleaned daily with 30 to 50% of the water replaced, if the static-renewal system is used. New PLs may grow four to more than six times its initial stocking weight in three weeks depending on stocking density.

Fig. 36. Net substrate inside nursery tanks. Substrate provides additional surface area for the PLs.

Secondary Nursery

If further nursing is needed, ponds and cages in ponds or open water bodies (Fig. 37), in addition to concrete tanks, may be used as secondary nurseries. Prawns from PL 15 may be stocked in these secondary nurseries at 100 to 200 pcs/m^2 for extended rearing of one to two months. PLs with initial stocking weight of 0.05 g may grow to 0.3 to 0.8 g after 60 days in secondary nursery, depending on initial stocking density. Survival rates of 80% or better are typical for a well-managed secondary nursery.

After the secondary nursery phase, the prawns are now considered early to advanced juveniles (Fig. 38) ready for stocking in grow-out ponds and cages.

Fig. 37. Secondary nursery facilities for PLs may be ponds (left) or hapa net cages (right) in open water.

PACKING AND TRANSPORT

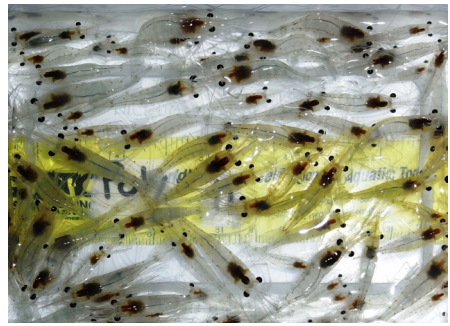

Fig. 38. Juvenile *M. rosenbergii*

PLs and juvenile prawns for transport to the site for grow-out are conditioned before packing. The prawns are starved for at least 24 hours prior to packing to minimize excretion and fouling of the water during transport. During the conditioning period, feces are siphoned out to prevent reingestion. The PLs are counted and packed inside extra strength clear transport bags (Fig. 39). The number of juveniles per bag will depend on the size of the prawn and the travel time. Typically 500 pcs well conditioned 15 day-old post-larvae (PL 15) may be packed in a 50 x 75 cm in plastic bag containing 3 to 5 liters of water for 3 hours transport time. Bigger PLs and longer travel time will require lower stocking densities in the transport bags. To minimize extreme temperature fluctuations, the plastic bags are placed inside insulated boxes.

Fig. 39. Packing of giant freshwater prawn PLs in plastic bags (left) and filling the bags with oxygen (right).

HEALTH MANAGEMENT AND DISEASES

Integral to the success of seed production of *Macrobrachium rosenbergii* is the health management aspect. Health management involves routine practices such as disease prevention, detection, diagnosis and control.

Culture Management

Maintenance of good water quality in the hatchery and nursery is the first step to ensuring the health of prawn larvae, PLs, and juveniles. These stages are particularly vulnerable to unfavorable water quality conditions. For instance, egg hatchability is very sensitive to pH changes. Highest egg hatching rate at 12 ppt salinity is obtained in pH 7. At pH 6.5 and 7.5, hatching rate is drastically reduced and beyond these, zero rates have been reported. Survival, growth, feeding, and molting of juveniles are also affected by unfavorable pH. Nitrite, NO_2-N, levels higher than 2 mg/L have been shown to delay larval development and lowers survival in the hatchery. Curiously, late zoeal stages of larval development are more sensitive to high ammonia and unfavorable pH compared with earlier stages (up to Stage VIII). Total ammonia nitrogen levels of 5 mg/L or higher also has significant effect on survival even of late juveniles. Regular tank cleaning and water replacement have been mentioned previously.

Sanitation

It is recommended that after each batch in the hatchery and nursery, the culture facilities should be cleaned and disinfected whenever feasible before a new run is started. Chlorine solution is a good disinfectant. Hatchery and nursery personnel should practice good hygiene as well to prevent introduction or reintroduction of pathogens.

Antibiotics, probiotics and immunostimulants

The use of antibiotics is common not only in penaeid hatcheries but also in giant freshwater prawn hatcheries. However, this is strongly discouraged because of human health implications. Products treated with antibiotics have limited market acceptability with the implementation of strict regulatory standards on antibiotic and other chemical residues.

The use of probiotics or useful microorganisms to control pathogens is widely accepted. Immunostimulants, or products that enhance the immune response of the prawns to disease and pathogens is also an alternative to the use of restricted drugs.

Viral Diseases

Compared with penaeid shrimps, *M. rosenbergii* is considered moderately disease resistant. Among the pathogens, viruses are very important and responsible for huge losses, especially in the hatchery and nursery phases.

- ***Macrobrachium* Hepatopancreatic Parvo-Like Virus (MHPV).** This is the first reported virus in *M. rosenbergii*. The virus resembles hepatopancreatic parvovirus (HPV) found in penaeid shrimp but did not cause mortalities and even in stress conditions, no increase in intensity and prevalence was found with this virus.

- ***Macrobrachium* Muscle Virus (MMV).** This disease is similar to idiopathic muscle necrosis (IMN) and has been reported in PLs. Infected PLs showed opaque areas in the abdominal segment with reduced feeding and swimming activity. It is found only in *M. rosenbergii*'s striated muscle.

- **Infectious Hypodermal and Haematopoietic Necrosis Virus (IHHNV).** This virus can cause up to 100% mortality and has been reported in post-larvae.

- **White Spot Syndrome Virus (WSSV).** This virus is highly pathogenic and responsible for huge economic losses in penaeid shrimp culture. This virus is also highly pathogenic to crabs and freshwater prawns. Although *M. rosenbergii* is not a natural host of this virus, experimental infections have been conducted and resulted in significantly lower mortalities (5%) compared with 95% in penaeid shrimps.

- ***Macrobrachium rosenbergii* Nodavirus (MrNV) and Extra Small Virus-like Particle (XSV).** These two viruses have been known to cause white tail disease (WTD) (Fig. 40) in *M. rosenbergii*. The viruses have been reported to cause 100% mortality in post-larvae. Lethargy and opaque abdominal muscles, as well as degeneration of the telson and uropods, are the overt symptoms of infection.

The first three viruses described above are not common and have been reported once in Malaysia in the case of MHPV and Taiwan, for both MMV and IHHNV. Few studies are being pursued on these viruses. However, further research are being conducted on WSSV, MrNV and XSV.

Fig. 40. Prawn post-larvae showing white tail disease (Photo: AS Sahul Hameed).

Bacterial Diseases

Several opportunistic bacteria have been identified in the culture water of *M. rosenbergii*. These have also been found in the gut microflora. These bacteria may become pathogenic under adverse rearing conditions. Species of *Vibrio* have been isolated from eggs, larvae, and post-larvae. Very few studies have been conducted to document bacterial pathogens in freshwater prawns.

Fungal Diseases

Heavy losses have been reported by hatcheries infected by the fungi *Lagenidium* spp. and *Fusarium solani*. Larvae infected with *Lagenidium* can be diagnosed under the light microscope. Extensive mycelia (root-like growth) in the shell of dying or dead infected larvae. *Fusarium solani* is an opportunistic fungus and takes advantage of openings due to damage or lesions in the animal.

Adequate training in disease diagnosis is needed to effectively diagnose the various diseases which may affect seed production.

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Manuel A. Laron has worked with SEAFDEC/AQD as a Technical Assistant (1980-2003) and Associate Researcher (2004-2010) at its Binangonan Freshwater Station wherein he conducted studies on larval rearing; nursery rearing and grow-out cage culture of freshwater fishes and freshwater prawn; and broodstock management and nursery rearing of Asiatic carp, catfish, and tilapia. Mr. Laron likewise worked on various research on the *Macrobrachium* species such as the evaluation of different live food organisms as starter food for *M. rosenbergii* (De Man) larvae and the refinement of broodstock and hatchery management methods for the commercial production of the giant freshwater prawn seedstock and of the bioeconomics of freshwater prawn hatchery production in different larval rearing systems. Furthermore, Mr. Laron made refinements on broodstock and nursery technology for the commercial production of bighead carp *Aristichthys nobilis* (Richardson) fingerlings in cages in Laguna de Bay. He performed supportive breeding and restocking of indigenous freshwater fishes in selected Philippine inland water system such as the cage farming of silver therapon, *Leiotherapon plumbeus*, also in Laguna de Bay. Mr. Laron obtained his B.Sc. in Fisheries, major in Inland Fisheries, from Don Mariano Marcos State University in La Union, Philippines and M.Sc. in Animal Nutrition from Universiti Putra Malaysia, Selangor, Serdang, Malaysia.

Emiliano V. Aralar has worked with SEAFDEC for almost 30 years. He is a Senior Technical Assistant and currently the Head of the Binangonan Freshwater Station of SEAFDEC/AQD. He finished his B.Sc. Chemical Engineering at Adamson University, Philippines with M.Sc. units at Universiti Putra Malaysia. He has undergone training on Integrated fish farming in China, Aquaculture health management in Japan, and Giant freshwater prawn hatchery, nursery, and culture in Thailand and has since been invited as resource person on water quality, carp breeding, and design and construction of aquaculture facilities.

Ursan C. de la Paz is a Technical Assistant at SEAFDEC/AQD's Binangonan Freshwater Station and is working on the breeding of freshwater fishes, particularly, the giant freshwater prawn. He earned his B.Sc. Inland Fisheries degree from the University of Rizal System in 2006.

ABOUT SEAFDEC

The Southeast Asian Fisheries Development Center (SEAFDEC) is a regional treaty organization established in December 1967 to promote fisheries development in the region. The member countries are Brunei Darussalam, Cambodia, Indonesia, Japan, Lao PDR, Malaysia, Myanmar, the Philippines, Singapore, Thailand and Vietnam. The policy-making body of SEAFDEC is the Council of Directors, made up of representatives of the member countries.

SEAFDEC conducts research on fisheries problems; generates appropriate fisheries technologies; trains researchers, technicians, fishers and aquafarmers, and managers; disseminates information on fisheries science and technologies; and recommends policies pertaining to the fisheries sector.

SEAFDEC has four departments that focus on different aspects of fisheries development:

- The Training Department (TD) in Samut Prakan, Thailand (1967) for training in marine capture fisheries
- The Marine Fisheries Research Department (MFRD) in Singapore (1967) for post-harvest technologies
- The Aquaculture Department (AQD) in Tigbauan, Iloilo, Philippines (1973) for aquaculture research and development, and
- The Marine Fishery Resources Development and Management Department (MFRDMD) in Kuala Terengganu, Malaysia (1992) for the development and management of fishery resources in the exclusive economic zones of SEAFDEC member countries

SEAFDEC/AQD is mandated to:

- Conduct scientific research to generate aquaculture technologies appropriate for Southeast Asia
- Develop managerial, technical and skilled manpower for the aquaculture sector
- Produce, disseminate and exchange aquaculture information

SEAFDEC/AQD maintains four stations: the Tigbauan Main Station and Dumangas Brackishwater Station in Iloilo province; the Igang Marine Station in Guimaras province; and the Binangonan Freshwater Station in Rizal province. AQD also maintains a Manila Office in Quezon City.