

Black Tiger Shrimp

(Penaeus monodon)

HATCHERY OPERATIONS USING ENHANCED BIOSECURITY MEASURES

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Foreword

When SEAFDEC/AQD was established in 1973, its primary research focus was on shrimp culture, specifically the breeding of the black tiger shrimp, *Penaeus monodon*, and the production and securing of quality seedlings. Not long after, in 1975, the research center successfully completed the life cycle of the shrimp in captivity, using eyestalk ablation for inducing maturation. In the following years, the mass production of spawners in marine pens and fry in small scale barangay hatcheries were achieved, along with regular training courses done beginning 1977.

These, and other milestones by SEAFDEC/AQD in the areas of shrimp nutrition, contributed to a burgeoning shrimp industry in the Philippines that peaked at over 90 thousand metric tons in 1994. However, unrestrained intensification triggered the worldwide spread of shrimp diseases in the 1990s that plague the industry to this day.

With renewed vigor, SEAFDEC/AQD launched the “Oplan Balik Sugpo” program in 2018 to push for the revival of the black tiger shrimp industry in the Philippines which is now only a fraction of its size when it peaked. The program aims to exclude shrimp pathogens to produce high-quality postlarvae in the hatchery phase, and to use environment-friendly approaches in the grow-out phase.

This manual is a welcome summary of the best practices that have been developed at SEAFDEC/AQD’s biosecure shrimp hatchery complex. We hope other hatchery operators adopt these protocols, along with good aquaculture practices, for them to produce quality shrimp seeds and contribute to the responsible re-expansion of the shrimp industry.



Dr. Sayaka Ito
Deputy Chief, SEAFDEC/AQD

About the Manual

This manual, titled “Black Tiger Shrimp (*Penaeus monodon*) Hatchery Operations Using Enhanced Biosecurity Measures,” includes modifications on shrimp hatchery operations done by the Aquaculture Department of the Southeast Asian Fisheries Development Center to provide high-quality postlarvae for shrimp farming. Discussed also in this manual are the necessary protocols and biosecurity measures that shrimp hatchery operators can use as their guide.

Main sections included in this manual: 1. Site Selection, 2. Biology, 3. Hatchery Layout, 4. Facilities and Equipment, 5. Biosecurity Standard Operating Procedures, 6. Hatchery Operations, 7. Diseases of Shrimps, and 8. Economic Analysis.

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1

Introduction

Shrimp aquaculture is a lucrative industry bringing in substantial profits every year, as evidenced by its contribution to the aquaculture production of Southeast Asia and the world. The production of the black tiger shrimp (*Penaeus monodon*) started when it was discovered as an incidental crop in milkfish culture. It was in 1975 when SEAFDEC/AQD successfully spawned penaeid shrimps in captivity through eyestalk ablation for inducing maturation. This resulted to the mass production of fry from ablated breeders in small-scale barangay hatcheries. Training courses and technology transfer programs were also conducted. Since then, a lot of research and development activities were made that led to boost the production of this commodity.

The increasing demand for shrimps resulted into the intensification of farming which negatively impacted the environment. Cultured shrimps are vulnerable to diseases owing to their lack of an adaptive immune system. Irresponsible aquaculture practices, which are typically involved in farm intensification, could give way to the emergence of new diseases or the recurrence of existing ones. Disease outbreaks in farms are often serious and costly and may lead to the collapse of the local industry.

The shrimp industry was first affected in the 1990s by the devastating Luminous Vibriosis, when it entered into pond culture systems and led to massive losses of stocks and disruption of a majority of pond operations from key producing areas. The Philippines was at the forefront of *Penaeus monodon* production, with its peak production from 1993–1995 (**Figure 1**). However, the industry started to collapse from 1996 onwards largely due to over intensification and unsustainable farming practices. Pollution and disease outbreaks, specifically the White Spot Syndrome Virus (WSSV) and Yellow Head Virus (YHV), negatively impacted the aquaculture environment.

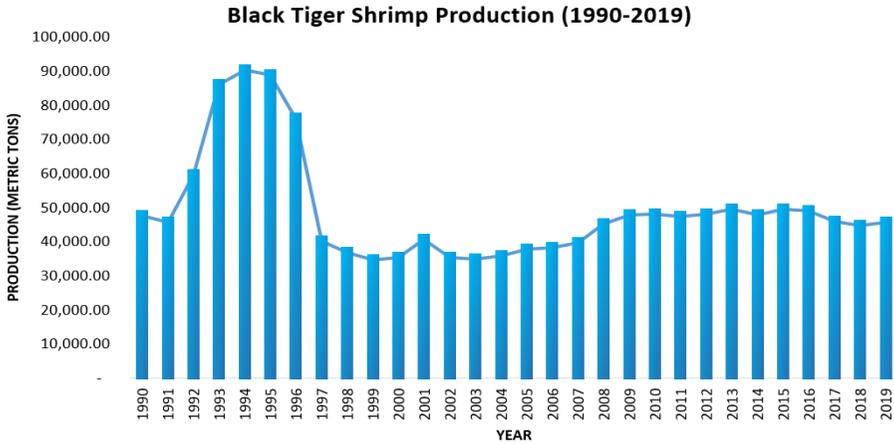


Figure 1. Philippine black tiger shrimp production from 1990 to 2019

Until now the shrimp industry in the country, and Asia in general, has not fully recovered yet. With this, SEAFDEC/AQD initiated the “Oplan Balik Sugpo” program which aims to revive the industry and to supply shrimp farmers with high-quality postlarvae (PL) to be used in grow-out culture. In this bid SEAFDEC/AQD introduced some modifications in its hatchery operations, primarily on enhancing biosecurity measures and good aquaculture practices (GAqP). This manual will guide the hatchery operators in the necessary protocols that should be incorporated with biosecurity measures to have a successful shrimp fry production.

2

Site Selection

The hatchery should be situated in an area suitable for the culture of black tiger shrimp. It should be far from possible areas of pollution such as domestic, industrial, and agricultural discharges or effluents. The following are criteria in selecting the site for the establishment of a shrimp hatchery.

Water Supply

The shrimp hatchery should have a clean and ample supply of seawater and freshwater. Seawater is mainly used for the culture of shrimps while freshwater will be utilized for the washing and rinsing of tanks and materials, and for the washing needs of the staff. The site should be far from mouths of rivers or streams with flowing water that can contribute to the abrupt change in the salinity. Aside from salinity, other water quality parameters such as temperature, pH, dissolved oxygen, ammonia and nitrites should be considered. **Table 1** shows the ideal range of water quality parameters in the hatchery.

Table 1. Ideal range of water quality parameters in maturation/hatchery

Parameter	Ideal Range
Salinity	29–36 ppt
pH	7.8–8.2
Temperature	28–32 °C
Dissolved oxygen	>4 ppm
Heavy metals/pesticides	minimal level
Iron	<1 ppm
Ammonia (NH ₃)	<0.1 ppm
Nitrite (NO ₂)	<0.1 ppm
Nitrate (NO ₃)	<10 ppm
Hydrogen sulfide (H ₂ S)	<0.003 ppm

Source of Spawner or Broodstock

It is recommended that the hatchery should be near the source of spawners or broodstock to minimize the stress and lessen the transport cost. It is important to make sure that the source has no record of disease occurrence.

Accessibility and Availability of Electric Power

The hatchery must be located near roads for easy transport of feeds, materials, and equipment. It must be accessible for buyers or grow-out farms for faster disposal of fry. Most importantly, it should be supplied with good and stable electric power to have a continuous operation. The electric power supply will run the equipment and other life support systems needed in the hatchery.

3 *Biology*

Black tiger shrimp (*Penaeus monodon*) is considered as one of the largest penaeid shrimps in the world and a commercially important species because it has a strong demand with high prices in the national and international markets. It is widely distributed throughout the Indo-West Pacific Region, particularly in tropical countries such as Indonesia, Malaysia, and the Philippines where the main fishing grounds are mostly located. Surface waters such as the shore areas and mangrove estuaries are inhabited by the fry, juveniles, and adolescents while most of the adults inhabit waters down to about 160 meters. **Figure 2** shows the life history of *Penaeus monodon*.

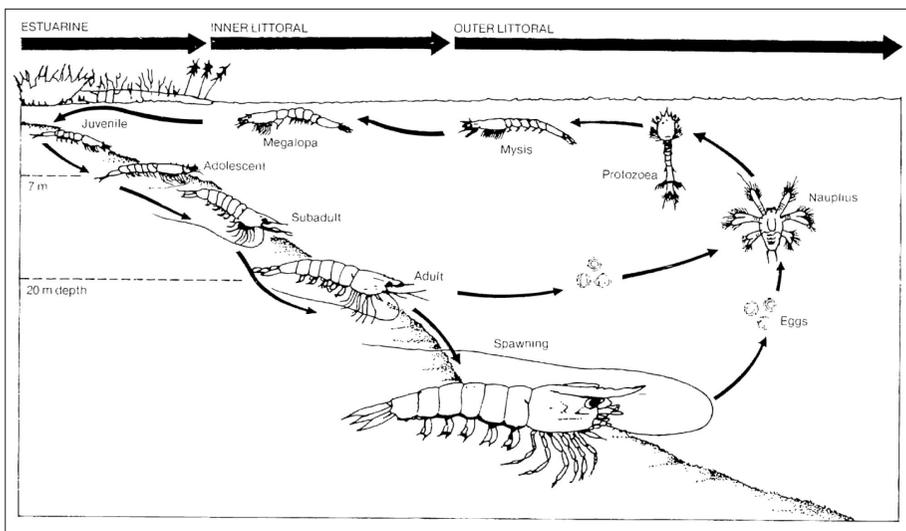


Figure 2. Life cycle of *Penaeus monodon*

Figure 3 shows live black tiger shrimp characterized by having striated bands of red and white, and greyish brown antennae. The pleopods and pereopods are brown with crimson fringing setae. In shallow brackish waters or when cultured in ponds, the color changes to dark brown to blackish brown.



Figure 3. Adult *Penaeus monodon*

The rostrum is sigmoidal in shape and extends beyond the tip of the antennular peduncle. It has 6–8 dorsal and 2–4 ventral teeth, mostly 7 and 3, respectively. **Figures 4** and **5** show the external anatomy of the shrimp and the methods of measuring the shrimp.

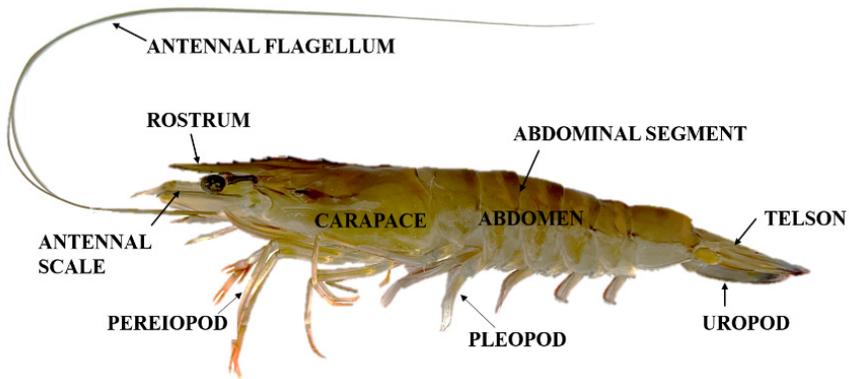


Figure 4. External anatomy of *Penaeus monodon*

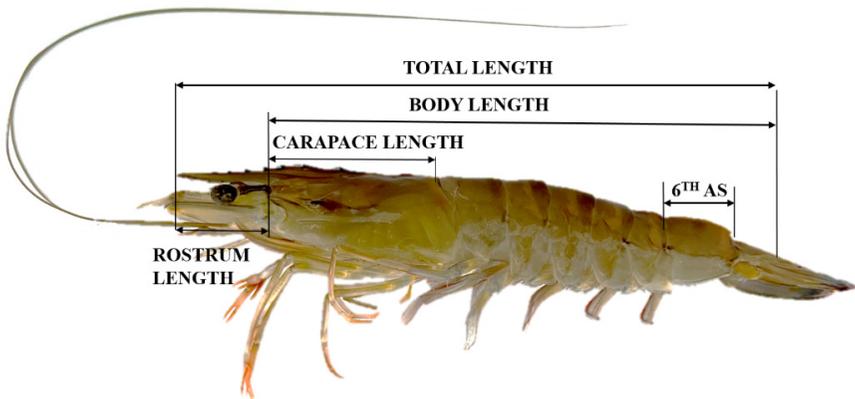


Figure 5. Methods of measurement of *P. monodon*: RL - rostrum length; CL - carapace length; TL - total length; BL - body length; 6th AS - length of the 6th abdominal segment

P. monodon is heterosexual and has separate sexes. Male shrimps are distinguished by having a petasma and an appendix masculina, while females are distinguished by their thelycum. The petasma is located between the 1st pleopods and the appendix masculina is on the exopods of the 2nd pleopods.

Mating happens at night, when the female has newly-molted. During mating, the male positions below the female in parallel swimming. The pereopods of the female hold on to the carapace of the male and maintains this position while swimming continues. The male then turns ventral side up and attaches to the female, and aligns the thoraco-abdominal junction with the posterior thorax of the female. The male turns perpendicular to the female, rotating at the point of the posterior end of the thorax. It curves

its body in U-shape around the thorax of the female and flicks both head and tail simultaneously. The male deposits the spermatophore inside the thelycum of the female. The deposited sperm remains inside the thelycum for a few weeks until the female releases them together with the eggs. **Figure 6** shows the courting and mating behavior of *P. monodon*.

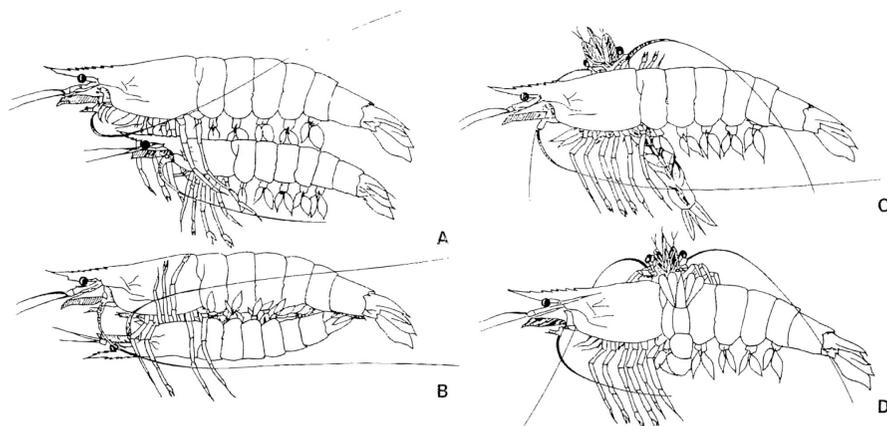


Figure 6. Courtship and mating behavior of *P. monodon* (Motoh, 1984). A. Female above-male below in parallel swimming, B. Male turns ventral side-up and attaches to the female, C. Male turns perpendicular to female, D. Male curves body around female and flicks head and tail simultaneously

Eggs

Eggs of *P. monodon* are spherical, yellowish green in color and somewhat translucent. The size ranges from 0.27 mm to 0.31 mm with an average of 0.29 mm diameter. Without aeration, they tend to sink slowly to the bottom. They develop into the nauplius stage after approximately 11 hours after spawning. The stages of egg development are shown in **Figure 7**.

Nauplius Stage

This is the stage after hatching and consists of six developmental substages (**Figure 8**) which take about 1.5–2 days to complete. Nauplii of shrimps are very tiny with its total length measuring from 0.30 mm to 0.58 mm. They are phototactic, which means that they are attracted to light, and without aeration, they concentrate in the most lighted portion of the tanks. They swim intermittently upwards in a “bat-like” manner using their appendages.

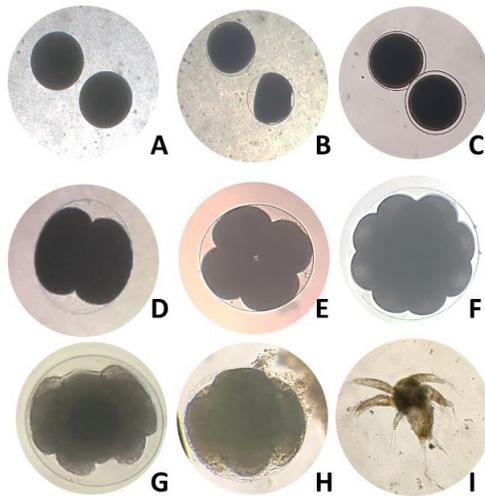


Figure 7. Egg development of *P. monodon*: (A) newly spawned egg; (B) separation of external membrane; (C) completion of external membrane; (D) fertilized egg, 2-cell stage; (E) fertilized egg, 4-cell stage; (F) fertilized egg, Morula stage; (G) early embryonic nauplius; (H) late embryonic nauplius; (I) nauplius

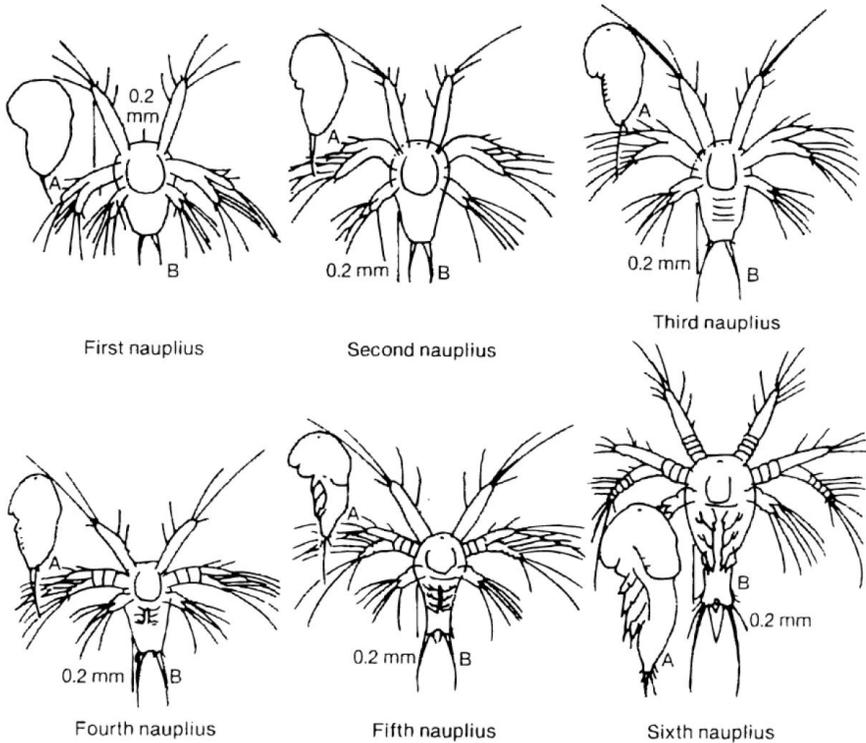


Figure 8. The development of the six nauplii substages of *P. monodon* (Motoh, 1984)

Protozoa Stage

The protozoa stage undergoes three substages and takes around 5 days to complete. It is distinguished from the nauplius stage by its more elongated body that measures from 0.96 mm to 3.30 mm total length and by its movement, which is characterized by swimming vertically and diagonally towards the water surface. When viewed under the microscope (**Figure 9**), the protozoa I (Z_1) can be observed to have unstalked eyes or having two dark spots in the upper portion of the carapace. At protozoa II, the eyes become stalked and the dorsal median spine appears at the sixth abdominal segment at protozoa III.



Figure 9. Developmental substages of the protozoa

Mysis Stage

The mysis is characterized by having a head pointing downward. It measures from 3.28 mm to 4.87 mm in total length and is composed of three substages: mysis I, II and III. At this stage, the telson and the uropods are already developed. Swimming is characterized by thoracic propulsion or by bending the abdomen backwards. As seen in **Figure 10**, the most prominent development on this stage is the appearance of the pleopods. Pleopods appear as buds at MI, protrudes at MII, and become segmented at MIII.



Figure 10. Developmental substages of mysis

Postlarval Stage

The postlarval stage of the black tiger shrimp is characterized by the presence of the plumose hairs on the swimming legs, and already resembles an adult shrimp (**Figure 11**). The age of the postlarvae corresponds to the number of days since the beginning of this stage.



Figure 11. Black tiger shrimp postlarvae with plumose hairs on the swimming legs

4 Hatchery Layout

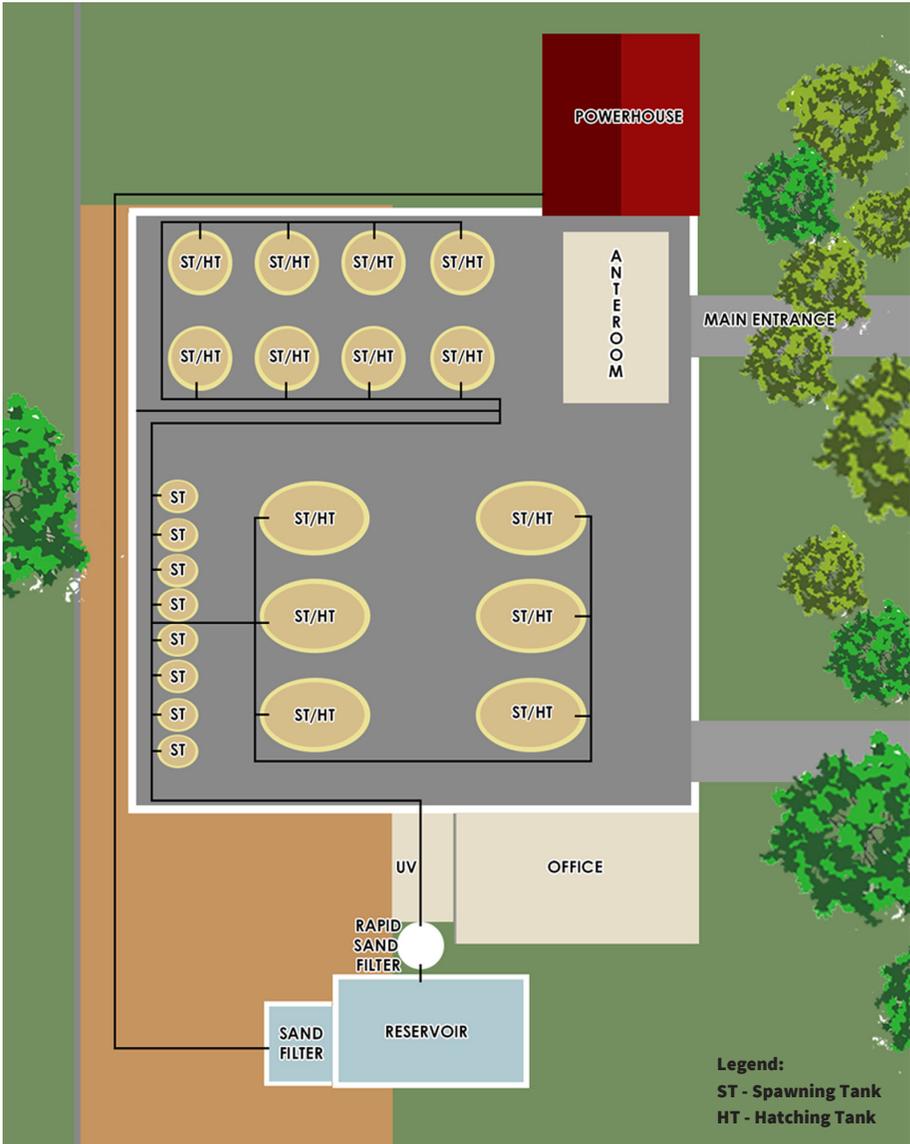


Figure 12. Layout of the Spawner/Broodstock Facility at SEAFDEC/AQD

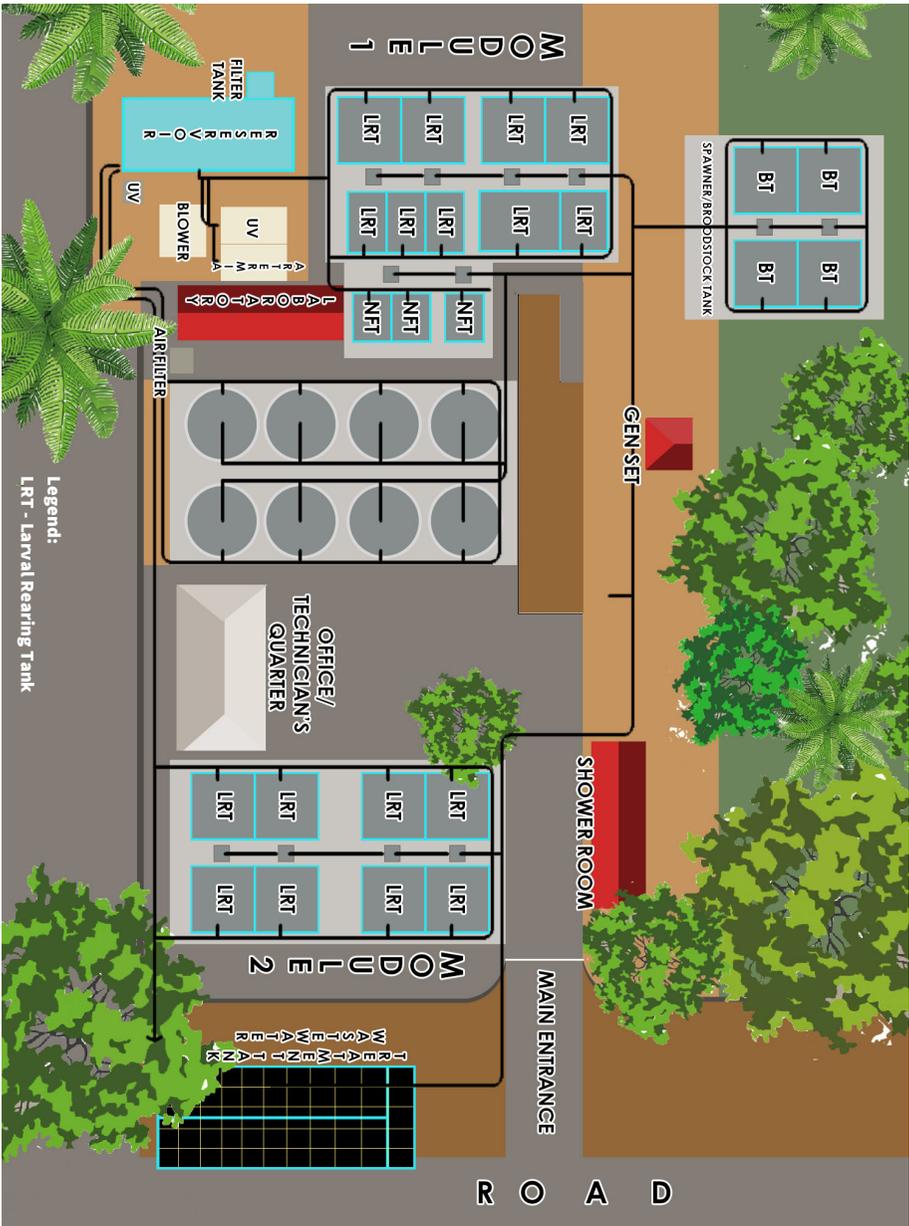


Figure 13. Layout of the Shrimp Hatchery Complex at SEAFDEC/AQD

5 Facilities and Equipment

Filtration System, Seawater Reservoir, and UV Sterilization System

Water from the source will undergo several filtration processes to ensure that the rearing water that will be used for the operation is free from disease-causing organisms. Maintaining good water quality enables us to predict the level of production that could be attained under existing conditions. The hatchery must practice filtration and UV sterilization to clean and disinfect the rearing water.

1. Seawater must pass through the sand filter (Figure 14) before reaching the covered reservoir (Figure 15).

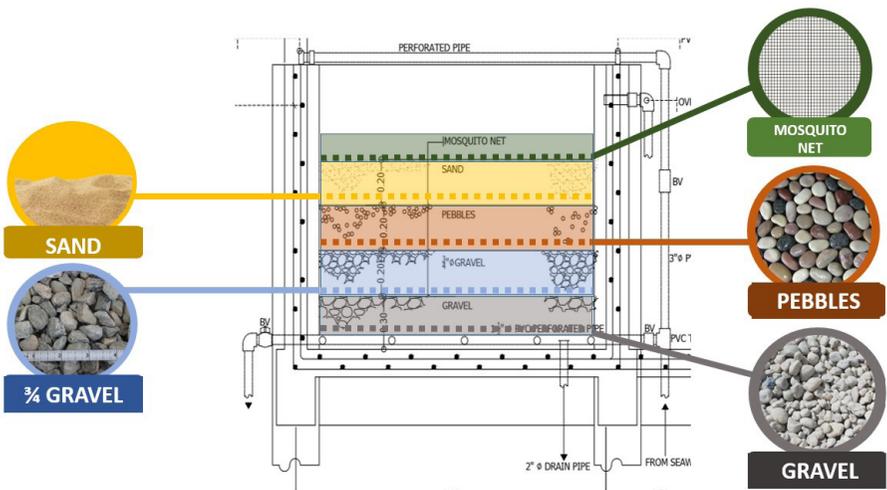


Figure 14. The components of a sand filter tank



Figure 15. The covered reservoir (A), the sand filter box (B), and the reservoir tank (C)

2. Seawater from the reservoir will pass through the rapid sand filter and UV sterilizer. Seawater outlet pipes on tanks will also be installed with 5 μm filter bags (**Figure 16**).



Figure 16. Seawater from the reservoir passes through various filtration and sterilization stages before reaching the larval rearing and natural food tanks.

Aeration System

Aeration is important to keep the feed and natural food suspended in the water column, and maintain sufficient dissolved oxygen levels. It will be supplied by a blower, and should pass through a 0.45 μm cartridge filter before reaching the tanks (**Figure 17**).



Figure 17. The aeration system: **A.** UV sterilizer, **B.** 0.45 μm cartridge filter, **C.** larval rearing tank with aeration

Spawning/Hatching Tanks

Spawning and/or hatching tanks (**Figure 18**) will be stocked with the spawners or eggs. The volume ranges from 0.25 ton to 1 ton, and preferably with tapered bottom to allow homogenous aeration necessary for hatching.



Figure 18. The hatching/spawning tanks at SEAFDEC/AQD

Larval Rearing Tanks

Larval rearing tanks will be covered to avoid contamination (**Figure 19**). These must be manageable, preferably with capacities ranging from 10–12 tons, and a maximum of 20 tons, depending on the operator’s preference and financial capability. Water depth should be about 1 m.



Figure 19. The larval rearing facility at SEAFDEC/AQD: **A.** exterior view of the facility, **B.** tanks completely covered with laminated black sack, and **C.** divisions provided between tanks.

Natural Food Tanks

The hatchery must have its own natural food laboratory to supply diatoms (*Skeletonema* sp.) and live feed (*Artemia*) during the whole operation of the hatchery. Starters of diatoms will be cultured here before scaling up to larger concrete outdoor algal tanks. Indoor culture of diatoms could utilize 1 L–20 L culture containers while outdoor culture could use concrete tanks. For *Artemia* culture, 250-L circular fiber glass tanks can be used. The tanks must have a conical bottom for faster draining and harvesting of *Artemia*.

Generator Set

The generator set will be used to provide electricity when there are cases of brownouts or blackouts. The hatchery will be equipped with a generator that automatically turns on during low or no power supply. This is to ensure that the electricity and aeration systems remain functional so that the stocked larvae/fry will not be affected.

Office and Technicians' Quarters

Hatchery staff will be provided with the technicians' quarters where they could stay during feeding schedules especially at night.

Wastewater Treatment Tank

All the effluents from the hatchery will be released into a wastewater treatment tank (**Figure 20**). After each operation, wastewater will be disinfected with 75 ppm chlorine and aerated for 24 hours to make sure that all discharges are safe to be released to the sea.



Figure 20. The wastewater treatment tank covered with black net

Other Materials and Equipment

A refrigerator is required to store feeds, vitamins, fertilizers, and other materials that are needed in the hatchery. A microscope and haemocytometer will be used to monitor and count the algal cells of diatoms for feeding, as well as to monitor the stages of development of the shrimp. A refractometer will also be needed for the determination of the salinity of rearing water, and a thermometer for the temperature monitoring. A weighing scale will be used for weighing feeds and chemicals.

6

Biosecurity Standard Operating Procedures

Shrimps are vulnerable to diseases; hence, biosecurity measures should be strictly implemented. Following strict biosecurity protocols prevents the exposure of healthy stocks to diseases and avoids contamination. The following are standard operating procedures (SOP) that a shrimp hatchery must follow:

1. It is recommended to have a spawner/broodstock facility. It will be a separate area from the shrimp hatchery that will be used to quarantine spawners while they are being processed and sampled for pathogen detection.
2. Prior to the arrival of the spawners or broodstocks, the whole area will be prepared, cleaned and disinfected, including the facilities and equipment. Two hundred (200) ppm chlorine solution will be prepared and splashed into the tanks and reservoir. It will then be left in the tanks for at least one day before being washed with detergent and rinsed with freshwater.
3. Other materials such as air hoses, air-stones, pails, basins, dippers, filter bags, filter nets, and other paraphernalia will be soaked in chlorine solution prior to the start of the operation.
4. After disinfection, the spawning tanks will be prepared by installing the aeration lines and covering them individually with black nets and black sacks. It is recommended that each tank must be provided with all necessary materials, such as filter bags, scoop nets, pails, and dippers, separately from the other tanks to prevent any cross-contamination.
5. Signages, foot baths, and hand sanitizers will be provided at the main entrance of the hatchery (**Figure 21**). Disinfectants such as alcohols, bleach, and chlorine must be available inside the facility. Slippers or rubber boots will be provided and will be used solely inside the facility (**Figure 22**).
6. Only authorized staff or hatchery personnel will be allowed to enter the facility and handle the stocks. The use of gloves is highly recommended to avoid contamination.
7. A “one-way in and out” scheme will be practiced for entrance and exit.



Figure 21. Biosecurity measures practiced inside the Spawner/Broodstock Facility: **A.** signages on doors, **B.** foot bath, **C.** hand sanitizers, and **D.** changing/wearing of laboratory gowns and boots



Figure 22. Entry-level biosecurity at the Shrimp Hatchery Complex: **A.** slippers and scrub suits, **B.** changing/wearing of scrub suits and slippers, **C.** use of hand sanitizers before entry, **D.** laboratory gowns inside the anteroom

Tank Preparation and Disinfection

To prevent disease outbreaks, tanks and other facilities will be thoroughly cleaned and disinfected to eliminate pathogens. Disinfection will be done by preparing 200 ppm of chlorine and splashing it into the tanks and reservoir. The tanks will then be left for at least three days before it will be rinsed with freshwater. Other materials such as air hoses, air stones, pails, basins, dippers, filter bags, filter nets, etc. will also be soaked in chlorine.

After disinfection, these tanks will be cleaned by scrubbing the sides and bottom with a mixture of water, detergent, and sodium hypochlorite. Tanks will be washed thoroughly using freshwater. Seawater inlet pipes will be fitted with 5 μ m filter bags before filling in with UV-treated seawater.

All tanks, including each division, will be covered with black sacks to avoid entry of other organisms and to avoid cross-contamination within the tanks.

Natural Food Production

Indoor Culture of Diatoms (Skeletonema sp.)

For indoor culture, UV-sterilized seawater will be used to culture the diatoms. The fertilizer that will be used for diatoms is the F-medium (composed of EDTA, trace metals, vitamin stock, disodium phosphate, ferric chloride, and disodium silicate) and TungKang Marine Research Laboratory medium (TMRL) (composed of sodium nitrate, disodium phosphate, ferric chloride and disodium silicate). Culturing the diatoms in the laboratory is shown in **Figure 23**.

1. Examine the starter under the compound microscope to check if the cells are suitable for culture.
2. Add appropriate volume of UV-sterilized seawater in clean glass containers.

3. Add fertilizer at 1 ml per liter to the container and to be followed with the addition of the starter.
4. Provide aeration and illumination until the culture is ready to be fed or scaled up to larger containers.



Figure 23. A hatchery staff conducting the culture of diatoms (*Skeletonema* sp.) inside the laboratory. **A.** checking of diatom cells under microscope, **B.** addition of UV-sterilized seawater, **C.** addition of F-medium fertilizer, **D.** measuring of *Skeletonema* sp. starter, **E.** addition of *Skeletonema* sp. on UV-sterilized seawater, **F.** addition of aeration and illumination

Outdoor Culture and Harvest of Diatoms (Skeletonema sp.)

1. Prepare the concrete algal tanks for the outdoor culture of *Skeletonema* sp. (**Figure 24**).
2. Place a ton of UV-sterilized seawater and supply with aeration.
3. Two types of fertilizers will be used: urea (46-0-0) and ammonium phosphate (16-20-0). Completely dissolve urea (75 g) and ammonium phosphate (20 g) in a pail of freshwater prior to application.
4. Filter the fertilizer solution using a 90 μ m filter net, and add the diatom starters.
5. The diatoms will be expected to bloom for 3–4 days before they will be harvested.



Figure 24. Outdoor culture of *Skeletonema* sp. **A.** tank for *Skeletonema* culture, **B.** urea and ammonium phosphate, **C.** mixing of fertilizers, **D.** filtration of the fertilizer

Harvesting of Diatoms (*Skeletonema* sp.)

1. To harvest diatoms, attach a double-lined harvesting bag at the end of the drain pipe of the algal tank (**Figure 25**).
2. Slowly remove the stand pipe to allow the diatoms to flow, thereby concentrating in the harvesting bag.
3. The concentrated diatoms will be placed in pails and will then be divided for feeding. The recommended feeding density for algae is 20,000–50,000 cells/ml.



Figure 25. Harvest of *Skeletonema* sp. from outdoor algal tanks. **A.** concentration of diatoms using double-layer harvesting bag, **B.** draining of *Skeletonema* sp. from culture tank, **C.** concentrated *Skeletonema* sp. in 10-L pail

Artemia Culture and Harvest

1. Weigh the desired amount of *Artemia* cysts (**Figure 26**).
2. Hydrate the *Artemia* cysts in a pail supplied with aeration and UV-sterilized seawater for an hour.
3. Disinfect the cysts with 20 ppm sodium hypochlorite for 15 minutes.
4. Rinse the cysts with running UV-treated seawater until the smell of sodium hypochlorite disappears.
5. Incubate the cysts in a 250 L incubation tank filled with UV-sterilized seawater with aeration for 24 hours.



Figure 26. *Artemia* culture: **A.** weighing of cysts, **B.** hydration in a 10 L pail with UV-sterilized seawater, **C.** disinfection using sodium hypochlorite, **D.** rinsing, **E.** transferring the rinsed cysts to the incubation tank

Harvesting of Artemia

1. Prior to harvest, remove the aeration and cover the top portion of the incubation tank for at least 30 minutes to separate the unhatched cysts from the nauplii. The unhatched cysts will float while the nauplii will accumulate at the lower portion of the tank (**Figure 27**).
2. Slowly open the outlet of the incubation tank to avoid the mixing of unhatched cysts and nauplii.
3. Collect the nauplii using a filter box and wash with running UV-sterilized seawater. The nauplii will then be concentrated in a pail and fed to the shrimps.



Figure 27. Harvesting *Artemia* nauplii: **A.** incubation tank with harvesting box, **B.** draining of *Artemia* nauplii, **C.** rinsing, **D.** transferring from harvesting box to 10 L pail with UV-sterilized seawater, **E.** harvested *Artemia* nauplii

Selection and Processing of Spawners

Acclimatization and Disinfection

Penaeus monodon spawners will be delivered from the source to the spawner/broodstock facility. The spawners must be packed in transport/broodstock bags with ample amount of seawater and aerated using battery-operated aerators.

Processing of Spawners

1. Upon arrival, acclimatize the spawners by placing them in white basins with aeration (**Figure 28**).
2. Check the salinity of the transport water and seawater on site to determine the salinity difference between the two. The salinity inside

the basin should be slowly adjusted to equalize with the salinity of the water in the transport bag.

3. When the salinities of both waters have already equalized, the spawners will then be allowed to acclimate for two hours.
4. After two hours, disinfect the spawners using 50 ppm povidone-iodine (added to the water in the basin).



Figure 28. A. acclimatization of the spawners in basin upon arrival, B. adjusting the salinity of the water, and C. disinfection of the spawners using povidone-iodine

Checking of Gonadal Maturity and Stocking

1. After disinfection, individually place the spawners in the spawning tanks.
2. While they are being transferred to the spawning tanks, check their gonadal maturation (Figure 29).
3. Individual scoop nets will be used to transfer each spawner to avoid contamination.
4. Each tank will be covered with black nets and black sacks.
5. The spawners will be allowed to stay overnight for spawning.

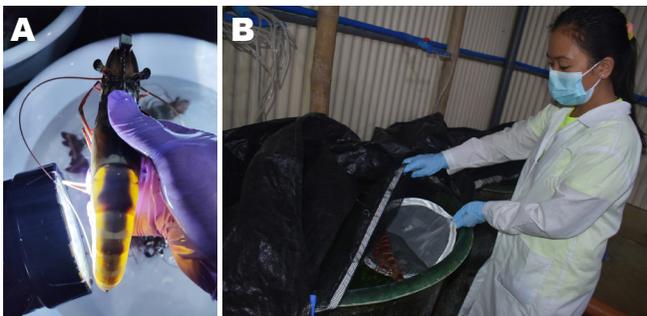


Figure 29. A. assessing a spawner's gonadal development by shining a beam of light on its dorsal side and B. stocking at the spawning tank.

Stages of Gonadal Maturity of Spawners

Stage I (Immature) — Ovaries thin, transparent, not visible through the dorsal exoskeleton. On dissection these appear as colorless strands without visible eggs. Some hatchery technicians refer to this as Stage 0.

Stage II (Early maturing) — Ovaries observed through the exoskeleton as a linear band as these start to increase in size, particularly in the anterior and middle lobes. Color of dissected ovaries ranges from cloudy white to light brown and grayish-green. Some hatchery technicians refer to this as Stage I if the band is thin, and Stage II if the band has grown thicker.

Stage III (Late maturing) — Ovaries visible through the exoskeleton as a thick, solid, dark linear band as these expand considerably from the anterior thoracic to the posterior abdominal region. A somewhat “diamond” or “butterfly” outline can be seen at the level of the first abdominal segment. Dissected ovaries are mostly light olive-green, firm and granular in texture, and with visible clumps of eggs.

Stage IV (Mature or ripe) — The diamond-shaped expansion at the first abdominal segment is larger and more distinct; the linear band is thicker. Upon dissection, the ovaries appear dark olive-green and are so distended as to occupy nearly all available space in the body cavity.

Stage V (Spent) — Completely spent ovaries are limp and thin and outwardly appear similar to Stage I (immature) ovaries. Dissected ovaries are yellowish but become more and more white as regression continues. Females that are partially spent have either the front or rear portion of the ovary still distended.

Eyestalk ablation

P. monodon females, unlike males, do not attain maturity in captivity unless they undergo ablation or destruction of one eyestalk. The eyestalk is where the production and storage sites of a gonad inhibiting hormone is found, which prevents the maturation of ovaries. In nature, some environmental factors cause the decrease of this hormone as the shrimp migrate from estuaries to offshore areas where they normally spawn. Eyestalk ablation eliminates this substance or at least reduces it to a level at which maturation of the ovary can take place. Shrimp are ablated only when hard-shelled, never when newly molted (soft-shelled) or ready-to-

molt (with whitish spots on shell). Only the healthy animals with clean shells, intact legs and tails, and uninfected gills must be ablated. The procedure is as follows:

1. The shrimp will be gently but firmly held with one hand. The sex will be checked, and only females will be ablated. Shrimps with broken or diseased external sex organs (petasma or thelycum) will not be used.
2. The ovarian maturation stage will be checked by external examination (**Figure 30**). Only immature (Stage I) and early maturing (Stage II) females will be ablated. Late maturing (Stage III) ripe (Stage IV) females are ready to spawn.
3. The thelycum will be closely examined for presence (bulging, with a whitish vertical streak on each side) or absence (depressed, evenly colored with no whitish streak) of sperm sacs. Only females which appear to have sperm deposited in the thelycum will be ablated; the rest will be returned to the holding tank for mating with males.
4. Ablation will be performed on either the left or right eye. However, an already infected or otherwise damaged eye will be ablated to leave one unablated healthy eye.
5. Ablation will be performed through the following ways:
 - a. **Pinching** — An incision is made on the eye with a sharp blade, the contents are squeezed out, and eyestalk is crushed 2–3 times to destroy the tissue. This is the preferred method because one person can do it alone. The eyestalk heals even without the use of antibiotics; the external (corneal) layer forms the scar tissue in a week's time.
 - b. **Ligation** — The eyestalk is tied with a piece of string at the base close to the carapace, to fall off in a few days. This process needs two persons - one to hold the prawn while another ties the eyestalk.
 - c. **Cautery** — The eyestalk is ablated by squeezing with a pair of red-hot forceps or by using an electric cauterizer (nichrome wire, 5 volts). This requires a cauterizer which may not be easily available.

- d. **Cutting** — The eyestalk is cut off with a pair of sharp scissors about 3–5 mm from the base. This is inconvenient because it requires additional sealing by cauterizing; otherwise, loss of blood from the open (cut) eyestalk may lead to mortality.
6. Ablation will be performed quickly and with the shrimp underwater to minimize stress. After ablation, the shrimp will be immediately released. Mortality due to ablation stress should not be more than 10 %. Ovarian maturation follows a few days or weeks after ablation, and spawning may occur as quickly as three days after ablation. If ablation is done during the inter-molt, maturation and spawning will immediately follow. When ablation is during the early premolt, the females will first molt before they start to mature.

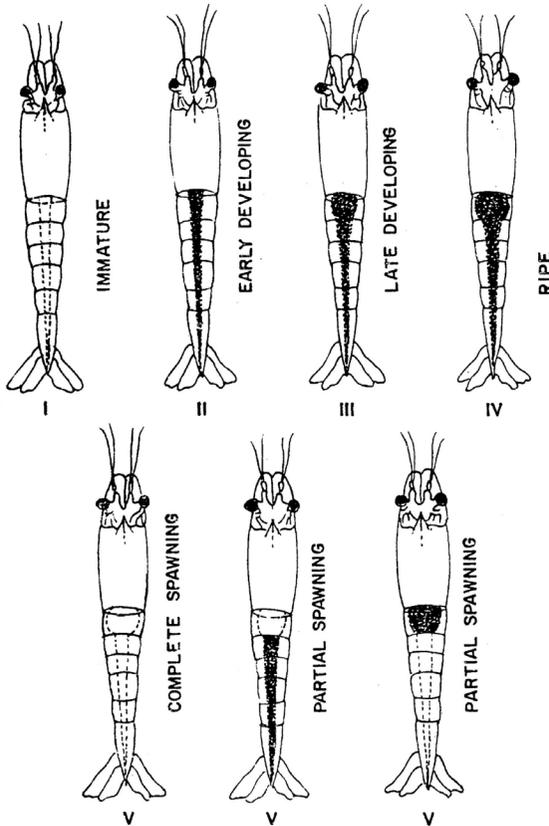


Figure 30. Stages of gonadal development and maturity of *P. monodon* (Primavera, 1989)

Post-Spawning Sampling

1. In the following morning, remove the spawners from the spawning tank and check if they have spawned. Those that were unable to spawn will be allowed to stay for another night.
2. Place the spent spawners in a labelled resealable plastic bag and send to a fish health laboratory for PCR tests (**Figure 31**).

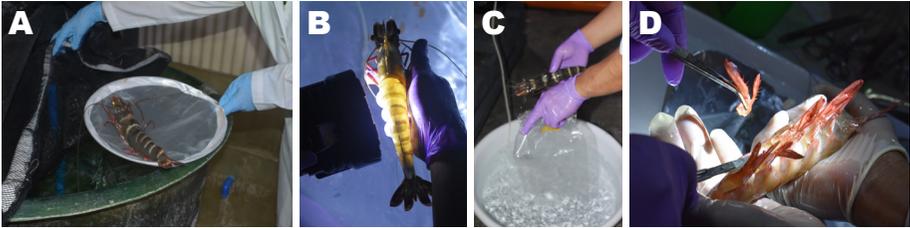


Figure 31. Spent spawners (A) are checked (B), placed in labelled plastic containers (C), and sent to a fish health laboratory for PCR tests (D)

Egg Disinfection and Treatment

Washing of eggs reduces the accumulation of bacteria and viruses when the eggs hatch into nauplii. The capsule that encloses the egg might contain bacteria and viruses during spawning. During hatching, the capsule breaks and the eggs hatch into nauplii. When the eggs are not washed and disinfected, the nauplii may engulf the bacteria and viruses.

Processing of Eggs

1. Remove the spent spawner from the spawning tank.
2. Clean the tank by removing the scum and dirt that were released with the eggs during spawning using a scoop net.
3. Harvest the eggs by siphoning the water from the spawning tank and allow to flow in the 90 μm harvesting box placed in a basin with aerated UV-sterilized seawater.
4. Three white basins will be prepared beforehand. One basin will be used for the first washing of the eggs. The second basin will be for the disinfectant, and the third basin will be for the second washing or rinsing of the eggs (**Figure 32**).

5. The disinfectant will be prepared by mixing 1 ml of povidone-iodine solution in 20 L of water in the basin. The water will then be aerated for homogenous mixing.
6. After the first washing (5 minutes), harvesting box with the eggs will be placed in the second basin supplied with UV-sterilized and aerated seawater with disinfectant for 1 minute.
7. The eggs will be placed again in the third basin for rinsing for another five minutes.
8. The washed eggs will then be stocked in another clean tank supplied with UV-sterilized and aerated seawater to facilitate hatching.



Figure 32. Washing of eggs with UV-sterilized seawater (**left**); treatment of povidone-iodine (**middle**); and rinsing (**right**)

Harvesting and Stocking of Nauplii

The following morning, the hatching tanks will be checked to monitor if the eggs have hatched into nauplii. The processing and stocking of nauplii will be based on the results of the PCR analyses made on the spent spawners. Pathogens are transferred from mother to offspring via vertical transmission. Hence, only the nauplii from the PCR-negative spawners will be stocked.

Harvesting and Transporting of Nauplii

1. Gradually release the water from the hatching tank to the harvesting box which is placed in a basin with UV-sterilized seawater (**Figure 33**).

2. Scoop-out the nauplii slowly using a dipper, and place in a pail with aeration.
3. Estimate the nauplii in the pail to have an initial count before stocking.
4. The nauplii will then be transported from the Spawner/Broodstock Facility to the shrimp hatchery using clean and covered pails. The nauplii will be acclimated for 30 minutes before gradually releasing to the tanks.

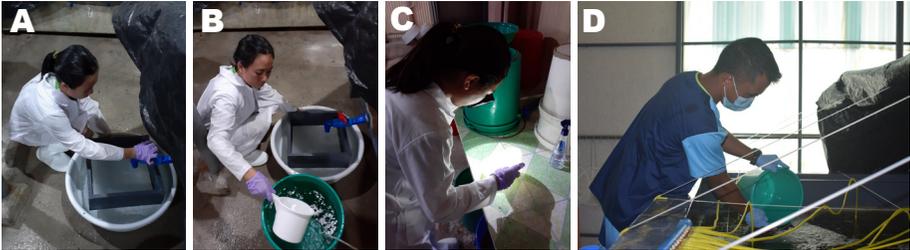


Figure 33. Nauplii are gradually harvested from the hatching tank (A), placed in a pail (B), counted (C), and released into the larval rearing tanks (D)

Feeding

Feeds consist of natural food, egg yolk, and artificial diets (**Table 2**). To prepare the feeds:

1. The required amount of feed will be weighed using a weighing scale.
2. A filter and pail will be prepared. The feeds will be placed on a filter net and the filter net will then be positioned on top of the pail.
3. Flowing treated seawater will be introduced gradually through the filter while dissolving the feeds.
4. The dissolved feeds will be divided and will be fed to the larvae inside the tanks.
5. Vitamins will also be incorporated in the diet by dissolving the vitamin tablet with water and mixing the solution with the prepared boiled egg yolk.

Table 2. Feeds and feeding scheme for tiger shrimp hatchery production

Stages	Feeding	Feeding in a Day
Nauplii	—	—
Zoea	<i>Skeletonoma</i> sp. (20,000–50,000 cells/ml)	2 times
	egg yolk (5–10 g for one million larvae)	1~4 times
	artificial diet (5–10 g for one million larvae)	1~4 times
Mysis	<i>Skeletonoma</i> sp. (20,000–50,000 cells/ml)	2 times
	egg yolk (10–20 g for one million larvae)	3~4 times
	artificial diet (10–20 g for one million larvae)	3~4 times
Postlarvae	<i>Skeletonoma</i> sp. (20,000–50,000 cells/ml)	2 times
	egg yolk (25–200 g per million)	4~6 times
	artificial diet (25–200 g per million)	4~6 times

Water Management

Regular water change could help lessen the concentration of toxic substances present in the water. The water will be changed when the larvae have totally metamorphosed to the PL stage. Water change will be done every other day and depends on the water quality and fry condition. Water change at PL 1 usually starts at 10 % and gradually increases up to 50 % until the shrimp fry is of harvestable age.

Water and Fry Quality Monitoring

Daily monitoring and checking of larvae will be done to detect occurrence of any problem and diseases. Bacterial analyses will be done twice a week starting from PL 1, while PCR analyses and fry quality monitoring will

be conducted at PL 5, 10, and 15. Shrimp larvae will also be sampled to determine the estimated count during different stages of shrimp.

Collection of Water Samples

1. A clean sampling bottle with proper label that will be used in collecting water from larval rearing tanks should be prepared.
2. Separate the fry from the water using a scoop net for easy sample collection.
3. Place the water sample into a sampling bottle.
4. The bottle will then be sealed with the cover and sent to a fish health laboratory (**Figure 34**).



Figure 34. Collection of water samples for bacterial analysis

Collection of Fry Samples

1. Collect fry samples from the different areas of the tank using a scoop net.
2. The collected fry will then be placed in a white basin with UV-sterilized seawater (**B**).
3. Slowly swirl the water in the basin (**C**).
4. The fry that accumulated at the center will be collected, placed in a pre-labeled plastic container (**D**), and submitted to a fish health laboratory for bacterial and PCR tests (**Figure 35**).



Figure 35. Collection of fry samples for bacterial and PCR tests

On the other hand, fry in the tanks will be observed and monitored regularly to determine their stage and condition, as well as to monitor their survival at every stage. One liter of water will be taken from the tank using a beaker and the fry will then be observed (**Figure 36**). They will be manually counted by placing them in a white bowl and will be returned to the tanks after counting. Sampling of fry will be done every 2–3 days.



Figure 36. Fry sampling and monitoring

Harvesting, Packing and Transport of Shrimp Fry

Harvesting of Postlarvae

1. The harvesting net will be prepared inside the harvesting pit (**Figure 37**).
2. UV-treated seawater will be added inside the pit.

3. Before harvesting, the water level in the tanks will be reduced to about 1/4 of the total volume to lessen the pressure on the drain pipe during the release of water thereby minimizing stress on postlarvae.
4. After reducing the water level, the stand pipe will be partially removed and placed in a slanting position, and the remaining content of the tank will be allowed to flow in a controlled manner into the harvesting net inside the harvesting pit or box.
5. The PL will be collected and concentrated using a clean scoop net and will then be transferred into a basin supplied with UV-sterilized and aerated seawater.
6. The harvested fry will later be distributed in white basins for counting and packing.



Figure 37. Shrimp postlarvae are harvested by draining the tank (A), concentrating the fry in a harvesting net (B), and transferring them in a basin with UV-sterilized and aerated seawater (C)

Packing and Transport of Fry

1. The harvested fry will be placed in basins with UV-sterilized seawater and supplied with aeration. They will be counted based on the desired packing density per bag during transport.
2. The estimated fry will be distributed to the other basins based on the counted fry density.
3. After estimating, the fry will be transferred from the basins into doubled polyethylene (PE) bags. The inner bag will contain 1/3 UV-sterilized seawater and 2/3 oxygen by volume, and the inner and outer bags will be separately tied with rubber bands (**Figure 38**). If the travel time exceeds 6 hours, water temperature will be lowered to 25 °C by placing wrapped ice on top of the inner plastic bags.

Packing of fry will also depend on the destination and duration of transport. For short-distance transport like 2–4 hours, fry will only be packed in double-lined PE bag and placed in carton boxes. For transport

to farther places that would take 5–12 hrs, fry will be packed in double-lined PE bags, inserted with wrapped ice and placed inside styroboxes. For long distance transport or transport via plane, fry will be packed in double-lined PE bag with wrapped ice on the side of the first bag, placed inside the cartons and styroboxes, and sealed with packing tape (**Figure 39**).



Figure 38. Packing fry for transport: **A.** concentrated fry from a scoop net are placed in a basin, **B.** counting of required fry for packing, **C.** distribution of estimated number of fry, **D.** transferring of fry to doubled polyethylene bags, **E.** oxygenation, **F.** tying



Figure 39. Shrimp fry are packed using different methods depending on transport duration. **A.** fry bags inside carton boxes, **B.** sealed styroboxes, **C.** loading of styroboxes, **D.** transport of fry

8

Diseases of Shrimps

Table 3. List of common shrimp diseases, the life stages they affect, clinical signs, and prevention/control measures

Disease	Stages Affected*	Signs	Prevention/Control
<i>Viral</i>			
Infectious Hypodermal and Hematopoietic Necrosis Virus (IHHNV) Disease	PL	Erratic swimming behavior, weakness and loss of appetite, delayed molting, white opaque abdominal muscles	Strict hygiene, use SPF stocks, disinfection of facilities, screening or filtration of inlet water
White Spot Syndrome Virus (WSSV) Disease	M, PL	Presence of white cuticular spots at the exoskeleton and epidermis, red discoloration and loose cuticle, surface swimming, reduction in food consumption and empty gut, rapid onset and high mortalities up to 100 % in 3 to 10 days	Use SPF stocks, strict hygiene, disinfection of facilities, egg washing and disinfection
Yellow Head Virus (YHV)	N, Z, M, PL	Light yellowish, swollen cephalothorax; whitish, yellowish or brown gills; abnormal high feed intake and rapid growth prior to cessation of feeding and onset of rapidly accelerating mortality	Use SPF stocks, impose strict hygiene, use of only dry commercial feeds, fine screening of inlet water
<i>Bacterial</i>			
Luminous Bacterial Disease	N, Z, M, PL	Larvae become weak and opaque-white, continuous greenish luminescence when observed in total darkness, presence of highly motile bacteria on the internal tissues when viewed under the microscope, luminescent bacterial colonies on nutrient agar medium streaked with infected larval tissues	Series of proper filtration system for incoming water, use of chlorinated or UV-sterilized seawater, immediate removal of spent spawner from spawning tank to prevent colonization of luminous bacteria before hatching

Disease	Stages Affected*	Signs	Prevention/Control
Acute Hepatopancreatic Necrosis Disease (AHPND)	N, Z, M, PL	Pale to white discolored hepatopancreas	Use SPF stocks, implement biosecurity measures, treat influent water or use UV-sterilized seawater
Shell Disease, Brown/Black Spot, Black Rot/Erosion, Blisters, Necrosis of Appendages	N, Z, M, PL	Brownish to black erosion on the carapace, abdominal segments, rostrum, tail, gills, and appendages; blister containing gelatinous fluid may develop on the carapace and abdominal segment; affected appendage shows a cigarette butt-like appearance	Maintain good water quality, provide adequate diet, minimize handling and avoid overcrowding
Filamentous Bacterial Disease	N, Z, M, PL	Presence of fine, colorless, thread-like growth on the body surface and gills as seen under the microscope	Maintain good water quality with optimum dissolved oxygen (> 5 ppm) and low organic matter levels
Fungal			
Larval Mycosis	N, Z, M, PL	Infected eggs, larvae, and PL whitish, become weak, and may eventually die; presence of fungal filaments and their reproductive structures within infected tissues when the disease is widespread	Siphon sediments and dead shrimps, reduce stocking density, increase water circulation, observe rigid water management and sanitation, egg washing and disinfection, use 0.1 ppm Treflan or trifluralin as prophylactic treatment every 2–3 days for 24 hrs
Microsporidian			
Enterocytozoon hepatopenaei (EHP)		Retarded growth, variation of shrimp size or weight	Proper broodstock selection, egg washing and disinfection, good water quality, monitoring by PCR

***B** - broodstock, **N** - nauplii, **Z** - protozoa, **M** - mysis, **PL** - postlarvae

Shrimp Fry Production*Small-scale Hatchery***Table 4.** Assumptions used in the computation of costs and returns in a shrimp hatchery in the Philippines. Money values are in Philippine Pesos (PHP)

Project duration (years)	20
A. Broodstock	year-round
Number of spawners/million nauplii (2–3 spawners/ million nauplii)	3
Total number of spawners required	30
B. Hatchery	
Larval tank capacity (tons)	100
Stocking density of nauplii/ton	100,000
Total number stocked nauplii	10,000,000
Survival	25 %
Total fry production	2,500,000
Number of runs/year	6
Total fry production/year	15,000,000
Fry selling price (PhP/fry)	0.25

Table 5. Investment items, costs, depreciation and re-investment requirements in Shrimp Hatchery Operations in the Philippines. Money values are in Philippine Pesos (PHP)

Investment Items	Total Cost	Economic Life	Annual Depreciation Cost	Reinvestments on Year 6, 11 and 16	Reinvestments on Year 11
A. Spawner/Broodstock facilities and Hatchery facilities					
Land, 500 m ²	750,000				
Broodstock facility	200,000	20	10,000		
Larval rearing tanks, 10 units, 10-ton capacity	350,000	20	17,500		
Natural food rectangular tanks, 4 units 6-ton capacity	260,000	20	13,000		
Larval rearing tank roofing (canvass, bamboo & nylon)	30,000	10	3,000		30,000
Reservoir tank, 20 tons capacity	250,000	20	12,500		
Pre-filter seawater intake pipe system	60,000	10	6,000		60,000
Technicians' quarters	100,000	20	5,000		
Harvesting area, pit, and drainage	80,000	20	4,000		
Treatment tank, 30-ton capacity	80,000	20	4,000		

Investment Items	Total Cost	Economic Life	Annual Depreciation Cost	Reinvestments on Year 6, 11 and 16	Reinvestments on Year 11
Water and aeration system (plumbing)	100,000	20	5,000		
Smart UV sterilizer, 1 unit	58,000	5	11,600	58,000	
Ring blower, 2 HP, 1 unit	33,000	5	6,600	33,000	
Roots blower, 2 HP, 1 unit	61,000	5	12,200	61,000	
Submersible pump, 1 unit	15,200	5	3,040	15,200	
Centrifugal pump, 2 HP, 1 unit	30,800	5	6,160	30,800	
Refractometer	12,100	5	2,420	12,100	
Refrigerator	25,000	10	2,500		25,000
Generator 6 KVA	60,000	10	6,000		60,000
Total investments on breeding and hatchery facilities	2,555,100		130,520	210,100	175,000

Table 6. Cost and return analysis of Shrimp Hatchery Operations (1 year) in the Philippines. Money values are in Philippine Pesos (PHP)

Item	Quantity	Unit Cost	Total Cost
Gross income			
Shrimp fry PL 15 sales, 3 % mortality allowance as discount	14,550,000	0.25	3,637,500
Production Cost			
Variable Cost			
Spawners	180	1,500	270,000
<i>Artemia</i> (can)	105	2,800	294,000
Artificial diets (kg)			
Diet 1	24	660	15,840
Diet 2	24	864	20,736
Diet 3	10	1,152	11,520
Fertilizers and other chemicals			18,000
Electricity (kWh), 95 kWh/day	25,650	10	256,500
Transportation and other consumable materials (2 % of revenue hatchery)			72,750
Repair and maintenance (10 % of hatchery life-support equipment)			29,800
Food allowance for personnel during operation	6	10,000	60,000
Labor & personnel (40 % of net profit)			1,035,342
Subtotal (variable cost)			2,084,488
Fixed Cost			
Depreciation			130,520
Permits and License			5,000
Interest on loans to variable cost, 6.35 % per annum			132,365
Opportunity cost of own capital, 1.07 % per annum			27,493
Subtotal (fixed cost)			295,378

Item	Quantity	Unit Cost	Total Cost
Total Cost (Shrimp hatchery operations)			2,379,865
Total annual net income			1,257,635
Variable cost per fry			0.14
Total cost per fry (break-even cost)			0.16
Payback period (years)			1.84
Return on investment (ROI) (%)			49.22
Net Present Value @ 10 % (NPV)			11,052,169
Internal Rate of Return (IRR) (%)			57.52
Discounted Benefit-Cost Ratio			1.67
TOTAL INVESTMENT REQUIREMENT (Investment Cost + 1 year Operation Cost)			4,804,445

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11 Glossary

- Ablation** – incision and removal of the contents of the eye and the eyestalk to induce gonad maturation
- Acclimatize** – to gradually adapt to the environment
- Aerate** – to supply with air
- Algae** – refers to microscopic marine plants
- Ammonia** – a metabolite excreted by shrimps and aquatic animals
- Appendages** – include swimming and walking legs and also the mandibles in nauplii
- Biosecurity**- procedures intended to protect humans or animals against disease or harmful biological agents
- Broodstock** – adult male or female prawn which can be induced to sexually mature
- Chlorination** – treatment with chlorine (bleach) solution
- Cyst, Artemia** – dormant stage of brine shrimp (*Artemia*) where eggs have a hard, thick protective outer layer; this may be hatched under suitable conditions
- Decontamination** - is a term used to describe a process or treatment that renders a medical device, instrument, or environmental surface safe to handle. Sterilization, disinfection, and antisepsis are all forms of decontamination
- Density** – number of individuals or units per volume
- Density, algal** – number of cells of algae per volume
- Diatom** – microscopic algae with siliceous cell walls
- Disease** – an abnormal condition affecting growth, function, or appearance of the animal
- Disinfect** – to rid of harmful microorganisms
- Dorsal** – referring to the top side of the prawn
- Formalin** – a 37 % formaldehyde solution used as disinfectant
- Hatchery** – a place for artificial breeding, hatching, and rearing through the early life stages of animals—finfish and shellfish in particular

- Incubation** – to maintain under favorable environmental conditions to aid egg development and hatching
- Induce** – to simulate and cause
- Molt** – to shed off the shell
- Monitor** – to check, record, and keep track
- Pathogens** – disease-causing organisms
- Rapid sand filter** - is a container of granular media, normally following settling basins in conventional water treatment trains
- Rear** – to culture
- Reservoir** – large tank which stores or holds water
- Salinity** – the concentration of dissolved salts
- Seawater** – water with salinity of 30 to 40 ppt or ocean water
- Spawners** – mature females which are ready to spawn
- Starter (algal)** – inoculum; small volume of pure culture of algae used for starting mass production
- Stock** – to place in a tank
- Toxic** – poisonous
- UV sterilizer** – UV disinfection or ultraviolet germicidal irradiation (UVGI) which effectively inactivate microorganisms by damaging the DNA of cells

12 Appendices

Appendix A. Preparation of Disinfectant for Spawners and Eggs

Needed Concentration of Disinfectant: 50 ppm

1. Prepare the povidone-iodine and basin.
2. Mix 1 ml povidone-iodine in a basin with 20 L of UV-sterilized seawater.
3. Provide aeration for homogeneous mixing.

Appendix B. Packing densities (postlarvae per liter) for shipping shrimp postlarvae of different ages over various transit times

Postlarval Age	Hours in Transit				
	4	8	12	24	48
PL-6	4,000	3,700	3,500	1,800	1,000
PL-8	3,200	2,800	2,500	1,200	800
PL-10	2,500	2,000	1,800	900	600
PL-12	2,000	1,600	1,300	700	500
PL-16	1,800	1,400	1,000	600	400
PL-18	1,200	900	500	500	300
PL-20	1,000	800	400	400	250

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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, Philippines, Singapore, Thailand, and Viet Nam.

The policy-making body of SEAFDEC is the Council of Directors, made up of representatives of the member countries.



SEAFDEC has five 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
- 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, and
- Inland Fishery Resources Development and Management Department (IFRDMD) in Palembang, Indonesia (2014) for sustainable development and management of inland capture fisheries in the Southeast Asian region.

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

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 has an office in Quezon City.

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