AQUACULTURE EXTENSION MANUAL NO. 69 JULY 2022

Hatchery Production of Sea Cucumbers
(Sandfish *Holothuria scabra*)

Jon P. Altamirano
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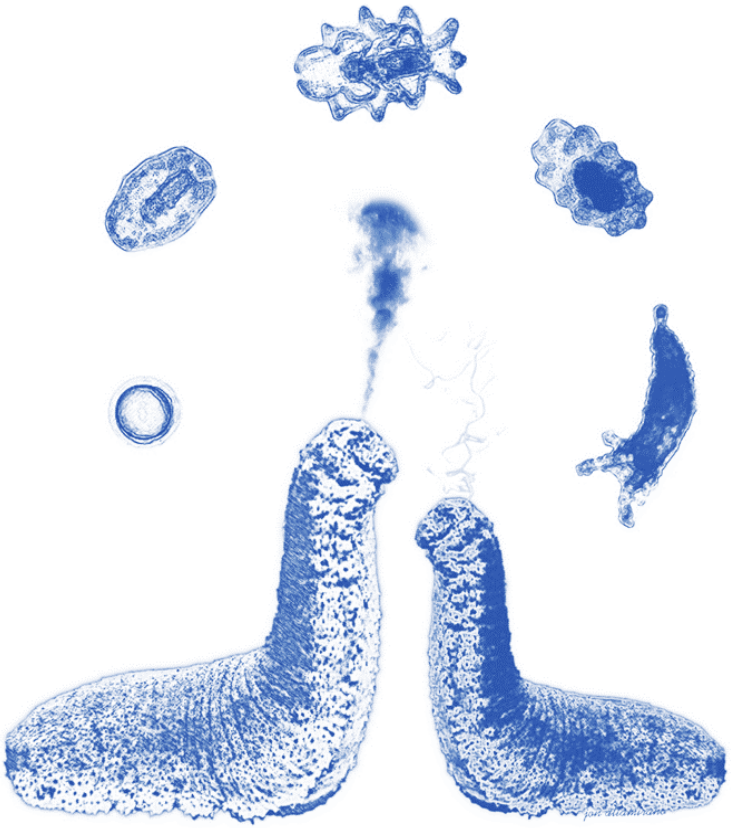

Southeast Asian Fisheries Development Center
AQUACULTURE DEPARTMENT
Tigbauan, Iloilo, Philippines

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Foreword

Sea cucumbers are highly valued marine commodities traditionally collected from the wild. However, lucrative market prices promoted increased trade that led to their overexploitation in many countries. Some species have even been classified as endangered or vulnerable under the IUCN Red List of Threatened Species.

Fortunately, the aquaculture of sea cucumbers is making progress, which can help reduce the pressure on wild stocks and contribute to the market supply. It was in 2007 when SEAFDEC/AQD began work on the hatchery production of sea cucumber, specifically the sandfish *Holothuria scabra*, mainly for stock enhancement. In 2010, a small-scale sea cucumber hatchery was established at the research center's main station in Tigbauan, Iloilo, Philippines. At this same year, the first training course on seed and nursery production of sandfish was conducted.

After developing initial seed production techniques and producing hatchery-bred juveniles, SEAFDEC/AQD tested the potential of sandfish mariculture in community-based sandfish sea ranching sites in central Philippines from 2013. One pilot site in Negros Occidental, Philippines successfully sustained a stock of cultured sandfish up to the present, some even reached market size in as early as 2017. There, sandfish ranching is viewed to be a potentially sustainable source of alternative livelihood for coastal communities.

Fifteen years since sea cucumber research began at SEAFDEC/AQD, Dr. Jon Altamirano and his team have accumulated a substantial understanding of sandfish reproductive biology and developed significant refinements on the practical procedures of seed production of the species. This manual is a compilation of those techniques and technologies that continue to be developed at SEAFDEC/AQD's small-scale sea cucumber hatchery.

We hope that the hatchery technology described in this manual will be a useful guide for hatchery operators, technicians, researchers, and other stakeholders, and lead to a flourishing sandfish industry and the recovery of wild sandfish populations.

DAN D. BALIAO
SEAFDEC/AQD Chief

About the Manual

This manual, entitled Hatchery Production of Sea Cucumbers (Sandfish *Holothuria scabra*) includes the consolidated methods, practical protocols and good practices in sea cucumber breeding that were established within the past decade of research and development at the small-scale sea cucumber hatchery in the Tigbauan Main Station of SEAFDEC Aquaculture Department. Hence, this manual focuses only on demonstrating the technology, requirements and procedures in operating a small to medium-scale tropical sea cucumber hatchery.

This manual is written for potential sea cucumber hatchery operators, technicians, enthusiasts, researchers, and students with some basic experience and knowledge on aquaculture. However, even beginners may find this manual informative and useful, as it highlights the importance of sea cucumbers and their potential as an aquaculture commodity, particularly that of the tropical sea cucumber *H. scabra*, commonly known as the sandfish. This manual also serves as the primary reference material of the Training Course on "Sandfish (*Holothuria scabra*) seed production, nursery and management" offered by SEAFDEC/AQD. Hence, some specific descriptions of the actual facilities and existing equipment are detailed here.

The manual describes the various hatchery production methodologies that were specifically optimized for the sandfish *H. scabra*. These methods were designed to be practical and easy to implement. The materials mentioned herein are those that can be easily procured, otherwise if unavailable, can be fabricated from common materials.

The following are the main sections included in this manual:

1. Broodstock selection, collection, and conditioning
2. Natural food (microalgae) cultivation
3. Spawning stimulation and fertilized eggs management
4. Larval rearing and settlement
5. Harvesting of early juveniles, packing, and transport

Contents

1 Introduction

1.1. Ecological importance of sea cucumbers

Sea cucumbers, also called holothurians, are marine animals closely related to other echinoderms like star fishes, sea urchins, and brittle stars. There are over 1,700 species or types of holothurians worldwide but only about 60 are considered to be commercially important. Majority of these species can be found in the warmer tropical regions, especially in Southeast Asia where they are distributed from the shallow shores to the deep oceans, depending on the species (see **Figure 1**). In addition, they can be of many different colors, body shapes, skin textures, and sizes. But most are typically characterized as having a leathery skin, elastic body, and a generally rounded and elongated shape. This is why they are referred to as the "cucumbers of the sea" or sea cucumbers.

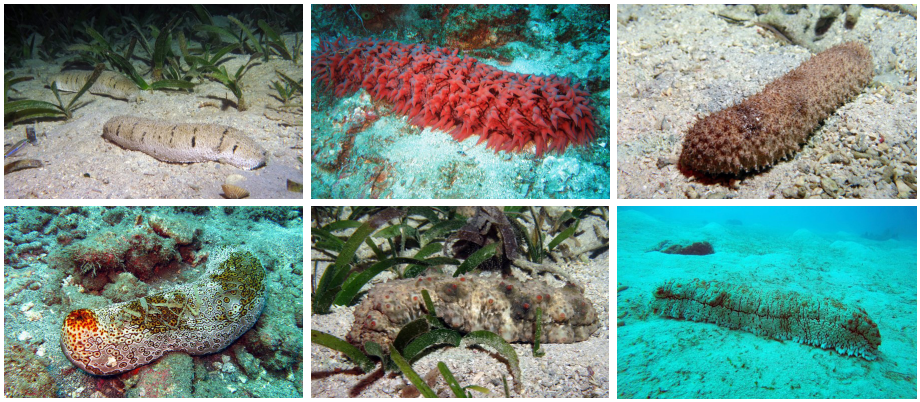


Figure 1. Different species of tropical sea cucumbers can be found in the shallow sea grass beds, stony coral reefs, and deep sandy sea floor

In the natural environment, sea cucumbers can ingest large quantities of sediments and particulate matter as they graze off the sea floor or filter-feed particles in the water. During these feeding processes, the organic matter of the ingested materials are reduced and nutrients recycled before they are expelled. In addtion, they also aid in the oxygenation of sediments through bioturbation as they "plow" into the sediments when they bury and emerge every day. In short, sea cucumbers help maintain a healthy and fertile marine environment for the benefit of other marine animals and as such, they are often referred to as the "earthworms of the sea."

1.2. Economic importance of sea cucumbers

Wild sea cucumbers have been traditionally harvested for centuries in many countries, with the highest demand coming from East Asia. Sea cucumbers are

most often gutted or eviscerated and the body wall or thick skin is dried into what is commonly called as *bêche-de-mer* (French), *trepang* (Indo-Malay), balat (Philippines) or *namako* (Japanese). Dried sea cucumbers are very expensive as a culinary delicacy ingredient because they are believed to have medicinal qualities, especially among the Chinese. Prices in Hong Kong can reach to as much as US$ 2,000 per kilogram when packaged lucratively, although regular average prices are around US$ 200 (see **Figure 2**).

Figure 2. Sea cucumbers are highly valued marine commodities, with prices ranging from US$ 200 to US$ 2,000 per kilogram in Hong Kong when processed and dried into *trepang* or *beche-de-mer*

The very high market prices and the increasing demand for sea cucumbers in the past decades have driven the over-exploitation of its wild populations in many countries, especially those located in the West Pacific and Indian Oceans. This dependency on wild stocks is causing a severe decline in global supply (see **Figure 3**). Fortunately, the aquaculture of sea cucumbers is beginning to progress well, and is expected to increase overall production while alleviating pressures on wild collection. The mariculture of sea cucumbers can offer a good livelihood option for coastal communities, while protecting the remaining wild stocks. The basic methods to propagate sea cucumbers in the hatchery are already established and will be discussed in detail in this manual.

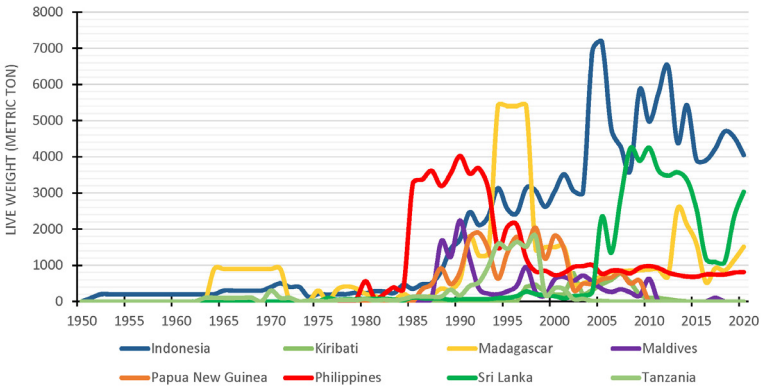


Figure 3. Sea cucumber capture production in selected countries (Source: FAO Fishstat J Database - Capture Production 1950–2020)

1.3. The sandfish (*Holothuria scabra*)

Sandfish is the common English name for only one particular species of sea cucumber, which is scientifically called *Holothuria scabra*. They are one of the most threatened tropical species because of their high price (reaching US$ 1,600 per kilogram, dried) and their being easily harvested from the shallow intertidal sandy-muddy shores. They are commonly associated with seagrasses but not with coral reefs. Like other sea cucumbers, the sandfish do not have a sturdy skeleton and the whole body can be very elastic. They can enlarge and bloat by sucking in water. Then they can shrink and harden by expelling water out of their anus while constricting their body wall. Sandfish can grow to a good adult size of 350 grams (about 20 cm long or 8 inches) in more than one year. They can grow to almost 2 kg (about 40 cm long) and naturally live for more than 10 years.

1.3.1. Physical characteristics

In most of Southeast Asia, adult sandfish have a greyish-gold skin color with some (8 to 12) dark brown to black irregular stripes across its rounded top side (dorsal). The underside (ventral) of the body is flat and much lighter in color to almost white. The outer skin is commonly rough and covered with numerous small protrusions called papillae that can retract when disturbed. The papillae on their underside are modified into tiny tube feet to aid in crawling or creeping on the sea floor.

Figure 4. A juvenile (top) and adult (above) sandfish, *Holothuria scabra*, in their natural habitat

1.3.2. Behavior, feeding, and defense

Sandfish are nocturnal animals, meaning they tend to bury into the sediments during daytime and start to emerge only in the late afternoon to feed through the night. They are deposit feeders and they graze on the surface layers of the substrate using some 20 short tentacles with flattened tips that grab and deliver food into their mouth or oral cavity. They can engulf large amounts of sediment and other organisms that go with it (including tiny mollusks, plankton, seaweed, decaying matter or detritus, and even bacteria). They digest and assimilate what they need and excrete the sediments in pelleted form.

Aside from burying into the sediments, the sandfish does not have much of defenses against predators. Unlike other sea cucumbers, sandfish do not expel white sticky filaments or cuvierian tubules when agitated. However, they can eject all their internal organs when threatened, in a process called evisceration. Predators like crabs, shrimps, and fish will then nibble on the eviscerated organs while the sandfish crawls away and buries in the sediment. Sea cucumbers will then regenerate their internal organs in a month or two.

1.3.3. Reproduction and life cycle

Sandfish are dioecious invertebrates, meaning females and males are separate individuals. However, both sexes look very similar, and differentiating an adult male apart from a female by physical scrutiny can be very difficult. A way to tell for sure is to identify the gametes (whether egg or sperm) contained in their gonads by biopsy (surgically taking small samples) or by observing them during a spawning event. Males would have milt with sperm while females would have eggs.

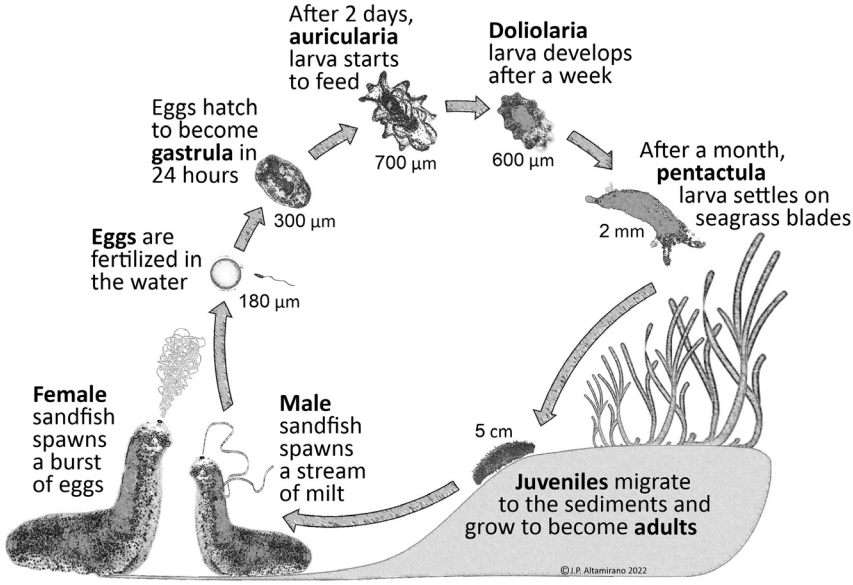


Figure 5. The life cycle of sandfish *Holothuria scabra*

During reproduction, males and females spawn their gametes out into the water column where external fertilization occurs. In the wild, a group of sandfish would naturally congregate (more than 10 individuals at about 5 m apart) before spawning synchronously to ensure the highest possible fertilization rates. Fertilized eggs will develop into planktonic *auricularia* larvae that continuously feed on suspended microalgae. After about two weeks, they will shrink into the

doliolaria stage before settling to adhere onto the leaf blades of seagrasses as they metamorphose into crawling *pentactula* and become early juveniles. At this stage, they will be nursing by grazing off the biofilm that coats the leaf surfaces. In another week or two, the young sea cucumbers will migrate down on the sediments and forage on to deeper areas as they grow and mature as adults.

2 *The Sea Cucumber Hatchery*

In order to produce sea cucumber juveniles, a hatchery facility is required. This is where the spawning of adults and larval rearing are performed. The general design and cost of the hatchery depends on the scale of the production target and budget. This can range from a small-scale practical hatchery that may cost less than one million Philippine Pesos (less than US$ 20,000) to a multi-million advanced large-scale facility. A small-scale sandfish hatchery similar to that being operated at the SEAFDEC/AQD Tigbauan Main Station (TMS) can have an optimal rearing capacity of five million larvae per spawning batch.

Figure 6. The SEAFDEC/AQD sea cucumber hatchery established in 2010

2.1. Location and structural layout

A suitable site for a sandfish hatchery is that which is close to the sea shore for easy access to seawater. The ideal location should be on a stable elevated coast with a steep slope, far from rivers, and protected from storms.

As an example, the main sea cucumber hatchery can measure 10 × 26 m, covering a floor area of 260 m^2. The concrete floor should be elevated by 1 m above ground level to allow the easy provision of harvesting pits and drain canals. Roofing can be with opaque corrugated sheets, with some translucent sheets every 5 meters to allow natural diffused lighting. Galvanized iron pipes can be used for posts and roofing frames.

Similar to that of the SEAFDEC/AQD facility, the hatchery can be divided into two main sections, along its length. The first section is the enclosed larval rearing area, holding 10 concrete tanks of 3-ton capacity each (2.3 × 1.3 × 1.2 m). The other section holds 4 concrete tanks (8-ton each, 3.2 × 2.3 × 1.2 m) with sediments which are used as primary broodstock holding tanks. These can also be used as primary nursery rearing tanks whereby fine-meshed hapa bag nets can be suspended in. A duplex concrete tank (3-ton each) can be used for algal culture like benthic diatoms (Ex. *Navicula* sp.) to be used as feed for settled sandfish juveniles.

A space for spawning is provided with tanks which can either be rectangular (1.6 × 0.5 m) or cylindrical (1 m diameter), made of wood or fiberglass material. Cylindrical tanks (4 to 6 units) with 250 L capacity are used to culture live microalgae (Ex. *Chaetoceros* sp.) as natural food during sandfish larval culture. Nursery tanks (1 × 2 m) with sediments are also provided for the rearing of late sandfish juveniles or fingerlings.

It is recommended to, at least, include a 10-ton elevated water reservoir with a gravity-driven sand filter. Another option is to have at least one 10-ton concrete raceway tank for broodstock conditioning. This raceway tank can also be used for wastewater treatment when needed. In this area, 4 wooden tanks (1 × 1 m) can be added to be used as juvenile conditioning tanks or as quarantine tanks for unhealthy animals.

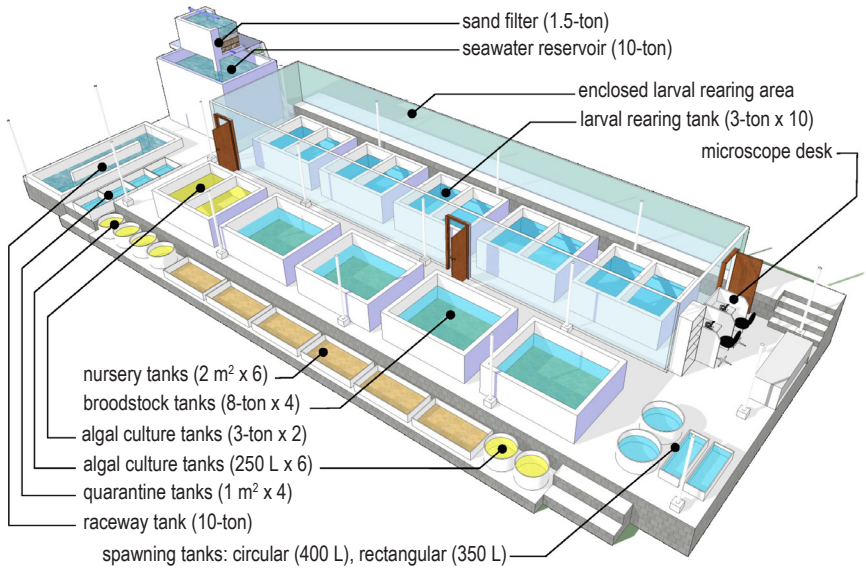


Figure 7. The basic structural design of a sandfish hatchery based on the existing facility at SEAFDEC/AQD (roofing not shown)

2.2. Facilities, equipment and technical manpower

The basic requirements for a hatchery include sufficient volume of clean sea water, uninterrupted aeration supply, stable source of larval food, and hatchery technicians that have the skills and experience to operate the equipment and monitor spawning and larval development.

2.2.1. Primary seawater intake system

The primary intake system will allow sea water to flow through a pipe (at least 15 cm diameter) laid horizontally straight at 1 m below sea level and extending out to the sea. The seaward end must be fitted with perforation and screen filters, while the landward end connects to a concrete sump pit or a hollow well near the shore (about 2 m below sea level) that collects sea water (see **Figure 8**). Water from the sump pit will then be pumped up to a primary reservoir, passing through a multi-granular sand and gravel filtration system. The pump's power can range from 3 horsepower (HP) to 60 HP, depending on the volume required, depth and size of sump pit, and the tidal conditions in the area.

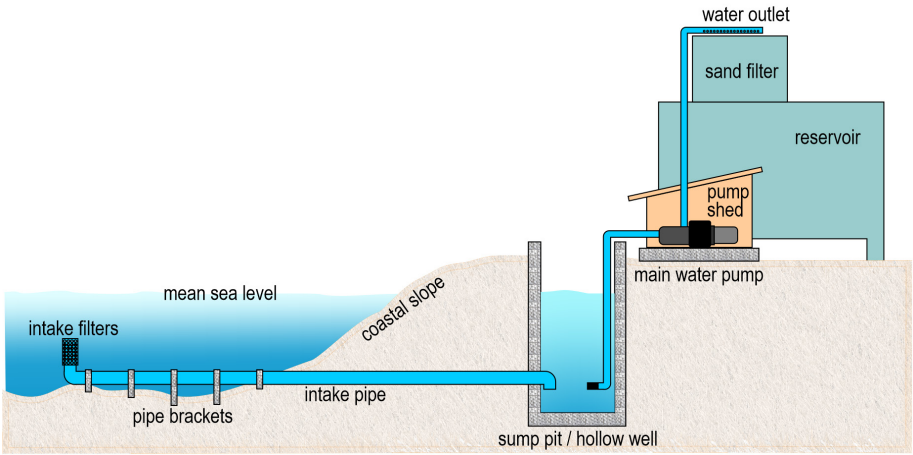


Figure 8. Schematic diagram of seawater intake system

2.2.2. Secondary seawater pump, sand filter tank, and reservoir

Depending on the seawater source, water from the primary reservoir may already be used in the hatchery. However, if turbidity is high, a secondary reservoir and filtration system may be needed. In sites receiving seasonal high turbidity, seawater that has settled in the primary reservoir can be pumped to the hatchery through an elevated (4 m from the ground) auxiliary sand filter tank (1.5-ton capacity) using a 1-HP (single phase) electric pump. This sand filter tank is a multi-granular filtration system whereby seawater is sprayed from the top and allowed

to seep through layers of fine sand, pebbles, gravel, charcoal, and stones (see **Figure 9**). Filtered seawater then pours down by gravity into a secondary reservoir (~10 ton) below. Another 1-HP pump will draw water from this reservoir to be passed through a series of micro-filters (described below) and a UV-treatment system before being distributed into the larval rearing tanks, algal culture tanks, and spawning tanks, as needed.

2.2.3. Microfilter array and UV sterilizer

Seawater that will be used for the spawning tanks, larval rearing tanks, and algal culture tanks must be as clean as possible. Sand-filtered seawater from the reservoir is pumped through an array of microfilter cartridges (10 – 5 – 1 micron series) to further filter off particulates from the water. Then, water passes through the UV (ultraviolet light) sterilization chamber to eliminate microbes that cannot be filtered out. The capacity of the UV system, which is measured in terms of liters/minute (lpm) or gallons/minute (gpm), should match or exceed the required volume of water needed to fill the tanks at a given time.

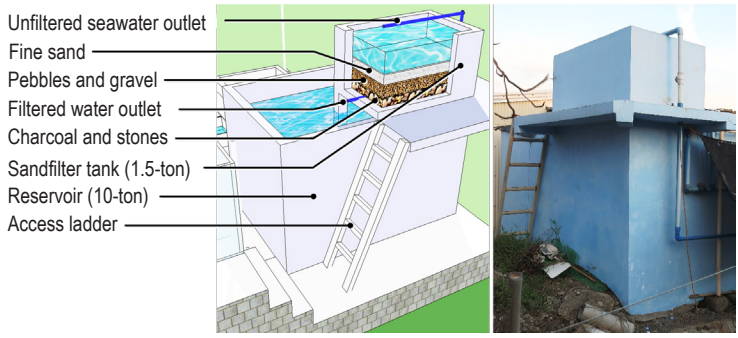


Figure 9. The elevated 10-ton seawater reservoir with a 1.5-ton sand filter tank

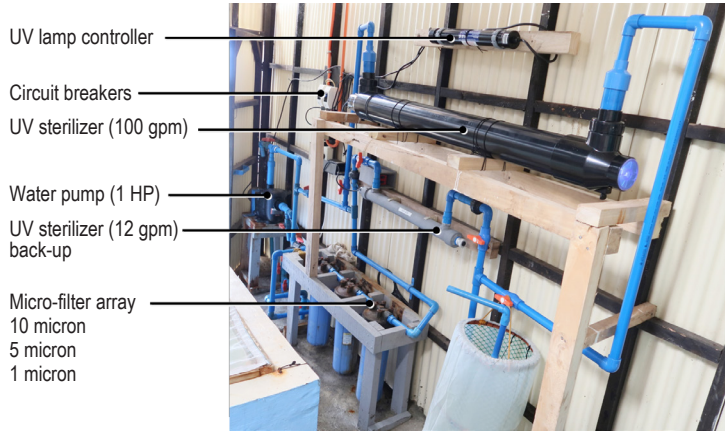


Figure 10. The seawater micro-filtration and UV sterilization system

2.2.4. Aeration lines and air filters

The hatchery can be supplied by a 1 HP electric roots-type air blower. Since aeration has to be constantly provided, it is recommended to have 2 blower units operating alternately every 6–12 hours. Aeration lines can be made from polyvinyl chloride (PVC). The aeration lines leading to the larval rearing tanks and algal culture tanks are fitted with a series of 5-micron and 0.45-micron microfilter cartridges. This is to minimize potential contaminations that may come with the air.

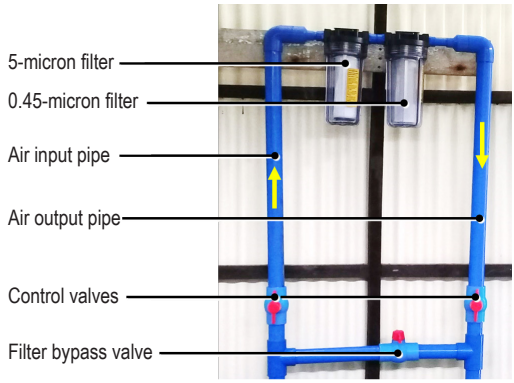


Figure 11. Air filtration system with a 5-micron and 0.45-micron series filters and control valves

2.2.5. Microscopes and implements

The daily operation in the hatchery requires at least two sets of microscopes. One is a standard compound microscope with 40–100 times magnification which is used for counting microalgae and eggs, as well as for monitoring embryonic development. Another is a stereo microscope or dissecting microscope (10–40 X) used for counting sandfish larva. Important implements which are used to measure cell density and determine food quantity include a hemocytometer, Sedgewick rafters, glass slides, coverslips, Pasteur pipettes or micro-droppers, small beakers, and custom counting chambers.

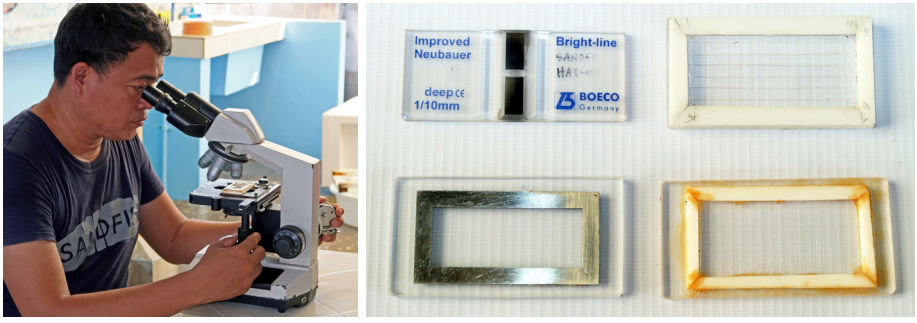


Figure 12. A microscope station or desk within the hatchery (left) and examples of microscope implements like hemocytometer and various custom counting chambers (right)

3 Microalgae as Larval Food

Hatchery production mainly involves the rearing of larval stages of the target aquaculture species in tanks. The most crucial requirement for a successful hatchery operation is the availability of natural food needed to feed the larvae. In the case of sandfish, microalgae are required which are preferably cultured live. There are some commercially available concentrated microalgae products that have been initially tested. However, these products can be expensive and still relatively difficult and impractical to procure, as most of these are imported from abroad. There are some locally-available concentrated products or "pastes," but these have not been very well tested and verified to be used for sandfish hatchery production. Therefore, we will mainly focus on the production of live microalgae in this manual.

3.1. Microalgae species for larval sandfish

As we will be discussing in more detail in later sections, there will be two main stages of larval culture for sandfish that will require different types of microalgal food. The first phase is when the sandfish larvae are small, planktonic, and mostly suspended in the water column (called as auricularia larvae). They feed by engulfing microalgae that they opportunistically encounter in the larval rearing tank. At this stage, the larvae will also require a type of microalgae that can be freely suspended or mixed in the water as the tank is mildly aerated. The second phase is when the larvae are settled on substrates and feed by grazing (called as pentactula larvae), which will then require microalgae or diatoms that can sink and adhere on the same substrate that larvae are grazing on.

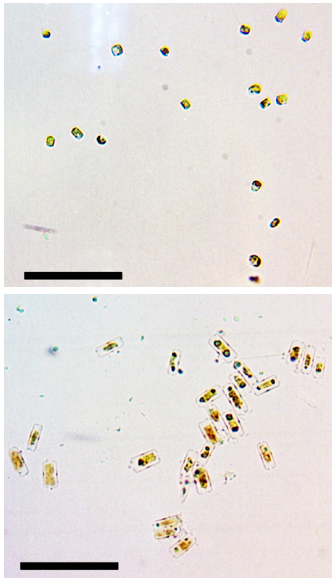


Figure 13. Images of *Chaetoceros calcitrans* (top) and *Navicula ramossisima* (above) under a microscope at 400 X magnification (scale bar = 50 microns)

Live microalgae and diatoms that have been tested as natural food for the auricularia larvae of sandfish are *Chaetoceros calcitrans*, *Chaetoceros muelleri*, *Rhodomomas* sp., and *Isochrysis galbana*. However, in order to simplify hatchery operations, only the *Chaetoceros* species (either *C. calcitrans* or *C. muelleri*, depending on availability) are currently being used. On the other hand, for sandfish pentactula larvae and early juveniles, the benthic diatom *Navicula ramossisima* is being used.

3.2. Live culture of microalgae in the hatchery

To sustain hatchery production year-round, live microalgae cultures have to be produced regularly. Large volumes and millions of cells of cultured microalgae will be needed for every larval rearing operation. This means that the live food will have to be propagated or "scaled up" to produce the needed number and volume.

The initial or starter stock of microalgae can be procured from algal laboratories that produce the pure cultures of desired species, like the Phycology Laboratory of SEAFDEC/AQD and similar facilities of the Bureau of Fisheries and Aquatic Resources (BFAR) in the Philippines.

3.2.1. Initial culture of microalgae to 10 L

The initial microalgae stock purchased from laboratories will usually be in 20 ml glass tubes. This can then be scaled up to 3–5 L in aerated glass bottles with continuous light from fluorescent or LED lamps in a dedicated cold room (air-conditioned). At this stage, the microalgae culture can be transferred to 10-L transparent containers or plastic carboys with aeration in dedicated algal culture shelves with continuous lighting using fluorescent tubes or LED lamps (see **Figure 14**). Algal cultures at this scale (>10 L) will require fertilizers to effectively grow and reproduce. The TMRL medium is commonly used at this stage. This is a mix of chemicals developed by Liao and Huang from the Tungkang Marine Laboratory in Taiwan (see **Table 1**).

Figure 14. Algal culture shelves with transparent carboys of *Chaetoceros calcitrans*

The prepared TMRL medium solution can be stored in 1 L glass bottles at ambient temperature. During live culture of microalgae, 1 ml of the prepared TMRL solution is added to every 1 L of the microalgae container. So, for a 10 L carboy, 10 ml of the TMRL solution will be needed only once at the start of culture.

3.2.2. Scaling up to 200 L and 1,000 L

To further up-scale the microalgae supply (both for *Cheatoceros* and *Navicula* species), the contents of the 10-L carboy can be transferred to outdoor 200 L tanks or larger. Here, the TMRL medium can still be used at the same concentration. However, it will be more practical to use commercial fertilizers which are much less expensive, especially for larger tanks of 1,000 L or more (see **Table 2**).

Table 1. Chemicals needed to produce 1-L solution of TMRL fertilizer medium

Chemical Name	Amount
Sodium metasilicate ($Na_2SiO_3 \cdot 9H_2O$)	1.0 g
Ferric chloride ($FeCl_3$)	3.0 g
Sodium nitrate ($NaNO_3$) or Potassium nitrate (KNO_3)	100.0 g
Disodium phosphate ($Na_2HPO_4 \cdot 6H_2O$)	10.0 g
Distilled water	1,000 ml

Table 2. Chemicals needed to produce a 1-L solution of commercial fertilizer

Chemical Name	Amount
Urea (46-0-0)	7.5 g
Ammonium phosphate (16-20-0)	25.0 g
Ammonium sulfate (21-0-00)	150.0 g
Sodium silicate ($Na_2SiO_3 \cdot 9H_2O$)	15.0 g
Ferric chloride ($FeCl_3 \cdot 6H_2O$)	5.0 g
Disodium ethylenediaminetetraacetic acid (Na_2EDTA)	5.0 g
Fresh water	1,000 ml

Because of the higher volume needed of the commercial fertilizer solution, it is practical to prepare batches of 20 L of this medium. During its use, 1 ml of this fertilizer solution will be needed for every 1 L of the cultured microalgae. So, for a 200 L tank, 200 ml will be needed and 1,000 ml (or 1 L) fertilizer solution will be needed for a 1,000 L or 1 ton tank.

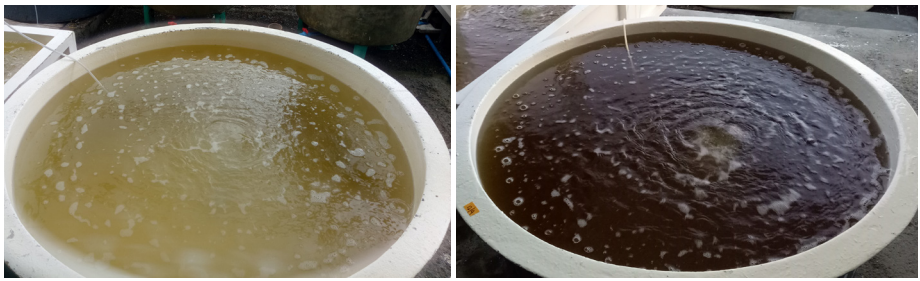


Figure 15. A 200 L tank with newly-transferred *Chaetoceros calcitrans* from carboy (microalgae density of 50,000-100,000 cells/ml) (left); *C. calcitrans* ready to be harvested after about 4 days of culture (microalgae density of 0.5–1.5 million cells/ml) (right)

4 Broodstock Management

4.1. Field collection, selection, transport

1. **Collect broodstock from intertidal shores.** Suitable sites for sandfish collection are flat intertidal shores with sandy-muddy to coarse sediments and often associated with seagrass (e.g. *Sargassum* sp., *Enhalus* sp., *Thalassia* sp., etc.).
2. **Schedule collection when low tide occurs late afternoon or early evening.** Because sandfish are nocturnal animals that emerge to feed at night, they are best collected from the wild between 5 and 7 PM. This is when most of them start to emerge from being buried during the daytime. At these practical conditions which is typical in most areas in the Philippines, collectors will not require diving. Many more sandfish will emerge towards nighttime and as the tide rises, but collection will require the use of flashlights, LED torch, or head-mounted lamps.
3. **Select broodstock that are preferably more than 400 g and with no signs of parasites and deformities.** The fecundity or the number of eggs of sandfish corresponds with the size of the adult female. So, ideally, bigger sandfish with sizes more than 400 g will be best as broodstock to optimize the number of potential eggs per individual. However, smaller adult sandfish of at least 150 g may also be collected if no larger individuals are available. Adult sandfish should be plump, heavy, and free from parasites and skin deformities.
4. **Collect broodstock in non-abrasive bags, baskets, or trays.** Sandfish can be temporarily placed out of the water for up to one hour but must be kept moist, shaded, and cool.

Figure 16. Selection and collection of sandfish broodstock from the field at late afternoon with rising tide

5. **Make sure that a proportional number of animals will be retained at the site to ensure sustainability.** In the wild, the distribution of males and females are about the same, meaning the ratio of male to female can be 1:1. So, random collection of broodstock will provide about similar chances of getting both sexes in a group. In a single collection batch, 30 to 60 individuals will be sufficient.
6. **Before packing, allow breeders to defecate in a separate container.** If the destination hatchery will take more than a day of travel, it is best to store them in a tub or basin with sea water to allow defecation or removal of gut contents for 12–24 hours before packing them for transport. This will reduce water quality degradation in the transport bags. For short duration transport (e.g. less than 4 hours), a few hours of defecation (1–2 hours) will be sufficient.

7. **Pack sandfish in polyethylene bags and place in styrofoam boxes for transport.** Pack the sandfish in thick transparent polyethylene (PE) transport bags (40 × 60 cm size). About 3 kg total weight of sandfish can be placed in one bag, with about 5 liters of clean seawater. No added oxygen is needed, especially for short duration transport of less than 12 hours. Bags are placed in styrofoam boxes in order to stabilize the temperature during transport. During hot weather, some ice packed in separate bags may be added in the box to lower the temperature, but not lower than 24 °C. During transport, it is ideal to minimize rocking and swaying motions. Too much vibration can cause stress to the animals, and can trigger evisceration.

Figure 17. Sandfish broodstock stored overnight in a plastic basin to allow excretion of gut contents before transport

4.2. Broodstock conditioning

1. **Acclimate the collected broodstocks to the water conditions in the hatchery.** Upon arrival from the field, allow acclimation of the animals to the water conditions in the hatchery. Do this by slowly transferring the animals from the bags into holding bins or tubs then minimally adding ambient sea water for 30–60 min. It is advised to disinfect the animals from the field by applying 1 ppm of povidone iodine solution into the holding bins for another 30–60 min, before rinsing with clean seawater and transferring to the final holding tanks.
2. **Thoroughly clean the broodstock tanks and disinfect prior to use.** The broodstock tanks may be made of concrete, fiberglass, wood, or canvass. Pre-washed, chlorinated, and sun-dried sand is provided inside the tanks at 2–3 cm thickness. Seawater level is maintained at a depth of at least 50 cm. This shallow depth will allow easy observation and monitoring of the animals. Aeration is provided to ensure proper water circulation and oxygenation throughout the tank. Seawater is exchanged at 100 % daily, using a slow flow-through or recirculating system.
3. **Stock broodstock from different batches separately.** A new batch of broodstock should not be mixed with other prior batches, especially when they came from different sites. This is to minimize potential contamination, and maintain easier monitoring of production performances of different batches. If the facility has enough tank space, biomass density of adult sandfish should be limited to <500 g per square meter of the tank floor. However, if tanks are limited, broodstock may be stored at <1,000 g per square meter. Higher biomass densities may result to body shrinkage after about 2–3 months of captivity. So, it is better to keep them at the lowest density possible.
4. **Maintain natural food and provide supplemental feeding to broodstocks.** Broodstock tanks are located in an area with translucent roofing to permit diffused natural light to allow growth of periphyton on the sediments which

serves as the primary food for the sandfish. In addition, broodstock can also be fed with a mixture of powdered formulated feed (like shrimp starter feed containing 50 % protein and 11 % fat) and ground marine macroalgae like *Sargassum* at a 50:50 ratio. This supplemental feed is sprinkled across the tank to ensure an even distribution. Aeration is temporarily stopped to allow the feed to sink and settle on the substrate sediments. Feeding rate is at 1 % of the total biomass of all sandfish broodstock in the tank. Feeding is commonly done in the late afternoon since sandfish more actively feed at night. Supplemental feeding can be every 3–4 days. Broodstock are conditioned in the tanks for 2 to 4 weeks before they are prepared for spawning.

Figure 18. Sun-dried (left) and ground (middle) *Sargassum*; powdered shrimp starter feed (right). Photos by J.C. Rodriguez, Jr.

5 Spawning

5.1. Spawning induction

1. **Allow broodstocks to defecate overnight prior to spawning.** A group of 20 to 40 mature adult sandfish, from the same batch and source, are selected from the broodstock tank. Ideally, adult sizes should be more than 200 g and free of any skin defects and parasites. They are transferred to a holding tank with clean sea water. They will be held here for 12 to 24 hours until they void their gut of sediments and excreta which can be sources of contamination (Ex. microbes, cysts, larva of other organisms, etc.). These excreted materials are siphoned out and the

Figure 19. A batch of broodstock are being prepared for spawning in a circular flat-bottom tank

water in the tank can be continuously exchanged to maintain good quality. After this defecation process, the animals are washed or rinsed with flowing freshwater and transferred into a separately-prepared shallow and flat-bottomed spawning tank (preferably 1–2 m^2 bottom area) with filtered and UV-treated sea water at 40–50 cm deep and provided with moderate aeration.

2. **Stimulate spawning through artificial induction.** There are various ways of artificially inducing sandfish to spawn, such as desiccation, hormone injection, *in-vitro* fertilization, and others. However, the most practical and convenient means of spawning induction is thermal stimulation and food stimulation, also called as "thermal shock" and "food shock," respectively. These methods are quite reliable, and easier to implement especially for small-scale and low-cost hatcheries. Sandfish can respond to thermal and food stimulation at any time of the day, but the most practical time for spawning can be done in the early morning when water temperature is relatively low.

3. **Induce spawning through thermal stimulation.** During thermal stimulation, the temperature of the water in the spawning tank is increased by 3–5 °C higher than the ambient temperature. To do this, the water level in the spawning tank is first reduced to 10–15 cm (from 40–50 cm) to lessen its volume for faster heating. Increasing the temperature is effectively and practically done by pre-heating enough seawater in a cauldron or vat then evenly mixing it into the spawning tank until a desired temperature is reached. Aeration in the spawning tank also helps in mixing. Commonly in the Philippines, the ambient water temperature in the morning (7–9 a.m.) can be between 26 to 29 °C. So, at an ambient temperature of 26 °C, the desired heated temperature should be at a maximum of 31 °C. While an ambient temperature of 29 °C will need a target heated temperature of 32–34 °C. It is also important to be cautious that the elevated temperature should not be more than 35 °C as this can adversely cause more stress to the animals. Heating can also be done using electric heaters but will be costly and achieving the desired temperature can take much longer than ideal. Broodstock are held in the tanks at an elevated temperature for 30 minutes. Then, they are abruptly transferred into another tank with clean seawater at an ambient temperature.

Figure 20. Mixing of pre-heated sea water into the spawning tank for thermal stimulation (left) with regular checking of water temperature (right)

4. **Apply food stimulation to increase spawning success.** Alternatively, food stimulation can be implemented as an independent (without thermal stimulation) spawning induction method or in sequence after the thermal stimulation. This is done by introducing some concentrated microalgae into the spawning tank. The most commonly used food is the commercially-available dried *Spirulina* powder being sold in a 500-gram can. Since the use of this powder is relative to the volume of water, it is more practical to transfer the broodstock into a smaller container or a basin that is wide enough to hold all animals without them piling on each other. Then, 0.2 g of *Spirulina* powder is added to every liter of water in this container. The animals are held in this "food bath" for 30–60 min. Then they are rinsed with water and transferred back to the spawning tank with clean seawater at ambient water temperature.

Figure 21. Food stimulation of sandfish broodstock in a "*Spirulina* bath" (left) using commercial *Spirulina* powder (right)

5. **Males spawn first by releasing milt, followed by females with a burst of eggs.** When the animals are in good condition and with mature gonads, spawning can occur within an hour after stimulations. But when the animals' condition is not optimal, spawning may take up to two hours or longer or not at all. While waiting for spawning to occur, it is important not to further disturb the sandfish in the tanks. Touching or moving about near the tanks should be avoided because vibrations, reflections and shadows can disrupt the spawning process. Males are expected to spawn first by releasing steady streams of white milt through their gonopore (a small opening at the top part of the anterior end or "head") that may last up to 3 hours. Females commonly spawn after the males by releasing quick bursts of eggs after a characteristic bulging of the "head." Females may perform 2–3 quick bursts at about 5 minutes interval. Mature and good quality eggs are characterized as being yellowish to light orange in color. If the burst of eggs is pale or whitish in color, then these are highly likely to be immature or of inferior quality. Mature female sandfish have high fecundity. A mature female sandfish weighing 250 g may release around 2 million eggs, and larger 450-gram females may release 3–4 million eggs each.

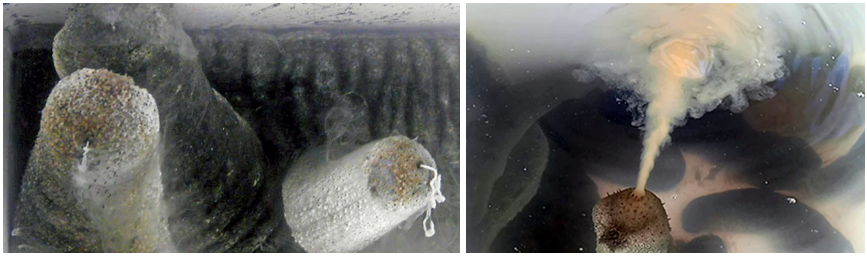


Figure 22. Sandfish males spawning steady streams of milt through their gonopore (left) and a female sandfish spawning a burst of yellowish eggs from its bulging "head" (right). Photos by C.P. Recente

6. **Avoid introducing too much milt in the tanks.** Commonly, there will be more males spawning than females. Additionally, males tend to spawn earlier and longer, thereby releasing a lot more milt in the water than what is desired. A single healthy male may spawn more than 1 million sperms within 10 minutes. Too many milt containing sperm cells in the tank may cause "polyspermy," which is a situation whereby a single egg is fertilized by multiple sperms. This can lead to abnormal embryonic development, non-hatching, and irregular larval development. To avoid this, spawning males may already be removed from the spawning tank before the tank gets very cloudy. A practical visual cue is when the water is just lightly cloudy and the bottom of the tank is still visible. In a spawning batch, not all individuals are expected to spawn. On the average, a batch of 40 animals will only involve successful spawning of 10–20 males and 2–5 females.
7. **Recondition the animals after the spawning induction activity.** Animals that have spawned successfully are placed in a separate broodstock recovery tank. Those that failed to spawn can be returned to the original conditioning tanks and may still be used on the next spawning activity. The same batch of broodstocks may be used for spawning after about one month of conditioning. However, holding of broodstock in tanks for the long term is not recommended. Commonly, adult sandfish will gradually shrink in size and lose good reproductive potential after about three months of captivity in the tanks, despite the provision of supplemental feed. Therefore, it is recommended to return these animals to the field, whether in marine ponds or sea pens, where they can recover more naturally while feeding on available food. Reproductive vitality can be regained after about two months of re-conditioning in the field.

Figure 23. Sandfish broodstock released for recovery (left) in 100 m^2 sea pens (right)

5.2. Egg collection and monitoring

1. **Immediately collect and rinse the newly-fertilized eggs.** Fertilization occurs instantly after the eggs are spawned by the females with sperms already present in the water. So, after the removal of all broodstock from the spawning tank, the fertilized eggs must be harvested immediately. Depending on the design of the spawning tank, eggs can be collected through a drain pipe, passed through hoses by siphon, or by manual scooping. As a practical method, scooping of the water from spawning tank can be quicker and more practical. This way, eggs are scooped out from the spawning tank and passed through a fabricated micro-filter scoop net (40-cm diameter) with <100-micron mesh. The brim of this scoop sieve is suspended on a frame above a basin or bucket. This set-up ensures that most of the filter net is constantly under water. This washing method will concentrate the eggs (that are expected to be >160 micron in size) inside the filter, while the excess sperm and debris are washed out. A continuous flow of clean filtered and UV-treated seawater is provided to ensure that the eggs remain in suspension and do not settle on the net. The concentrated eggs are scooped out regularly during this process and placed in a holding bucket with moderate aeration to keep eggs from clumping.

Figure 24. Harvesting and rinsing of fertilized eggs by micro-filtration (<100 micron net) with flowing UV-treated seawater

2. **Assess the viability of fertilized eggs.** Monitoring of eggs under a microscope is important to determine their quality. Upon fertilization, cell division and embryonic development occurs very quickly. The single egg cell will develop into a 2-cell and 4-cell stage within one hour after fertilization. It will develop into a 32-cell stage within three hours. During these multiple-cell development stages, it is easy enough to determine the “good” or viable eggs against the potentially “bad” or non-viable eggs using a microscope. Viable eggs are perfectly round with a diameter of 150–180 microns, while non-viable eggs are characterized by deformities like corrugations, protrusions, or irregular cell development. Clumped cells of the multi-cell stages should show evenly-sized and radially arranged cell components or blastomeres. Uneven clumping of blastomeres can indicate non-viability. A good spawning batch should show more than 80 % viable eggs.

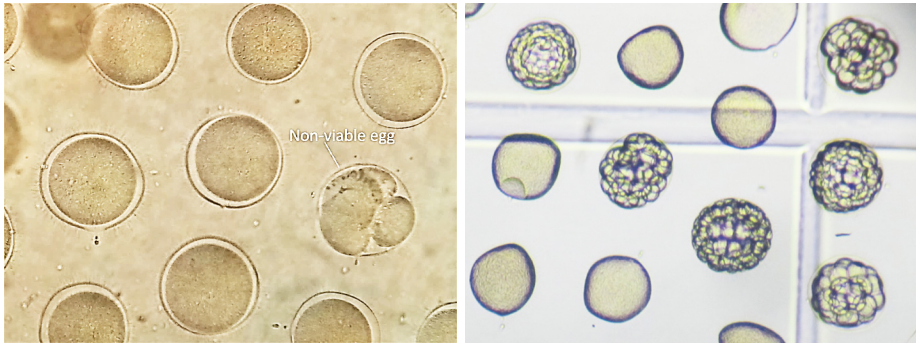
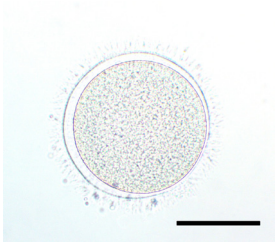
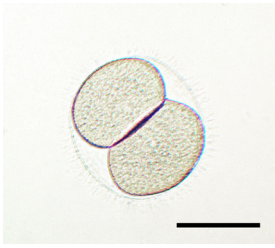
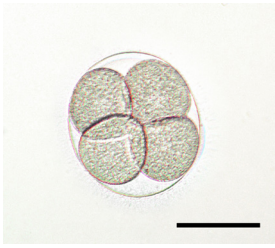
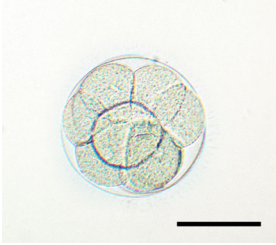
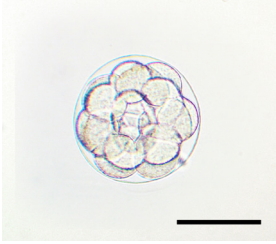
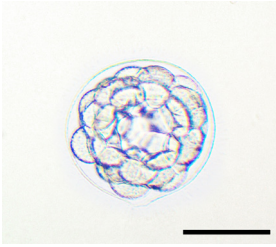
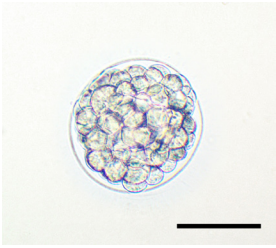
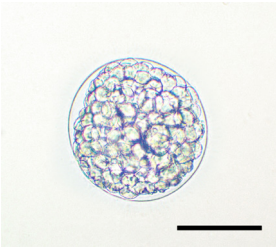
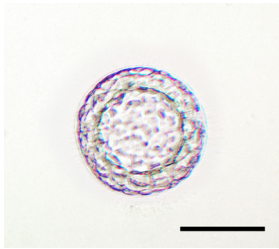
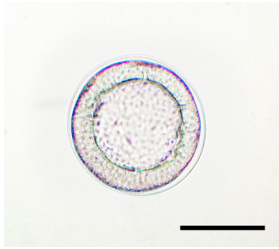
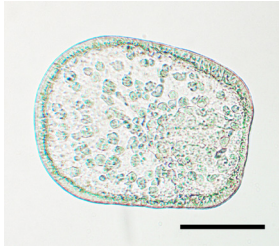
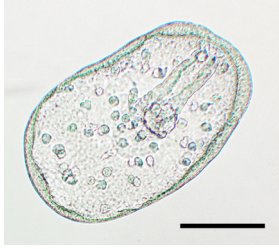


Figure 25. A non-viable egg amongst "good eggs" 10 minutes after spawning (left). Multiple-cell stage (clumped blastomeres) 3 hours after fertilization, together with some unfertilized and unviable eggs (empty cells) (right)

Table 3. Time schedule and embryonic development stages of sandfish *H. scabra*

Stage	Time from spawning	Actual Photo (scale bar = 100 µm)	Description
Newly Fertilized Eggs	10 min		• The fertilized egg becomes a single unsegmented **zygote**. • The zygote is coated with an outer layer called **fertilization membrane** which disallows other sperms from entering the egg.
2-cell Stage	30 min		• The **cleavage stages** begin when the embryo starts to divide and become segmented, starting with the 2-cell stage. • The fertilization membrane is present and excess sperms remain visible.
4-cell Stage	50 min		• The embryo continues to divide, now into 4 cells or **blastomeres**. • The overall size of the embryo remains the same (150–180 microns), so the dividing cells becomes smaller.

Stage	Time from spawning	Actual Photo (scale bar = 100 µm)	Description
8-cell Stage	1.5 h		• After 1.5 h, the embryo will have divided into 8 cells. • At this time, the eggs have been harvested from the spawning tanks and rinsed. So, most of the excess sperms have been rinsed off.
16-cell Stage	2.5 h		• At 2.5 h after fertilization, 16-cell embryo is formed. • These cells or blastomeres are **radially** arranged about the center.
32-cell Stage	3 h		• At 3 h, the blastomeres become smaller but double in number again to become a 32-cell embryo.
64-cell Stage	4 h		• After another hour forms the 64-cell stage.
128-cell Stage	6 h		• At about 6 h after spawning, the fertilized egg or zygote would have continuously doubled seven times to reach the 128-cell stage.

Stage	Time from spawning	Actual Photo (scale bar = 100 µm)	Description
256-cell Stage	9 h		• At 256-cells, the blastomeres become too small to be clearly distinguished. • At the center of the embryo, the **blastocoel** (a fluid-filled cavity) starts to form.
Blastula	12–14 h		• A **blastula** is now formed, which is still about the same size as the original egg. • The central blastocoel is clearly visible, creating what seems like two rings. • The outer wall is ciliated and the blastula can spin within the fertilization membrane.
Early Gastrula	20 h		• The embryo "hatches" out from the blastula and the cells are reorganized into multiple layers. • The early gastrula has a charateristic double-wall and now starts to grow and takes a generally oval shape.
Late Gastrula	28 h		• As the gastrula grows, it also continues to develop more functional cells. • The late gastrula now develops an **embryonic gut** or the **archenteron**. This will further develop an opening (called the **blastopore**) out of the outer wall, which will eventually form the early "mouth".

3. **Determine the total number and density of fertilized eggs prior to stocking.** After rinsing and placing fertilized eggs into a collection bucket, the total number of eggs for the batch needs to be determined. Firstly, the total volume of water with eggs in the bucket must be determined. For convenience, the holding bucket must have graduation lines marked on the sides. More seawater can be added to top-up the bucket to a manageable

volume (Ex. 10 L, 20 L, etc.). Then, continuous aeration is supplied to ensure that the contents of the bucket is well-mixed and evenly suspended. This even suspension is important to achieve a good representation of samples to be taken for measurement. Counting and observation of the eggs is done using a compound microscope and counting chambers such as a Sedgewick counting chamber. A 1-ml sample will be taken from the bucket using a pasteur pipette or micro-dropper. To estimate the total number of eggs in the bucket, multiple 1-ml samples (usually taken 5 times) will need to be counted.

Estimating the density (eggs/liter) of concentrated fertilized eggs in the bucket can be computed using the practical example below:

Let:

M = average density of concentrated newly-fertilized eggs (eggs/liter)

N = number of samples counted

L = 1,000, a constant multiplier to convert density to eggs/liter

A = number of eggs from 1st 1-ml sample

B = number of eggs from 2nd 1-ml sample

C = number of eggs from 3rd 1-ml sample

D = number of eggs from 4th 1-ml sample

E = number of eggs from 5th 1-ml sample

Formula:

$$\mathbf{M = \frac{A+B+C+D+E}{N} \times L}$$

For example, the actual total egg counts of five separate 1-ml samples are 395, 403, 438, 432, and 420, then:

$$M = \frac{(395+403+438+432+420)\ \text{eggs/ml}}{5} \times 1{,}000\ \text{ml/liter}$$

$$= 417.6 \times 1{,}000$$

$$= 417{,}600\ \text{eggs/liter}$$

If the estimated total volume of concentrated eggs in the bucket is 10 L, then the total number of eggs for this spawning batch is:

TOTAL EGGS = 417,600 eggs/liter X 10 L = 4,176,000 eggs

4. **Incubate the fertilized eggs and embryos in larval rearing tanks.** Since the total egg count and density in the holding bucket are known, the actual volume to be stocked into the incubation tanks can then be computed. Stocking at very high egg densities can lead to very high mortality. The optimal stocking density of sandfish eggs in incubation tanks should be less than 500 eggs per liter. In practice, the common stocking density shall be 200–300 eggs per liter.

Given the example above, wherein the 10-liter holding bucket contains a total of 4,176,000 eggs or at 417,600 per liter, the computation for the stocking volume needed for a 3-ton tank is as follows:

Let:

V = volume to stock or incubate from the bucket with concentrated fertilized eggs

M = average density of concentrated newly-fertilized eggs (eggs/liter)

= 417,600 eggs/liter

D = desired stocking density (eggs/liter)

= 300 eggs/liter

T = volume of water in the incubation (in liters)

= 3,000 liters

Formula:

$$\mathbf{V = \frac{D \times T}{M}}$$

For example, the capacity of the incubation tank is 3 tons or 3,000 liters, then:

$$V = \frac{300 \text{ eggs/liter} \times 3{,}000 \text{ liters}}{417{,}600 \text{ eggs/liter}}$$

= 2.155 liters or 2,155 milliliters

This example shows that 2.155 liters is needed to be stocked into a 3-ton incubation tank to achieve a target stocking density of 300 eggs/liter.

It is important that the incubation tanks have been cleaned, disinfected and filled with the target volume of filtered and UV-treated seawater hours before the intended stocking time. Aeration systems should also be already in place and running. Water parameters (like temperature, salinity, and pH) must be the same for both the holding bucket and the incubation tanks to minimize stress to the sensitive fertilized eggs and developing embryos.

Figure 26. The computed volume of concentrated eggs is carefully measured and stocked into incubation tanks

6 Larval Rearing

6.1. Hatching

Like other echinoderms, fertilized eggs of sea cucumbers, like that of the sandfish, will develop and hatch (out of the egg casing) into what is called as the **gastrula** (250–350 micron in length) of the embryonic development. This occurs between 16–20 hours after fertilization, depending on the water temperature. Warmer temperature tends to promote faster development and hatching. The optimal incubation temperature ranges from 27–29 °C. Mild aeration is provided inside the tank to promote just enough water circulation to keep the gastrula suspended and moving about the water column. The hatched gastrula will make use of its reserved nutrients and will continue the embryogenesis to develop into the **early auricularia** larvae (350–400 micron in length) that will occur 36–40 hours (or about 2 days) after fertilization. At this stage, the larvae will require feeding.

If enough tanks are available in the hatchery, the gastrula larvae can be transferred into separate larval rearing tanks when they are 2 days old after fertilization. However, for practicality, the incubation tank may already serve as the larval rearing tank without the need of transferring the gastrula, provided proper cleaning and water management is observed (as will be described later).

6.2. Larval development

There are three major stages in the larval development of sandfish, namely (1) the auricularia stage, (2) the doliolaria stage, and (3) the pentactula stage. Each of these stages involves 3 or 4 minor and intermediate stages.

6.2.1. The auricularia stage

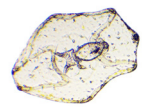
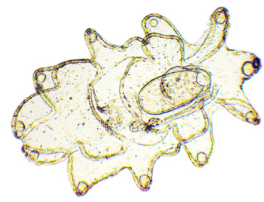
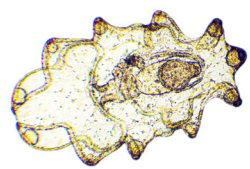
In about two to three days after fertilization, the gastrula will develop into **early auricularia** larvae (300–500 microns in length). At this stage, the larvae will already begin to feed on external food. This is the main reason why the microalgae feed is introduced into the larval rearing tank as early as Day 2 after fertilization, in anticipation that some auricularia larvae may develop early, depending on water temperature. The larvae would have developed its digestive system with a "mouth" or buccal cavity, a simple esophagus and intestine, a "waste chamber" or cloaca, and anus. The general shape of the early auricularia larva is generally smooth and is outlined by the ribbon-like structure called **ciliary bands**.

Between 6 and 12 days after fertilization, the larvae would be at the **mid-auricularia** stage of development. They are larger (600–1000 microns) and the digestive system is more pronounced and the intestine is enlarged. The outline of

the body would have five distinct lateral lobes (on both sides of the body) when viewed under a microscope. It is also at this stage when fat globules called **hyaline spheres** begin to emerge at the end of each lobe. These hyaline spheres are storage structures which hold neutral lipids of "fat reserves" which will eventually fuel the metamorphosis process in later stages of development.

Finally, the larvae will start to shrink (500–700 microns) as it enters the late auricularia stage. The lobes also begin to shorten, but the hyaline spheres will now be much larger and closer to the center of the body. Here, the neutral lipids will be converted to triacylglycerol and saturated fatty acids.

Table 4. Stages of auricularia larvae development

Stage	Time from fertilization	Actual Photo (scale bar = 500 µm)	Description
Early Auricularia	2–5 d		• The gastrula will grow and take about one day to become an early auricularia larva. • At this stage, the larvae will be about 300–500 microns. • The auricularia larva will begin to feed.
Middle Auricularia	6–12 d		• The **ciliary bands** (ribbon-like structures) are more distinct, as well as the **lateral lobes** become more prominent during the mid-auricularia stage. • The larva will be at its biggest size of 600–1000 microns. • The **hyaline spheres** at the tips of the lobes start to form.
Late Auricularia	10–18 d		• During the late auricularia stage, the lobes are shortened. • The whole larva begins to shrink (<700 microns) as it transitions to become a doliolaria larva. • The hyaline spheres become larger.

6.2.2. The doliolaria stage

When the larvae have accumulated enough nutrients and stored them in the hyaline spheres, they will begin to transform into the doliolaria stage, 14–20 days after fertilization. This stage is likened to the chrysalis or cocoon stage of the caterpillar of a butterfly or moth. At the **early doliolaria** stage, the buccal cavity or "mouth" will close and the larvae will stop feeding. The lateral lobes will be completely absent in **mid-doliolaria** stage, as the larvae become "barrel-shape" with a more reduced size (400–500 microns). The hyaline spheres will be at their biggest size. Because they are more compact and darker in color, they can now be visible as dark spots in a glass beaker when viewed by the naked eye.

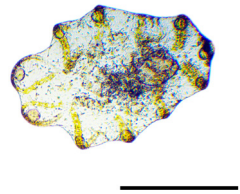
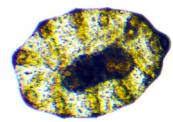
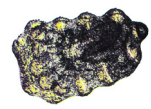
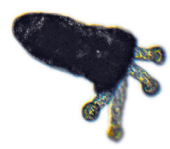
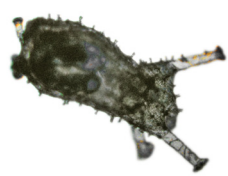
After three days of being doliolaria, the primary tentacles which are characteristic of the sandfish, will form to indicate the **late doliolaria** stage. This also signifies the start of the final metamorphosis process. The hyaline spheres would have decreased in size as the larva utilizes its nutrient reserve. The doliolaria larvae are active swimmers, aided by the minute hair-like cilia around their bodies. Eventually, they will begin to sink as they seek for ideal substrates to settle on. They will attempt settlement on various surfaces but will detach if food or conditions are not conducive and transfer to other more ideal substrates.

In nature, they will settle on seagrass leaves or blades that are coated with microalgae and diatoms. In the hatchery, when more than 50 % of the larvae have developed into doliolaria, artificial settlement plates coated with *Spirulina* powder are added into the larval tanks. This happens 12 to 16 days after fertilization. The presence of food on an ideal substrate will induce the settlement of the larvae.

6.2.3. The pentactula stage

Within two to three days of settlement, the doliolaria larvae will metamorphose into the third main larval stage – the **pentactula**. At this stage, the larvae cannot swim anymore and the mode of living will now be epibenthic. This means that they will be crawling using their newly developed tentacles to move. The tentacles are also used for grazing on the substrate for feeding. Occasionally, the pentactula larvae will also catch food particles that are floating about the water. They will still be very small (1–2 mm) and have very thin skin that will make them appear to be almost transparent to the naked eye. In about two weeks after settlement, the pentactula larvae will grow to 5–10 mm and develop thicker and darker skin as they become **early juveniles** or spats which can be easily identified on the settlement substrate.

Table 5. Stages of doliolaria and pentactula larvae development

Stage	Time from fertilization	Actual Photo (scale bar = 500 µm)	Description
Early Doliolaria	12–14 d		• The auricularia now becomes an early doliolaria and stops feeding. • The larva will continue to shrink in size; lateral lobes reduced
Mid-Doliolaria	13–16 d		• A mid-dololaria larva will have a characteristic **barrel shape** and appear darker in color. • Lateral lobes absent • Hyaline spheres will be at their biggest.
Late Doliolaria	14–18 d		• The **primary tentacles** start to form. • Hyaline spheres reduced as they are consumed for **metamorphosis**. • They have numerous hair-like **cilia** around their body, aiding movement in water.
Early Pentactula	15–20 d		• The larva will start to feed with its tentacles. • Body still very dark and compact. • The cilia around the body will disappear and the pentactula larva will begin to settle on an ideal substrate.
Late Pentactula	16–25 d		• Body wall and skin start to develop, but are still very thin and almost transparent. • The late pentactula larva will have more developed tentacles that it uses both for feeding and locomotion on the substrate.

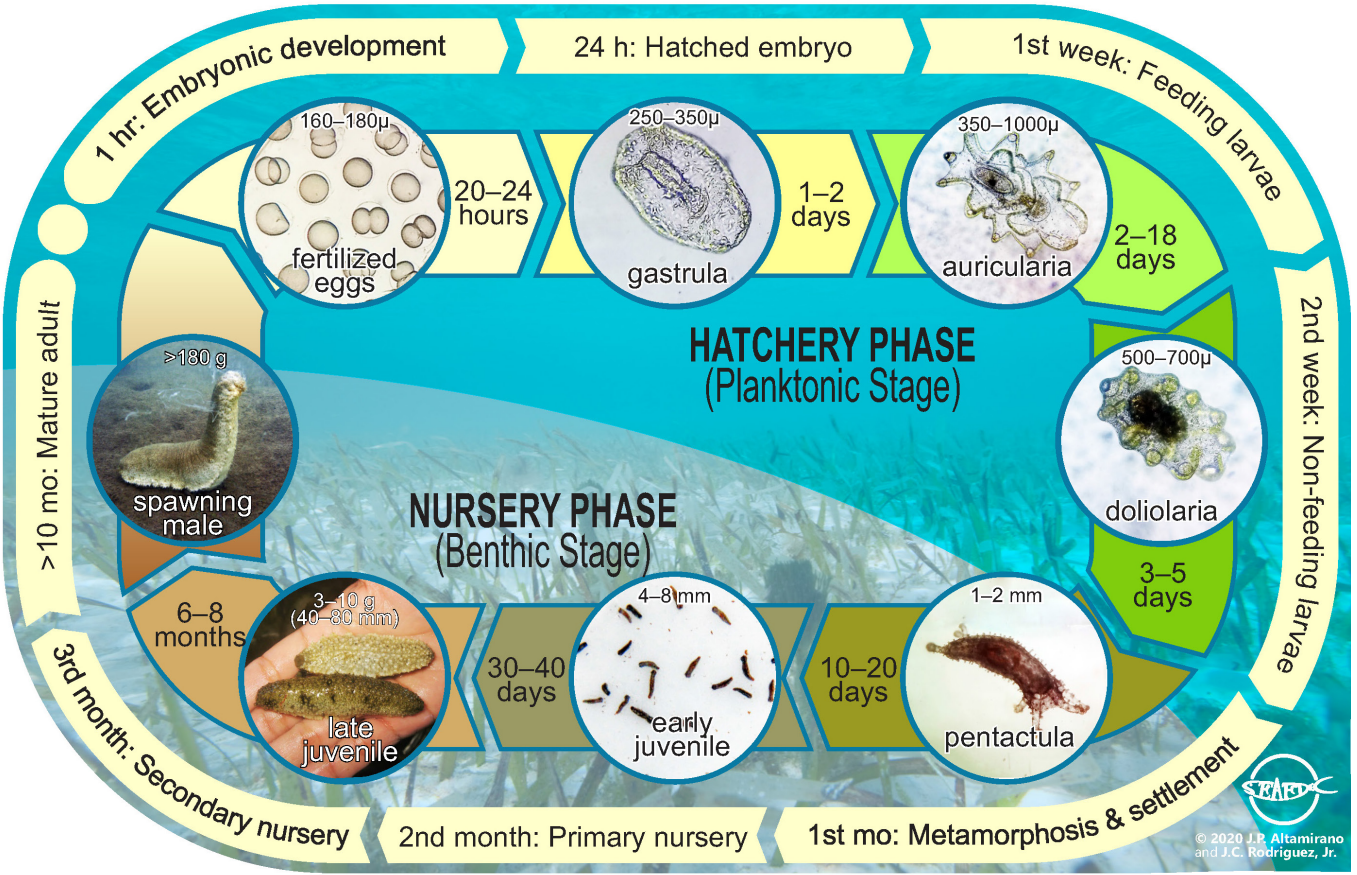


Figure 27. The life cycle and development schedule of sandfish *Holothuria scabra*

6.3. Water management during larval rearing

1. **Stop the aeration before cleaning the larval tanks.** About 15–20 minutes prior to cleaning of the tank floor, aeration in the larval rearing tanks must be temporarily stopped. This will allow the live and active auricularia larvae to move towards the upper portion of the water column, so they will not be sucked out from the bottom during the siphoning process. During the later days of larval rearing, however, some doliolaria larvae will tend to sink to the bottom when aeration is stopped, but these can be collected from the siphoned water by draining through a sieve of 90–100 microns.

2. **Clean the tank floor of debris by siphoning.** After stocking the fertilized eggs in the tanks, water can be held for a few days with mild aeration to allow undisturbed larval development. Water change can be initiated on the fourth or fifth day. At this time, the tank floor may show signs of settled debris that needs to be removed. A fabricated siphon tube fitted with a sponge at the suction tip can be used for this purpose (see **Figure 28**). This can be made from a common PVC pipe (13 mm or ½ inch diameter) with a perforated cross bar tip to allow for a wider suction coverage. The other end of the pipe is connected to a hose (19 mm or ¾ inch diameter) that drains to a harvesting pit.

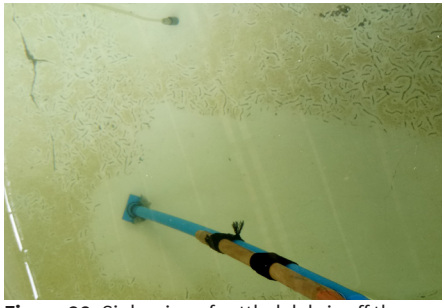


Figure 28. Siphoning of settled debris off the tank floor using a fabricated siphon device made of PVC pipe

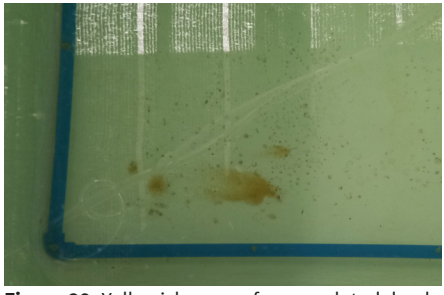


Figure 29. Yellowish mass of accumulated dead auricularia larvae on the tank floor. Photos by J.C. Rodriguez, Jr.

   Siphoning is done by sweeping all across the tank floor until all debris (that may include dead larvae, settled uneaten food, and others) are removed. Siphoning of the tank floor is done every two to three days, depending on the rate of accumulation of debris.

   Occasionally, dead larvae can accumulate and settle on the tank floor as a mass of yellow to orange in color (see **Figure 29**). If not immediately cleaned, this mass can quickly decompose and be colonized by bacteria. This is visually indicated by the color of the mass turning pinkish in color. Regular cleaning and siphoning of the tank can prevent this situation.

3. **Change water in the larval rearing tanks every 2 days.** Seawater inside the larval tanks is changed every 2 days during the auricularia stage (Day 5 to 18). This is done by siphoning out 20–30 % of existing water from the tank using

larger hoses (2.5 cm diameter) which drains to a harvesting pit. The siphoning end is fitted with a fabricated sieve drum (60 cm long, 40 cm diameter) covered with fine netting or plankton net (80–100 microns). This drum creates a wider surface area around the siphoning end of the hose which reduces the suction pressure to protect the sensitive larvae from being sucked in. The drum can be rotated about during siphoning to displace any larvae that may adhere to the sieve.

Figure 30. A fabricated sieve drum device used in water change in the larval rearing tank. Photos by J.C. Rodriguez, Jr.

4. **Reduce frequency of water change during the doliolaria phase.** During the peak of doliolaria development stage (Day 14–20), water change may be reduced or stopped to decrease disturbance and promote better settlement. At this stage, the larvae will be feeding less and the amount of feed is also reduced. The regular water change (20–30 % every 2 days) can be resumed during the pentactula stage (Day 15 onwards) until harvest. At this later stage, the sieve drum will not be needed for siphoning because the larvae would have already settled.

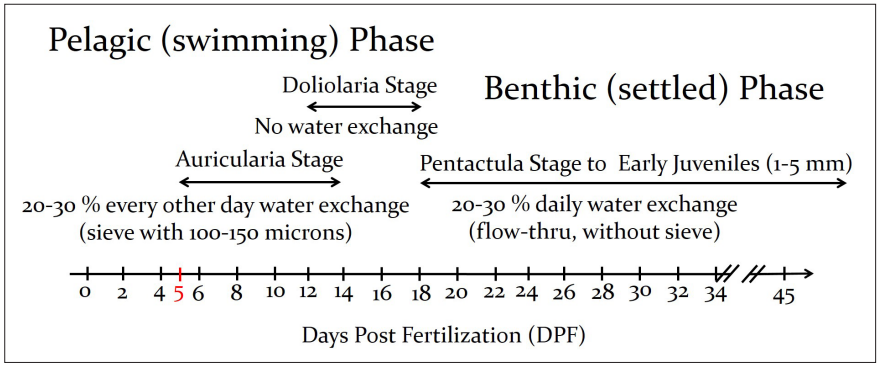


Figure 31. Water management schedule for the larval rearing tank

5. **Use clean and UV-sterilized seawater to top-up the tanks.** Refill the larval tanks with clean seawater by always using the micro-filters and UV treatment. Additionally, 1-micron filter bags may be used at the end of the water pipe or hose to filter out potential debris from the water lines.

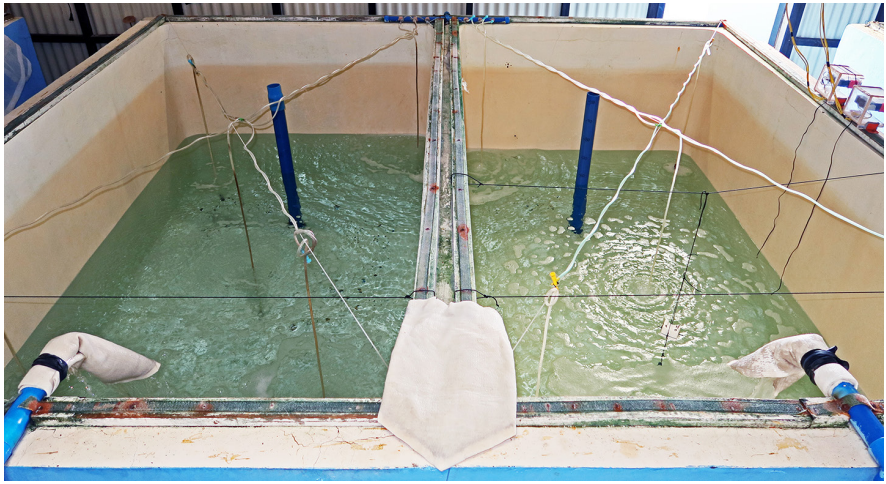


Figure 32. Filling the larval rearing tanks with clean seawater through a final stage 1-micron filter bag fitted on the water outlets

6.4. Feeding management of sandfish larvae

6.4.1. Feeding schedule for larval sandfish

For practicality, feeding of the larval sandfish may be done twice daily. The daily feeding ration may be divided into two parts, to be fed in the morning (8–9 a.m.) and the other portion is fed in the afternoon (4–6 p.m.).

Larval sandfish begins feeding during the auricularia stage when the larvae are in the **pelagic** or swimming phase. The primary larval food that is used for feeding at this phase is the suspended microalgae, *Chaetoceros calcitrans* (Cc). The pelagic feeding phase commonly starts at the 2nd day post-fertilization (DPF). However, some larvae develop earlier. So, the practical feeding in the hatchery begins in the afternoon of the first day, although at a smaller amount of 2,500 microalgae cells/ml. This is to ensure that food is readily available whenever some gastrula already develops into early auricularia larvae. The feeding rate increases to 5,000 cells/ml at 2–5 DPF. This increases further to 10,000 cells/ml at 6–14 DPF. This is the stage when the larvae feed the most. During the doliolaria stage (12–18 DPF), however, the feeding rate is reduced to 5,000 cells/ml since most doliolaria are non-feeding, but some auricularia are yet to develop.

During the **benthic** or settled stage, the pentactula larvae would have changed their mode of feeding to grazing on the substrates. Therefore, the food items are ideally those that adhere to the substrate as well. Thus, the benthic diatom, *Navicula* sp. is used to feed the settled pentactula larvae and early sandfish juveniles.

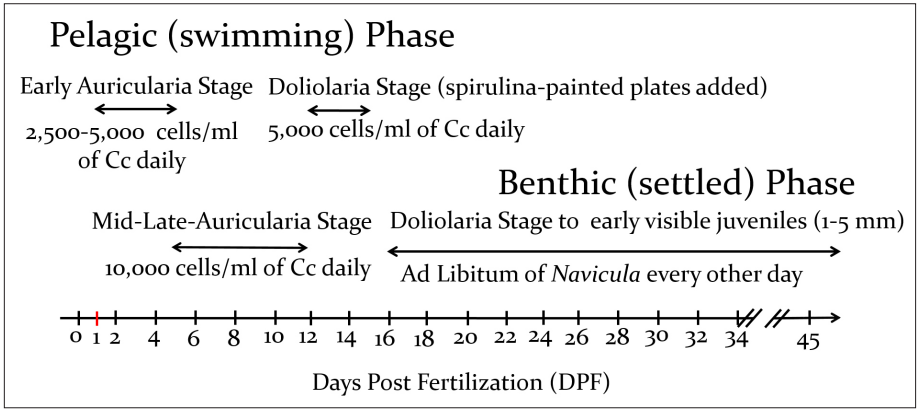


Figure 33. Feeding schedule for larval sandfish

6.4.2. Feeding rate calculation

Determination of accurate feeding rates is important in larval rearing. This is to optimize the use of live microalgae products by minimizing wastes from potential overfeeding and minimize problems on larval development that can be caused by potential underfeeding.

The desired feeding rates and densities of required microalgae are known based on various studies. However, algal cultures can have different densities, so it is important to determine how much volume of the microalgal culture will be needed during each feeding time. Therefore, computation of feeding volumes is dependent on the density of the microalgae stock (Ex. *Chaetoceros calcitrans*) in the culture tanks. This is determined using multiple small samples from the stock by a pipet or dropper, then counting using a hemocytometer and a microscope.

The calculation of the density (cells/ml) of the microalgae stock using a hemocytometer and computation of feeding volume are shown below with an example:

Let:

Dma = density of microalgae stock (cells/ml)
A = number of cells in the “A” block
B = number of cells in the “B” block
C = number of cells in the “C” block
D = number of cells in the “D” block
NB = number of blocks counted
HC = 10,000, a constant multiplier to convert density to cells/ml (note that only 0.1 microliter is counted in each block of hemocytometer counting chamber)

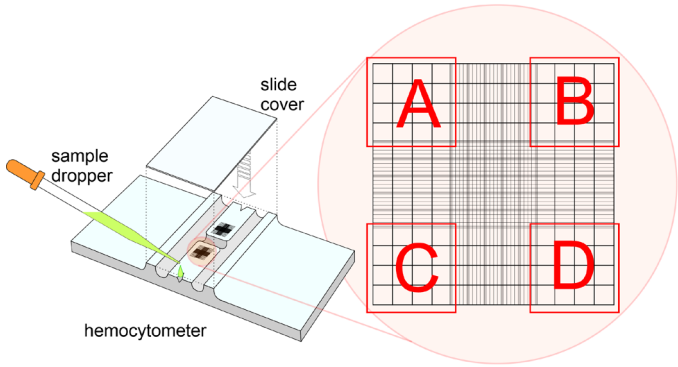


Figure 34. A hemocytometer and the four counting blocks (A, B, C, D)

Formula:

$$\textbf{Dma} = \frac{\textbf{A+B+C+D}}{\textbf{NB}} \textbf{ x HC}$$

For example, total cell counts from the four blocks are 123, 120, 118, and 129. Therefore, the calculation of the density of microalgae is as follows:

$$\text{Dma} = \frac{123+120+118+129 \text{ x } 10{,}000}{4}$$

= 122.5 x 10,000
= 1,225,000 cells/ml as the density of the microalgae stock

Now, in order to calculate the volume needed from the microalgae stock to feed the sandfish larvae at 10,000 microalgae cells/ml in a 3-ton tank (or 3 million milliliters), we assume:

Vms = volume needed from microalgae stock for feeding
Dma = density of microalgae stock (cells/ml)
DF = desired feeding (which is 10,000 cells/ml)
Tvol = tank volume in milliliter (which is 3 million ml)

Formula:

$$\textbf{Vms} = \frac{\textbf{DF x Tvol}}{\textbf{Dma}}$$

Example:

$$\text{Vms} = \frac{10{,}000 \text{ cells/ml X } 3{,}000{,}000 \text{ ml}}{1{,}225{,}000 \text{ cells/ml}}$$

= 24,490 ml or 24.5 L as the volume required for a 3-ton tank at 10,000 microalgae cells/ml rate

7 Early Juvenile Production

7.1. Settlement plates

Artificial settlement plates are required as the substrate where the pentactula larvae will settle on. In the sea cucumber hatchery, these are practically made from corrugated polyethylene roofing material, cut into smaller sheets. The dimensions of the plate will be proportional to the size of the larval tank, and the target number of plates to be used. This roofing material is usually smooth, so these are scratched using rough sandpaper to enhance their surface roughness. Before use, the plates are thoroughly washed and disinfected with chlorinated water.

Commercially-available *Spirulina* powder (same as that used for food stimulation during spawning induction) is prepared as paste and coated on the plates. The paste is prepared by mixing the *Spirulina* powder with clean seawater at a ratio of 1 g *Spirulina* powder per 3.5 ml of seawater. This paste is thinly coated on the plates by painting with an ordinary paint brush. Since most of the larvae settle on the upper side of the settlement plates, these plates are coated only on one side in order to economize the usage of the rather expensive commercial *Spirulina*. The *Spirulina*-coated plates are then air-dried for one day before using in the tanks. Direct sun-drying should be avoided.

Figure 35. *Spirulina* paste is lightly "painted" on the settlement plates (left), which are then air-dried indoor (right). Photos by J.C. Rodriguez, Jr.

Prior to use, the plates are half-rolled on the coated side and then temporarily fastened at one end. The plates are stacked haphazardly but horizontally on top of each other in the tank. In a 3-ton tank, 100 rolled plates (60 x 30 cm) can be

effectively placed. All the plates need to be always submerged in the water to receive the settling larvae.

The *Spirulina* coating on the plates will serve as the settlement cue for the doliolaria larvae. The plates are introduced into the larval rearing tanks when at least 50 % of the auricularia larvae have developed into doliolaria larvae. This happens at around 14–18 DPF, depending on weather conditions. In good conditions, 50 % doliolaria can be attained after 12 DPF. However, during non-ideal conditions, especially when temperatures are colder, this may occur longer at 18 DPF or more.

Doliolaria larvae will settle as pentactula as early as one day after the introduction of plates in the tanks. Some may also settle on the tank walls, below the water line. These can be carefully brushed off to allow them to settle on the plates below instead. Newly-settled larvae will look transparent and will be difficult to see. But they will quickly develop and become darker as they graze and feed off the settlement plates.

Most of the *Spirulina* paste may be consumed by the larvae within 3 to 4 days. At this time, food is supplemented by the benthic diatom, *Navicula*. The slurry of *Navicula* can be harvested from the live culture tanks and added into the larval rearing tanks. *Navicula* cells will settle evenly on the plates as long as continuous but mild aeration is provided. The supplemental feeding with *Navicula* is done every two or three days, depending on how fast it is consumed by the larvae and juveniles.

Figure 36. *Spirulina*-coated plates are rolled and submerged in the larval rearing tank.

Figure 37. Two to three days after settlement, early juveniles will appear as tiny black dots on the settlement plates.

Figure 38. Golden-brown or copper-brown live *Navicula* slurry being harvested for supplemental feeding in the larval tanks

Because larval development is not synchronous, feeding of *C. calcitrans* at 5,000 cells/ml per day is still maintained even after the plates are introduced. This is to allow the late-developing auricularia to catch up.

7.2. Water management after settlement

After the introduction of the settlement plates, water may become a bit turbid as some *Spirulina* powder can become dislodged from the plates. Water must then be carefully changed in the next day or two, until the water becomes clear. Maintain the water level to submerge all plates during water replacement because many larvae may have already settled. This is accomplished by slowly draining a portion of the water out from the tank floor using the built-in stand-pipe, while clean seawater is slowly filled in. The stand pipe is fitted with a larger pipe cover that is perforated at the bottom and covered with fine-meshed net (see **Figure 40**).

Figure 39. Early juveniles of sandfish grazing on benthic *Navicula* microalgae coating the settlement plates

Using this method, water exchange can be done up to 50 % per day in the first 2 days after the introduction of settlement plates. In succeeding days, the regular 20–30 % water change can be done until harvest, which is expected to be within 35–45 DPF.

Figure 40. Larval rearing tank with settlement plates showing the blue stand-pipe drain (left) and the perforated stand pipe cover (right)

7.3. Harvesting of early sandfish juveniles

Early juveniles of sandfish are ready to be harvested when they reach a size of 5–10 mm. This is commonly attained between 35–45 DPF. Settlement plates are slowly lifted up from the tanks and the juveniles are transferred into collecting bins or tubs with clean seawater. Juveniles are easily harvested by jigging (quick jerky, vertical motion) the plates into the collecting tubs. The remaining juveniles are gently brushed off the plates with a wet fine brush (see **Figure 42**).

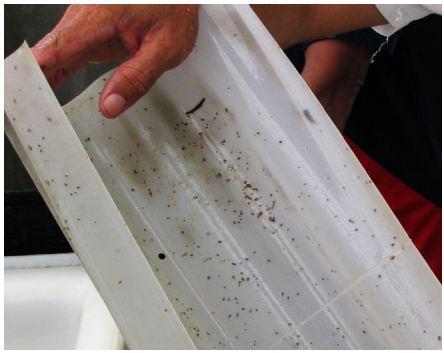


Figure 41. Harvestable early juveniles appear much bigger and darker on the settlement plates.

Juveniles on the tank walls and floor are collected by totally draining the tank water into a collecting sieve (90–120 microns) that is suspended in a basin with flowing seawater inside the harvesting pit.

Figure 42. Gentle brushing-off of the remaining juveniles from the plates. Photos by J.C. Rodriguez, Jr.

The collected juveniles can be sorted or graded according to size using net sieves with 1–2 mm mesh. Juveniles that are collected in the sieve are big enough to be transported for stocking in nurseries. These are counted and stored into separate bins (5–10 L capacity) at a desired density, depending on the nursery system where they will be transported to. A density of 500 or 1,000 juveniles per bin is common.

Figure 43. Collection of juveniles while draining water from the tanks during harvest

However, the smaller juveniles (<5 mm) will need to be grown some more. These smaller juveniles are placed in pre-prepared larval rearing tanks with plates and be continuously fed with *Navicula* slurry for a few days or weeks to attain harvest size.

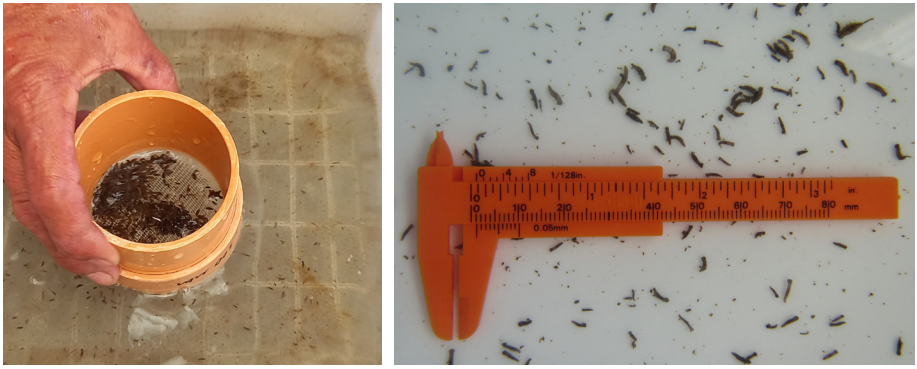


Figure 44. Newly-harvested early juveniles are size-graded using a sieve

Figure 45. A desired number of juveniles are temporarily stored in individual bins or basins with *Navicula* slurry prior to packing.

7.4. Packing and transport

Polyethylene bags (45 × 60 cm or 18 × 24 in) are mainly used for packing the juveniles. One bag with 2–3 L seawater and about 500 ml of *Navicula* slurry can safely support up to 2,000 juveniles for a travel time of less than 24 hours even without supplemental oxygen. The bags are sealed with as little air as possible to minimize splashing and wobbling during transport.

The bags should be maintained at 27–29 °C and abrupt temperature fluctuations must be avoided during transport. To achieve this, a styrofoam box is used to store the bags of juveniles. A 40-L box can hold 8–10 bags.

Figure 46. Early sandfish juveniles from the bin (left) are packed in a PE bag for transport (right).

Figure 47. A styrofoam box can help stabilize the temperature of the bags during transport. Photos by J.C. Rodriguez, Jr.

8 Common Problems and Solutions

8.1. Poor water quality

The hatchery operations are expected to run year-round. However, there may be seasons when the seawater source may become turbid, caused by intense rainfall or storms. The sand-filter system described in earlier sections (**Section 2.2.2.**) can help reduce this turbidity. Additionally, water stored in the reservoir can be held longer (1–2 days) to allow the remaining silt to settle, before the water is used in the tanks. Water exchange in broodstock tanks can also be delayed until the water source is clear of silt.

Figure 48. Storms and heavy rainfall can cause high turbidity of seawater source.

Water exchange in the larval rearing tanks can be adjusted and minimized. Although, any level of turbidity can effectively be removed by the micro-filter array (10–5–1 micron) in the hatchery. The problem is that the filter cartridges can get clogged with silt within a few hours of use. This drastically affects the flow rate of water and will require frequent replacement (every 1–2 days). The washable-type cartridges can be more economical in this case because they can be reused after cleaning and disinfecting. Silted cartridges can be cleaned by spraying with a strong water jet to remove accumulated silt, and then soaking in a chlorine bath for 1 to 2 days to disinfect and soften the filter. After soaking, another

spraying with freshwater is done to remove the remaining silt. Washable filter cartridges can be reused multiple times for up to 1 year. Because the cartridges need constant cleaning and replacement, a second parallel filtration array (filter cartridges) may also be installed.

Figure 49. A silted filter cartridge (left) and washed/disinfected filter cartridge (right). Photo by J.C. Rodriguez, Jr.

8.2. Limited microalgae supply

For sandfish larval rearing, live microalgae cultures are important, especially the *Chaetoceros* species. However, there may be seasons when the live cultures become limited and even collapse or die off because of unfavorable environmental factors. In this situation, feeding schedules of the larval sandfish will be affected and their development may be compromised. In most cases, having dedicated indoor microalgae culture shelves (see **Section 3.2.1**) that maintain various ages of live microalgae can ensure the supply of larval feed. Multiple batches of algal culture need to be maintained.

Alternatively, there are commercially available microalgae concentrates and pastes that may be used as back-up larval food. These concentrated products can be stored in a refrigerator for a few months and will be very useful during seasons when the supply of live microalgae cultures is low or unstable. However, most of these products were optimized for other aquaculture species like fish and shellfish and may not promote optimal growth and development for sandfish yet. Studies in optimizing the use of these concentrated products are being evaluated and developed, especially at SEAFDEC/AQD. So far, tests showed that live microalgae are still superior in promoting larval development and growth. However, commercially-available concentrated microalgae products, which includes *Chaetoceros* sp., showed good prospects as alternative larval food for sandfish, whether used purely or in combination with live cultures.

On the other hand, the commercially-available *Spirulina* powder that is coated on settlement plates was shown to be best for the induction of settlement of sandfish larvae. However, good-quality *Spirulina* powder are commonly imported products, and can be expensive and difficult to acquire. Alternatively, settlement plates coated with live microalgae cultures may also be used. Instead of painting algae paste on the settlement plates, these can be naturally cultivated with benthic algae, especially of the *Navicula* species.

Figure 50. Examples of commercial microalgae concentrates which can be imported (left) or locally-produced like the one by SEAFDEC/AQD (right)

Half-rolled settlement plates are placed in the *Navicula* culture tank for cultivation. Culture tanks must be exposed to diffused sunlight (through semi-opaque roofing) to promote algal growth. The initial stock of *Navicula* in a 10-L carboy (see **Section 3.2**) can be cultured in a 1-ton tank with clean seawater (filtered and UV-treated) and plates. After about four days of culture, *Navicula* cells will have settled on the plates and can then be harvested. These can be placed in the larval rearing tanks to induce the settlement of doliolaria larvae. Unlike the *Spirulina* powder, live *Navicula* does not dissociate from the plates and does not degrade to affect the development of auricularia larvae. So, *Navicula* plates may even be introduced into the tanks earlier. However, using the *Navicula* plates will require many algal culture tanks and will take several days before being ready for use. Therefore, this method may be used as a potential alternative, but using the commercial powdered *Spirulina* is still the better option if available.

Figure 51. Natural cultivation of *Navicula* on settlement plates at Day 1 (left) and Day 5 (right). Photos by J.C. Rodriguez, Jr.

8.3. Bloodworms

Bloodworms are the larval stage of the tropical midge fly or "non-biting mosquito," which are common in coastal areas. The worm-like larvae are called "bloodworms" because of their reddish body fluid that contains hemoglobin. Adult female midge flies lay eggs on water surfaces, even in seawater tanks in the hatchery. The egg sacks, which may contain thousands of eggs will sink and settle on the tank floor or walls (and settlement plates). The larvae will hatch out of their sacks and start to make their individual tubes as they grow. They will feed on organic material and algae, primarily competing with the sandfish juveniles for food. Bloodworms will also tend to nibble on the sandfish juvenile's skin, which can cause injury and mortality.

A simple solution to deter the adult female from laying her eggs in the larval tanks is to cover the tanks with a fine-meshed screen. In the hatchery, the screen cover can be fabricated with a velcro-type fastener to create a tight seal, while providing easy access to open the tanks when needed.

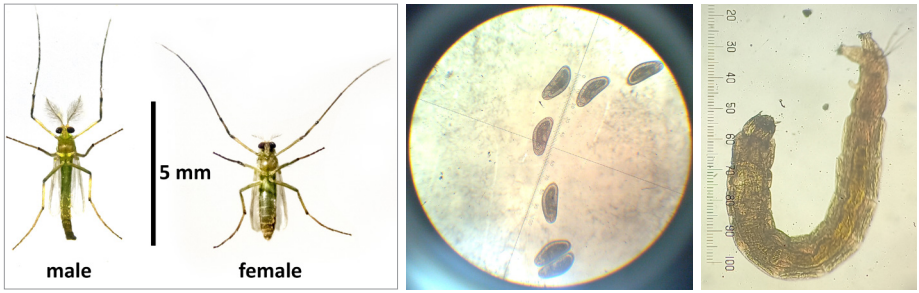


Figure 52. Adult midge fly (left) can lay eggs in sacs (middle) that hatch as bloodworm larvae (right).

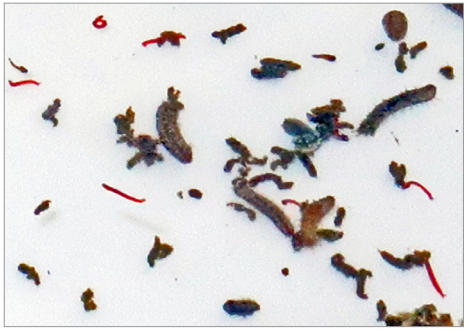


Figure 53. Bloodworms mixed with sandfish juveniles.

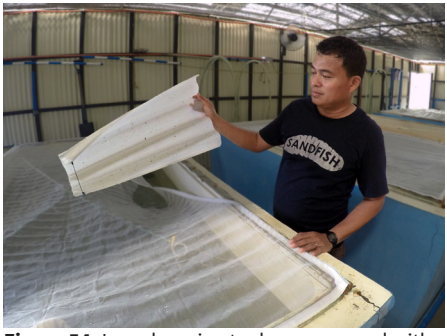


Figure 54. Larval rearing tanks are covered with a fine mesh screen to prevent midge flies from laying their eggs in the tank.

8.4. Copepod infestation

Harpacticoids are benthic copepods that are common in marine waters. They begin their life after hatching from eggs as nauplii. They can hitch a ride inside the gut of sandfish broodstock and mix with the sandfish eggs during spawning. If rinsing and cleaning of the fertilized eggs are not thorough enough, copepod eggs and nauplii can then find their way into the larval rearing tanks. Copepod nauplii can be about the same size as the developing sandfish eggs so they cannot be filtered through easily during rinsing. They also feed on phytoplankton and microalgae, so they can compete with sandfish larvae for food inside the larval tank. Because they are benthic in nature, they also settle and feed on the limited food on the settlement plates. They also nibble on the skin of newly-settled sandfish pentactula, causing injury and mortality.

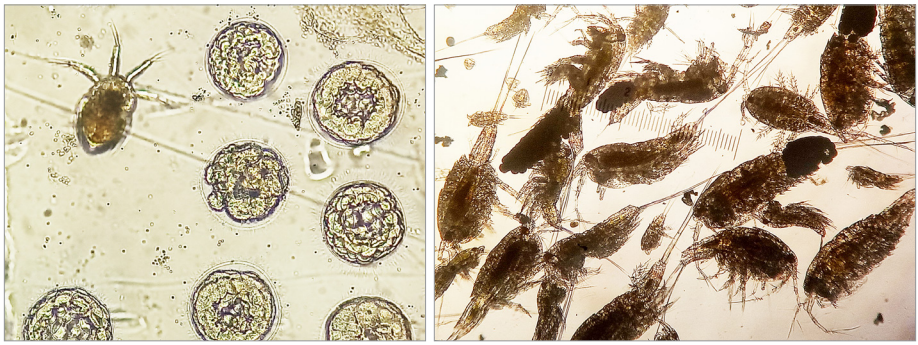


Figure 55. A copepod nauplius together with developing sandfish embyos (left) and adult copepods seen under a microscope (right)

Copepod infestation more commonly occurs during the settlement phase of larval rearing. When this happens, plates with settled early juveniles are transferred to a new larval tank filled with clean seawater. Plates from the copepod-infested tank are slowly rinsed with flowing seawater to remove most copepods while retaining the attached sandfish juveniles. The rinsed plates are then transferred to the clean tank. The remaining early juveniles on the tank walls and floor are collected with a sieve (90–120 micron) when draining. This sieve will allow most of the copepods to pass through while collecting the larger sandfish juveniles. The smaller juveniles are collected and allowed to settle in the collection bins while the floating copepods can be poured out while flushing with flowing seawater. However, while these practical and quick procedures can effectively minimize the number of copepods, all of the copepods may not be completely removed. Oftentimes, the settled sandfish juveniles will be able to grow until harvest size before another copepod bloom occurs.

Some hatcheries use pesticides and chemicals to control copepods in the tanks, if allowed. One such product is called Dipterex, which is more commonly known as Trichlorfon – a kind of organophosphate. This is used at a concentration of less

than 3 ppm in the tanks and held for 1–3 hours before rapidly changing 100 % of the water. This insecticide can be effective in removing the adult swimming copepod but the smaller nauplii, especially the eggs, can still remain viable.

At the SEAFDEC/AQD hatchery, we do not recommend and use such chemicals, especially since the practical rinsing procedures described above can have a similar level of effectiveness.

The best practice in controlling copepod infestation is prevention. This can be avoided by proper cleaning and disinfecting procedures described in earlier sections in this manual.

Figure 56. Draining and removal of settlement plates from a copepod-infested larval rearing tank

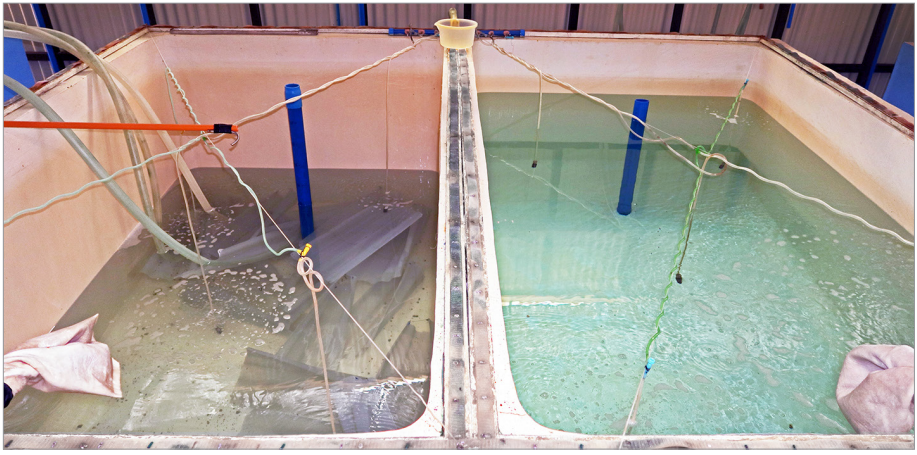


Figure 57. A new tank with clean seawater (right) is prepared to receive the rinsed plates and sandfish juveniles from the copepod-infested tank (left).

8.5. Unstable water temperature

As an optional equipment, an automated heating system is recommended to be installed to maintain a more stable water temperature in the larval rearing tanks during colder months. To manipulate water temperature in a 3-ton larval rearing tank, a submersible electric heater (2,000 watts, 220 V, 3-Phase) can be used. This heater is connected to a thermostat control unit (ST-1000) which can be set to automatically switch on and off at desired temperature settings. For example, a setting of 29±0.5 °C, will activate the heater if the water temperature drops to 28.5 °C and switch it off at 29.5 °C. This is achieved within 30 minutes at the SEAFDEC/AQD sandfish hatchery set-up.

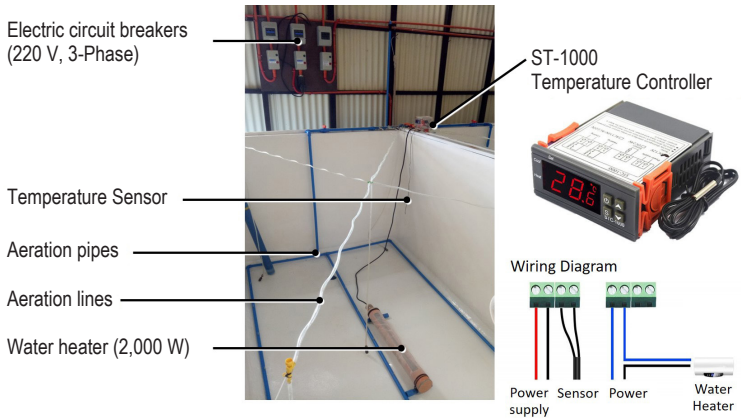


Figure 58. An automated temperature-controlled submersible heater with a net-covered PVC casing inside a 3-ton larval rearing tank

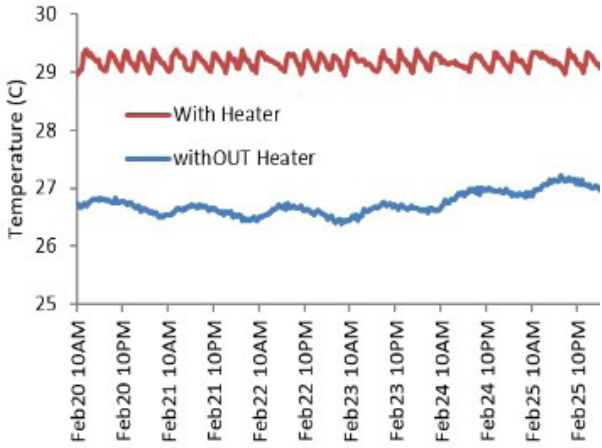


Figure 59. Water temperature profiles of larval rearing tank with heater is shown to be regulated around 29 °C, while the profiles of tank without heater remain at a cold temperature of <27 °C in the month of February

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10 Glossary

acclimation – a process of becoming accustomed or adapted to new environmental conditions, like to a new level of salinity or temperature.

ambient – the current state or level of natural conditions as affected by the surrounding environment.

beche-de-mer – French term for the dried product of sea cucumber.

benthic – relating to behavior of being attached to the bottom of the sea or of a tank

biomass – the total mass or weight of a group of animals or plants in a given area or volume

bioturbation - refers to the mixing and re-working of the upper layer of sea-floor sediments as the sea cucumber move, bury, and emerge

bloodworm – the red aquatic larva of a nonbiting midge fly that may infest larval rearing tanks especially during settlement phase

broodstock – mature stage of an animal used in the hatchery for breeding or spawning

carboy – commonly, a 10-liter capacity transparent plastic container with a narrow neck used in microalgae culture in a lighted shelf

cilia – microscopic hair-like vibrating structure found on planktonic organisms which provide propulsion or movement in the water

ciliary bands – densely packed cilia that help in larval movement

compound microscope – a binocular microscope with multiple lens options that can magnify or enlarge images of small specimens on a glass slide, commonly by 40x, 100x or 400x

conditioning – a period and process of becoming accustomed to a new environment which allows for the development of reproductive capacity

copepod – microscopic crustacean zooplankton found in aquatic habitats with certain species infesting larval rearing tanks

counting chamber – a modified microscope slide with grids and elevated borders made of glass or acrylic glass which can hold 1–3 ml of liquid sample to be counted.

cuvierian tubules – sticky white string-like tubes released by some species of sea cucumbers through their anus as a way of self defense

defecation – discharge of feces through the anus

deposit-feeder – a mode of feeding whereby the animal grazes or picks particulate materials on surfaces of the sediment on the sea floor, commonly using their oral tentacles

desiccation – the process of allowing the animals to be out of the water within a tolerable time as a part of spawning induction method

detritus – loose organic materials that usually settles to the bottom of the sea or tank

diatom – a single-celled alga which has a cell wall of silica, most of which are suspended in the water and some are attached to a substrate

dioecious – having the male and female reproductive organs in separate individuals of the same species

dissecting microscope – also called a stereo microscope and mainly used for observing and counting larvae (larger than 100 microns) with the use of counting chambers

echinoderm – marine invertebrate animals belonging to phylum Echinodermata characterized by a hard, thick, and spiny skin, and with a radial body symmetry

embryo – early stage of development of a multi-cellular organism after fertilization and prior to hatching

embryogenesis – the process of initiation and development of an embryo from a newly fertilized egg or a zygote through an organized sequence of cell division, cell differentiation, enlargement, and formation of organs

eviscerate – an intentional defensive strategy involving the ejection of guts or internal organs of an animal, common in sea cucumbers when threatened

evisceration – the process of releasing the internal organs from the inside of a body of sea cucumbers

fecundity – potential reproductive output of an individual, usually of the female

formulated feed – a thoroughly blended or combined mixture of quantified amounts of feed ingredients to meet specific nutritional requirement

gamete – a mature haploid male (sperm cell) or female (egg cell) germ cell which is able to unite with another of the opposite sex in sexual reproduction to form a zygote

genetic diversity – the variation in the genetic composition among individuals of a population of the same species

gonad – a reproductive gland that produces gametes

gonopore – also called a gonadopore, is a genital pore in many invertebrates, commonly located on the anterior end of the dorsal side of sea cucumbers

hemocytometer – a tool made of glass and microscopic graduation and grids used for visual counting very small cells such that of microalgae samples or other fluids under a compound microscope

holothurian – any echinoderm of the class Holothuroidea, comprising the sea cucumbers

hyaline spheres – round globules that serve as nutrient storage for larval metamorphosis

incubation – the process of nurturing fertilized eggs undergoing embryogenesis until hatching

intertidal – the area of a seashore which is submerged at high tide and exposed to the atmosphere at low tide

larvae – the early and immature form of an animal after embryogenesis and must undergo a series of developments before metamorphosing as early juveniles

mariculture – the cultivation, management, and harvesting of marine organisms in their natural salt water environment or in enclosures such as ponds, pens, cages, or tanks

metamorphosis – the process of physical transformation of an animal through its different stages in their life cycle from an immature form to an adult

microalgae – unicellular photosynthetic microscopic organisms that can grow in aquatic environments and utilize light, carbon dioxide, and water nutrients to convert to algal biomass

micron – a unit of length equal to one millionth of a meter

milt – the thick white semen containing multiple sperms released by a male animal during spawning

multi-cell stage – developmental phase for a single fertilized sandfish egg (or zygote) undergoing rapid cell division

nocturnal – an animal behavior whereby they become more active at night, commonly for feeding

non-viable eggs – unfertilized eggs or embryos with problematic development and malformations

opaque – property of a material whereby light cannot pass through or cannot be seen through

oral cavity – also called buccal cavity and refers to the opening through which many animals take in food

papillae – tiny protrutions on a part or organ of the body, common protrusions on the skin of some sea cucumbers

Pasteur pipette – a small glass tube device with narrow point and normally fitted with a rubber bulb at the other end used to collect small quantities of liquid samples

periphyton - microalgae and other materials that naturally grow on submerged surfaces like sea-floor sediments or artificial substrates

pH – short for "potential of hydrogen"; a measure of the acidity or alkalinity of a solution. Pure water has a pH of 7, acids have a pH less than 7, and alkalines have pH greater than 7

planktonic – relating to a behavior of being suspended or floating in the water column

polyspermy – fertilization of an egg cell by more than one sperm cell which may lead to mal-development of the embryo

pure culture – homologous or single species culture of organisms, commonly referred to microalgae species

raceway – a man-made structure like a narrow tank or canal which allows unidirectional water movement.

reservoir – in the hatchery, is a large container of water commonly made of concrete or fabricated plastic

roots-type blower – an electric machine used for compressing air or gas by the rotation of a meshing pair of lobed wheels in a closely-fitted case, commonly used for aeration systems

salinity – the concentration of dissolved salt in a given volume of water, commonly expressed in parts per thousand or ppt

scale-up – to produce more in higher volume

Sedgewick rafter – a proprietary counting chamber intended for counting of larvae and other planktonic organisms in water under a microscope

settlement – the process of settling or attaching unto a surface

siphoning – a physical process whereby a liquid is conveyed upward of a hose or tube by suction and then allowed to continuously flow towards a lower elevation by pressure and gravity

spat – refers to the younger stage or early juveniles of marine invertebrate animals that has settled onto a substrate

spawning – the process of releasing the male or female gametes into the water column to produce larvae and offspring in large numbers

spawning induction – artificial methods of promoting the release of gametes from breeders or broodstock

stereo microscope – a binocular microscope that gives stereoscopic view of the subject or specimen but at a relatively low-power or magnification, commonly used in counting larger plankton

supplemental feed – refers to alternative food material, like formulated feeds, given to animals being cultured or conditioned in addition to what they commonly feed on naturally

suspension/suspended – the state of being within the water column and not floating on the surface or not settling on the bottom

thermostat – a device with a temperature sensor and may be able to automatically regulate temperature levels by controlling the supply of electricity to a heating or cooling apparatus

translucent – property of a material that is not completely transparent, but still permit diffused light to pass through

trepang – Indo-Malay term for the dried product from sea cucumbers

UV-treatment – a process of sterilization of water supply by passing it through ultraviolet light in an enclosed device, whereby most microbes can be neutralized.

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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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