



# SANDFISH HATCHERY TECHNIQUES

By Natacha Agudo



SPC  
Secretariat  
of the Pacific  
Community





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Australian Government  
Australian Centre for  
International Agricultural Research



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Secretariat of the Pacific Community Cataloguing-in-publication data  
Sandfish hatchery techniques / by Natacha Agudo

1. Holothurians – Spawning – Handbooks, manuals, etc.

I. Agudo, Natacha II. Title.

639.8

AACR2

ISBN 978-982-00-0175-6

ACIAR, GPO Box 1571, Canberra, ACT 2601, Australia  
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Cover design and layout by Muriel Borderie  
Photos by Natacha Agudo, Cathy Hair and Steve Purcell  
Printed at SPC Headquarters, Noumea, New Caledonia



## ABOUT THIS MANUAL

Sandfish is arguably the most commercially valuable of the tropical species of sea cucumber that are processed into bêche-de-mer. It is widely distributed throughout the Indo-Pacific, occurring in shallow inshore areas where it is easily accessible to coastal fishers. A-grade bêche-de-mer processed from sandfish commands some of the highest prices on the international market. But these same attributes also make it vulnerable to overexploitation. Sadly, this has happened in most places where it occurs. While sandfish was an important component of bêche-de-mer fisheries 20 to 30 years ago, its contribution to bêche-de-mer exports is now relatively small, even trivial.

Not surprisingly, there is widespread interest in restoring the production of sandfish, especially where it promises to deliver benefits to coastal fishing communities with few other options for earning livelihoods. Although improved management of capture fisheries, through measures designed to safeguard the remnant spawning adults, will always be key to restoring production, aquaculture has the potential to help restore production of this valuable species in three ways:

- through production and release of cultured juveniles in restocking programmes to increase the number of spawners, but only where such releases are predicted to add value to other forms of management;
- through 'put and take' sea ranching operations, where cultured juveniles are placed in the wild to be regathered at a larger size with no intention of allowing them to spawn;
- through farming cultured juveniles in earthen ponds and sea pens.

This manual is designed to help government agencies and members of the private sector interested in implementing any of these ways of increasing production of sandfish by outlining the basic methods for spawning and rearing juvenile sandfish. It builds on the pioneering work done in 1988 at the Tuticorin Research Centre of CMFRI (Central Marine Fisheries Research Institute) in India and is based largely on methods developed and applied by the WorldFish Center (formerly ICLARM) in Solomon Islands, Vietnam and New Caledonia.

The information in the manual will enable hatcheries to produce sandfish suitable for release in the wild in relatively large numbers (tens of thousands) regularly. However, it does not pretend to be fully comprehensive. Rather, it is a reflection of current knowledge. We hope that it will soon be made out of date by those of you who apply and improve the methods described here.



Figure 1. Bêche-de-mer.

## ACKNOWLEDGEMENTS

The preparation of this manual was made possible through dedicated research on various aspects of the culture and ecology of sandfish by two groups of people: firstly, those who contributed to basic knowledge on spawning and larval rearing, and the ecology of sandfish – Stephen Battaglione, Chantal Conand, Jean-François Hamel, D.B. James, Claude Massin, Annie Mercier, Andrew Morgan, Rayner Pitt, Chris Ramofafia and J. Evizel Seymour; and secondly, my colleagues at the Worldfish Center, staff at the Northern Fisheries Centre, Queensland Department of Primary Industries and Fisheries, and trainees from Conservation International, Papua New Guinea (Priscilla Eka, Pamela Mua), who helped me to refine and develop the basic methods.

The work in New Caledonia arose from the early efforts by Deborah Gardner, and Eric Danty to establish the WorldFish Center hatchery at Saint Vincent, and to achieve our first successes there. For their commitment and perseverance I am grateful.

I would also like to thank Warwick Nash, Johann Bell, Steve Purcell, Cathy Hair, Gale Semmens and Richard Knuckey for their encouragement, comments on the content and editing, and Steve Purcell and Cathy Hair for their photos.

This manual was funded by the Australian Centre for International Agricultural Research (ACIAR), the Secretariat of the Pacific Community (SPC) and the WorldFish Center.

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# BASIC BIOLOGY OF SANDFISH

## *How to identify sandfish*

The body of the sandfish is elongated, cylindrical and stout. The dorsal body surface is relatively smooth and has small papillae (i.e. sensory tube feet) with black dots; colour varies from grey to black with dark transverse wrinkles. The ventral surface of the body is flattened and is generally whitish in colour. The mouth is on the ventral surface at the anterior end of the body. It is oval in shape and has 20 short peltate tentacles. The anus is located dorsally at the posterior end of the body.

Sandfish are Echinoderms, related to starfishes and sea urchins. The precise taxonomy of sandfish is:

Phylum Echinodermata

Class Holothuroidea (with tube feet)

Order Aspidochirotida (with tentacles peltate)

Family Holothuriidae (with body usually circular and gonads single)

Genus *Holothuria* (*Metriatyla*) Rowe, 1969

*Holothuria* (*Metriatyla*) *scabra* Jaeger, 1833



Figure 2. Sandfish.

Adult size range: 12-36 cm length

Adult weight range: 200-1500 g



## More about the biology of sandfish

Sandfish have the same general anatomy as other sea cucumbers. The gonads (ovaries or testes) lie in one tuft and open dorsally at the anterior end of the body through a single gonopore (i.e. genital orifice). The digestive system is composed of a mouth, oesophagus, stomach, intestine, cloaca and anus. Respiratory trees, which sandfish use to obtain oxygen, lie in the posterior of the body and open to the cloaca. The body wall that is processed into bêche-de-mer accounts for about 56% of total weight.

Sandfish move with the help of tube feet densely distributed on the ventral face, and through muscular action of the body wall.

Sandfish feed on detritus, i.e. organic matter in the mud or sand. They appear to feed continuously using the peltate tentacles surrounding the mouth to place sediment into the mouth.

Sandfish are usually observed partially buried in sediment. The daily burrowing cycle varies according to environmental conditions.

The growth rate of sandfish depends on environmental conditions and the time of year. At medium size, sandfish grow on average 0.5 cm per month, corresponding to 14 g per month. Under good conditions they grow to a size of 300 g in one year. We still do not know how long sandfish live, but it may be around 10 years.

Sea cucumbers have tiny calcareous plates called spicules in their skin. Microscopic examination of spicules is used to distinguish species. Sandfish have many spicules in the shape of tables and knobbed buttons.

Sandfish can be sexually mature at a size as small as 200 g. There is no apparent relationship between fecundity (egg production) and body size.

Like other sea cucumbers, sandfish can regenerate some of their organs. After spending long periods out of water, or being affected by the use of chemicals, being handled during collection and transport, or when stressed by predators, sandfish may eviscerate their internal organs. Regeneration of internal organs occurs within 2 months.

Sandfish and other tropical sea cucumbers can produce numerous toxins from their skin and viscera. These toxins inflict distress, loss of equilibrium and death in fish, but do not affect humans.

## Where do sandfish occur?

Sandfish are found in many countries in the Indo-Pacific, from east Africa to the eastern Pacific. They are usually found between the latitudes of 30°N and 30°S.

The preferred habitats of sandfish are shallow tropical waters, usually less than 20 m deep, such as sheltered areas with high levels of nutrients, including muddy substrata and seagrass beds. They can tolerate reduced salinity (20 ppt) for short periods and so are sometimes found in brackish water.



Figure 4. Wild sandfish in a seagrass habitat.

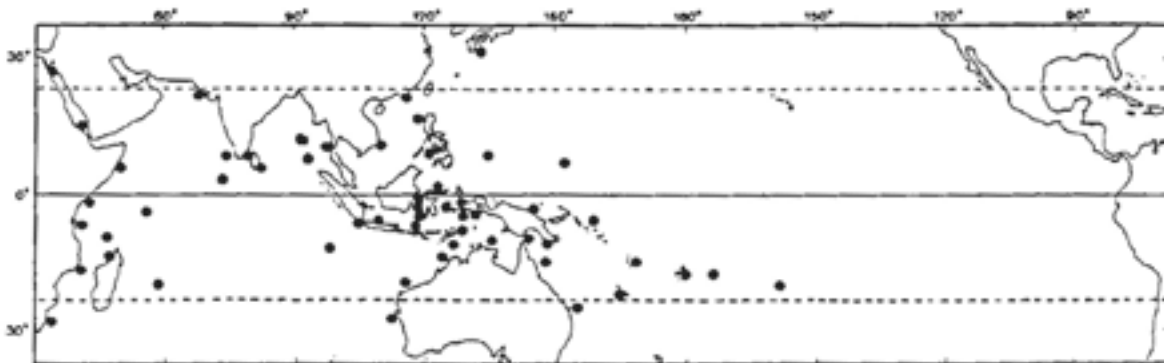


Figure 3. Geographical distribution of sandfish.



# BROODSTOCK

## When should broodstock be collected?

Broodstock should be collected during the reproductive season so that animals are ready for immediate spawning.

The spawning season for sandfish varies among countries. In countries close to the Equator, sandfish spawn throughout the year. As the latitude approaches 25°, spawning is restricted to a short summer period of 3 months. In some countries, there can be two spawning periods each year. It is important to determine when these periods occur.



Figure 5. Collection of broodstock.

## How many broodstock are needed? What size should they be?

Batches of 30–45 individuals are usually required to induce a small proportion of animals to spawn.

Ideally, the average weight of broodstock should be around 500 g. However, the size of broodstock may be smaller in some places, e.g. 250–415 g in Vietnam.

Broodstock should be undamaged, with no visible skin lesions. Skin appearance should be smooth and shiny, with a thin, transparent mucous layer. Animals should react by moving when you touch and disturb them.

*It is impossible to distinguish males and females externally. Sex can only be determined by biopsy or dissection of animals to examine the gonads, or by observation when they spawn.*

## Transport of broodstock to the hatchery

At sea, collected broodstock should be kept in seawater in insulated containers. Aeration should be provided if the animals are to be held this way for more than 2 hours. Preferably, the animals should be left in the transport containers to defecate before they are transferred to plastic bags.

To transfer the broodstock from the boat to a vehicle for transport to the hatchery, animals should be cleaned gently and packed individually in oxygen-filled bags with 1 L of seawater. The bags should be placed in insulated containers during transport. The containers should be protected against direct sunlight to maintain the temperature within the range of 27–30°C.



Figure 6. Broodstock packed individually in plastic bags in an insulated container.

Sandfish can tolerate low dissolved oxygen and high temperatures in static water (up to 30°C) for long periods (over 80 hours) without eviscerating. However, it is preferable to maintain them in optimal conditions to avoid stress, which can induce premature spawning.

*The transport of individual sandfish in damp tea-towels for many hours has been practised with varying success.*

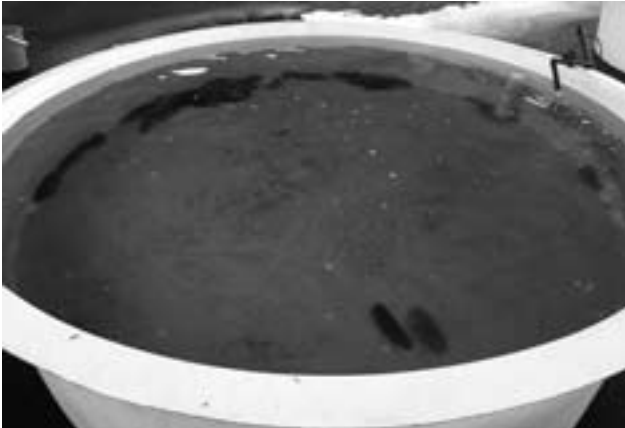
### Avoid

- Sudden temperature shocks and holding for long periods out of water, which can result in evisceration
- Damaging the skin of the animals – handle them gently
- Shocks during road transport

## Maintaining broodstock in tanks, sea pens and earthen ponds

Broodstock that are not yet mature can be kept in captivity until they ripen. Similarly, following spawning, broodstock can be maintained near the hatchery for future use. These animals can be held in tanks, sea pens or earthen ponds.

### *In tanks*



**Figure 7. Broodstock maintained in tank with sand.**

Broodstock tanks should have a flat bottom with a volume of 1000 to 4000 L and be placed outdoors in shade. The tanks should contain a 10–15 cm layer of sand or mud. Feed for broodstock should be added at the rate of 50 g/day. Suitable feed ingredients are: prawn head waste, soya bean powder, rice bran and seagrass powder. Care should be taken to ensure that broodstock do not lose weight. Broodstock should be stocked at a density of 15–30 animals per 1000 L tank in static aerated water. Exchange the water each day.

Broodstock held at low density in tanks with continuously flowing seawater, fed powdered dried algal preparations and ground-up prawn pellets, can often be spawned more than once.

### *In sea pens*



**Figure 8. Sea pen.**

Sea pens for holding broodstock should be located close to the hatchery so they can be monitored easily and regularly. Sea pens should be around 800 m<sup>2</sup> and the stocking density of the broodstock should be <200 g/m<sup>2</sup>. Additional feed is not needed. Survival in sea pens is often very high, but growth rates may be lower than in ponds depending on local environmental conditions.

### *In earthen ponds*



**Figure 9. Earthen ponds.**

Earthen ponds, typical of those used for maintaining shrimp broodstock (450 to 1500 m<sup>2</sup>), have proved suitable for holding sandfish broodstock. Pond sediments should be friable, sandy-mud without large rocks. Water depths of <1 m are best. Ponds should be filled at least 2 weeks before transfer of broodstock to ensure natural plankton development. The stocking density of the broodstock should be <250 g/m<sup>2</sup>. There should be daily water exchange or continuous flow. It should not be necessary to add any feed. Water quality parameters (temperature, dissolved oxygen and salinity) should be measured regularly, and daily if possible.



*Possible problems: Heavy rain can lead to stratification of water in the pond. Stratification can be detected by the presence of a thin layer of low-salinity water at the surface. The consequences of stratification can include an increase in temperature, fall in dissolved oxygen especially in the bottom layers, and development of anaerobic areas in the sediment. The combination of these extreme conditions can be dangerous for sandfish, which are benthic and slow moving. It can lead to total loss of broodstock in a few days.*

#### HOW TO OVERCOME THESE PROBLEMS

<p><i>High temperatures and low dissolved oxygen</i></p>	<p><i>Regularly check water temperature and dissolved oxygen (especially in the early morning). Increase water flow, preferably continuously 24 hours per day. Set up paddle wheels or other pond aeration equipment; installation should provide aeration on the bottom where the animals are located.</i></p>
<p><i>Heavy rain</i></p>	<p><i>Remove the surface layer of freshwater by adjusting the height of the outlet pipe. If a cyclone is predicted, transfer the animals from ponds to indoor tanks (where possible).</i></p>



Figure 10. Feeding broodstock.

#### Ways to increase spawning success

Animals held in ponds or tanks for several months to 2 years are easier to spawn than broodstock maintained in sea pens or animals taken directly from the wild. Another advantage of holding broodstock in ponds is that they generally spawn earlier in the season than wild individuals, presumably because of the higher temperatures in ponds. Note, however, that holding broodstock in ponds or tanks for several months before spawning is not essential. In Solomon Islands, for example, newly collected sandfish were often induced to spawn.

The advantages and disadvantages of keeping broodstock in captivity, or collecting individuals from the wild, should be evaluated for each location.

#### Summary

- Batches of 30–45 individuals (average weight 500 g) are needed to induce spawning.
- When relying on wild broodstock, ripe and healthy broodstock must be collected during the spawning season.
- Transport wild broodstock individually in oxygen-filled bags with seawater, using insulated containers at 27–30°C. Avoid changes in temperature and other shocks during transport.
- Broodstock can be conditioned by keeping them in: (a) tanks (15–30 animals/1000 L) with a sand or mud substratum, with flow-through seawater and a supply of food; (b) sea pens of 800 m<sup>2</sup> at densities of <200 g/m<sup>2</sup>; and (c) earthen ponds at densities of <250 g/m<sup>2</sup> with continuous water exchange.
- Ponds used to hold broodstock require management to avoid high temperatures and low dissolved oxygen caused by stratification due to heavy rain. Salinity must be maintained within the range of 28–36 ppt.
- The advantages and disadvantages of maintaining broodstock, or collecting them directly from the wild, should be evaluated for each location.



**Figure 11. Broodstock in a clean spawning tank before induction (30-45 animals are recommended).**





# SPAWNING

## Preparing broodstock for spawning

1. Use a bare, flat-bottomed tank up to 2 m<sup>3</sup>. Provide cover and heaters to maintain constant water temperature at night.
2. Clean the tank and disinfect with chlorine (sodium hypochlorite). Fill with 1- $\mu$ m filtered and UV-sterilised seawater at ambient temperature (<30°C), to a height of 30–40 cm. Aerate the water moderately.
3. Gently clean the 30–45 animals and place them in the tank.
4. Siphon the tank bottom, to remove any sediment and faeces, until spawning starts.
5. When spawning is complete, return broodstock to tanks, sea pens or ponds. Also return broodstock if they fail to spawn.
6. Use a different group of animals for each spawning.

### Notes

- Stress caused by collection and transport is often sufficient to induce spontaneous spawning. Spontaneous spawning usually occurs during the afternoon, evening and/or night on the day broodstock are collected.
- Spawning has often been observed just prior to the full moon and new moon, but can also be induced at other times.



Figure 12. Close-up of broodstock in spawning tank.

## Inducing sandfish to spawn



Figure 13. Cold shock treatment.

### Thermal stimulation

- Raise water temperatures by 3–5°C for 1 hour, either by adding warmed seawater to the spawning tank or using aquarium heaters.
- Keep water temperatures within the range of 28–32°C.
- Stir the water to maintain uniform temperature throughout the tank.
- If the ambient water temperature is >30°C, give a cold shock treatment for 1 hour before the heat shock. To do this, reduce the level of water in the spawning tank and add sealed plastic bags containing ice to quickly lower the water temperature by 5°C below ambient. Then apply heat shock as above.
- After thermal stimulation, replace water with new water at ambient temperature, keeping the animals covered.

### Gonad extraction method

- Dissect a few animals to extract gonads from 1 to 2 ripe males.
- Store the gonads at 5°C to extend the viability of the sperm.
- Use the sperm as a spawning stimulant by adding blended fresh male gametes to the spawning tank at ambient water temperature.

### Water pressure

- Leave broodstock to dry in the tank in the shade for about half an hour before subjecting them to a powerful jet of seawater for a few minutes.
- Return broodstock to the spawning tank at ambient water temperature.



Figure 14. Dry treatment.

*Dry treatment*

- Leave animals completely dry, or in ~ 2 cm of seawater in the tank for 30–45 minutes. Keep them in the shade.
- Refill tank with water at ambient temperature.

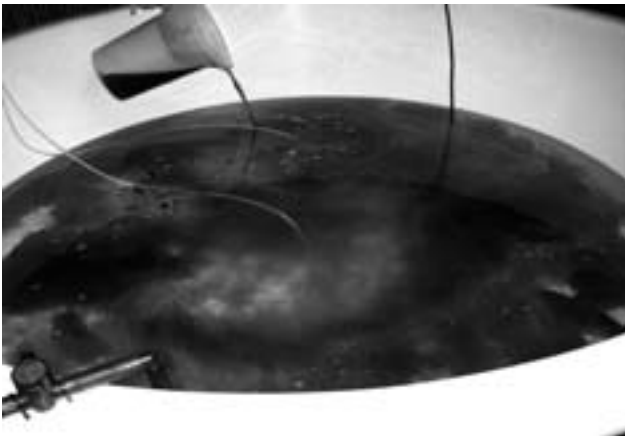


Figure 15. *Spirulina* bath.

*Food stimulants*

- Add dried algae (*Spirulina* at a rate of 30 g per 300–500 L, or Algamac 2000 at a concentration of 0.1 g/L) for 1 hour. Stir the water.
- After 1 hour, remove as much waste from the tank as possible and replace water with new water at ambient temperature.

*Combined treatments*

Often a combination of treatments is needed to induce spawning. The best combinations are given below:

*Treatment combinations*

A	B	C
1. Dry treatment	1. Hot shock treatment	1. Dry treatment
2. Cold shock treatment	2. <i>Spirulina</i> bath	2. Hot shock treatment
3. Hot shock treatment		3. <i>Spirulina</i> bath

Possible problem: After two days of spawning attempts, broodstock can lose their mucus and the skin may show white lesions due to stress and handling. Avoid handling the animals too much.

*Observing the behaviour of broodstock*



Figure 16. Pre-spawning behaviour.

The behaviour of sandfish often indicates that spawning is imminent. Pre-spawning behaviours include:

- rolling movements
- rhythmic contractions
- lifting and swaying of the front end of the body
- climbing the tank walls



Figure 17. Animal stands erect ready to spawn.



Males usually spawn before females, which start to release eggs 1 hour (sometimes sooner or later) after the first male releases sperm.

Spawning males are erect and sway from side to side, releasing a continuous stream of sperm. Males spawn for several minutes to hours, even when they are disturbed. Spawning females erect their body before releasing eggs in a short powerful spurt from the bulging gonopore (i.e. genital orifice). Females can spawn 2–3 times over a period of an hour or more, but often stop spawning if disturbed.

Record all information about spawning trials, including the unsuccessful attempts. This helps to improve future spawning success. Collect the following information:

- total number of males and females that spawned
- spawning time for each individual with comments
- observations of egg development (% regular and irregular shape, % fertilised, diameter)
- individual broodstock weight

*Natural fecundity ranges from 9 to 17 million eggs per female, but induced females generally release a fraction of this, i.e. 1 to 2 million, although some individuals can sometimes release up to 4 to 6 million eggs.*

### Steps to take when spawning fails

- If broodstock do not respond during spawning induction, try different methods of stimulation.
- When broodstock demonstrate pre-spawning behaviour but do not spawn, biopsy or dissect a few animals and examine the gonads under a microscope to determine if they are ripe. Ripe ovaries look translucent and ripe testes are milky white.
- Commence spawning induction later at night. Sandfish usually spawn at night in the wild.

*Reduce the risk of losing eggs from spontaneous or delayed spawnings at night by:*

1. *Maintaining broodstock in a spawning tank with a flow-through system.*
2. *Installing a second tank in series with an internal 100- $\mu$ m sieve at the outlet pipe, with moderate aeration, to retain eggs in suspension overnight until collection the following morning. Provide the tanks with air diffusers, heaters and covers to maintain constant water temperature overnight.*

### *The first female has just spawned! How should fertilisation be managed?*



Figure 18. A spawning sandfish.

### *Management of males*

Note that too much sperm in the spawning tank causes polyspermy (i.e. multiple fertilisation). Polyspermy can reduce the rate of fertilisation and cause damage to egg development and induce larval deformities.

- If the water in the spawning tank becomes cloudy due to excess sperm, reduce the amount of sperm by siphoning and adding new water, or use a flow-through system.
- To prevent excess sperm in the spawning tank, remove most males and transfer them to smaller containers shortly after they begin releasing sperm. Often they will continue releasing sperm.
- Leave one or two males spawning in the tank until the first female releases eggs. Retain the more vigorous spawners.
- Record the time that males start releasing sperm, and how many males spawn. Sperm stays active for several hours.

### Management of females

- Record the time that females begin spawning. This will be helpful in estimating the progress of egg stages.
- Increase aeration to moderate levels to keep eggs in suspension.
- Count and record the number of egg releases per female. Females usually spawn 2–3 times. Once females finish spawning (usually evident when they stop moving), remove all broodstock (males and females). Return the animals to their tank, sea pen or pond.

*Separation of males into a separate tank once they begin to spawn, and later addition of controlled amounts of sperm to the females' tank, is a way to avoid polyspermy while maintaining a larger number of fathers.*

#### Avoid

- *Disturbing or moving females when removing surplus males so that spawning of eggs is not interrupted.*

### Observation of egg development

- After the first eggs are released, sample them from the water column in a small beaker.
- Measure egg diameter, using a micrometer eyepiece placed in the lens of a microscope.
- Record the measurements of the eggs and other observations such as: egg stages and size, percentage of regular round eggs, and fertilisation rate.
- Repeat these observations every 20–30 minutes.

Recently shed eggs are white, spherical and visible to the naked eye. The diameter of the eggs ranges from 80 to 200  $\mu\text{m}$ , but this varies widely within the geographic range of sandfish.

Spermatozoa are not visible to the naked eye; they appear as small, active dark dots clustered together around the eggs.



Figure 19. Fertilised egg.

Fertilised eggs have a swollen membrane. If many sperm continue to cluster around the egg (i.e. polyspermy), irregular egg development will occur.

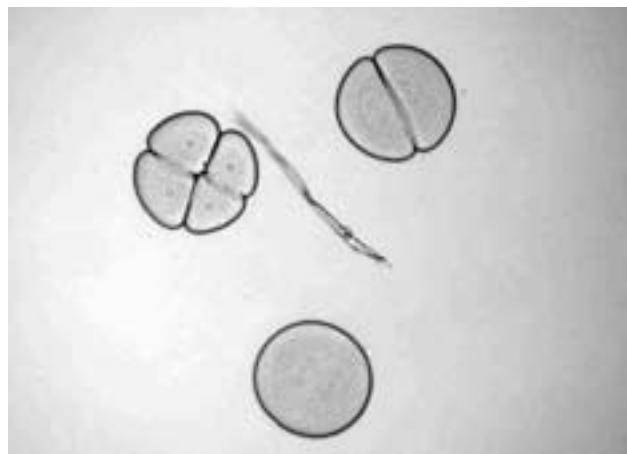


Figure 20. A fertilised egg, two cell, and four cell stages.

### Collection of eggs from the spawning tank

- Use a small beaker to collect a sample of eggs from the water column to estimate the fertilisation rate and percentage of eggs with advanced cell division.
- Ensure that the fertilisation rate is high and the majority of eggs are at the advanced cell division stage. This is at least 1 hour after fertilisation.
- Wait at least 1 hour after fertilisation before collecting all the eggs.



Figure 21. Egg collector.





- Siphon the eggs slowly from the tank into a 50–80 µm sieve placed in a bowl (Fig. 22). Make sure the water level is above the mesh of the sieve so that the eggs are not squashed onto the mesh.
- Introduce a gentle flow of filtered seawater, at ambient temperature, in the bowl. Rinse eggs to remove excess sperm and dirt. Maintain eggs in suspension in the sieve.
- Be patient! Collecting eggs takes time.



Figure 22. Method of egg collection.

#### Useful tips

- Carry out spawning in shallow water, and use several siphons with sieves in bowls to reduce the time needed to collect eggs. This requires more hatchery staff for a shorter period.
- Start by siphoning eggs from the water column; the eggs in the water column are cleaner (i.e. not surrounded by faeces or sediment). Distribute these batches of eggs in the larval tanks. If more eggs are required, then collect them from the bottom of the tank.

### Estimating egg density

- Transfer the collected eggs regularly and carefully, using beakers, into clean 10 L buckets, until the buckets are filled.
- Stir the water in the buckets gently to distribute the eggs uniformly.
- For each bucket, take three 1-ml subsamples. Estimate the egg density for each sample, using a counting cell (e.g. Sedgewick-Rafter chamber) under a microscope. Calculate the average density for each bucket. Estimate the fertilisation rate.
- Record all data.
- Transfer and distribute eggs from the buckets to the larval tanks quickly after determining the total number of eggs needed for each tank.
- Use egg densities from all buckets to estimate the total number of eggs spawned.

#### Avoid

- High densities of eggs in the sieve and buckets as this can damage eggs. Average egg density in the buckets should be <200 eggs/ml, i.e. <2 million eggs per 10 L bucket.



Figure 23. Transfer of eggs into a larval tank.



## Summary

- Batches of 30–45 clean broodstock are placed in a spawning tank, filled with 1- $\mu$ m filtered and UV-sterilised seawater.
- Thermal shocks, extracts of male gonads, water pressure, dry treatment, and food stimulants used alone or in combination can induce spawning.
- Pre-spawning behaviours include rolling movements, rhythmic contractions, and lifting and swaying of the anterior end of the body. Males usually spawn first and females usually start one hour after the first male.
- It is important to record spawning data, i.e. induction method used, number of males and females, spawning time, and observations of eggs.
- Females usually release eggs 2 to 3 times. Moderate aeration maintains the eggs in suspension. Samples of eggs are regularly taken from the water column to examine the stage of egg development.
- After > 1 hour post fertilisation, the eggs can be siphoned gently from the water column into a 50–80  $\mu$ m sieve placed in a bowl. Flow-through seawater should be used to rinse the eggs. The eggs are transferred to buckets for counting before being placed in the larval tanks.



Figure 24. Observation of eggs under a microscope.



# LARVAL REARING

## Transferring fertilised eggs to larval tanks



Figure 25. Larval tank.

- Prepare cylindrical tanks (up to 2 m<sup>3</sup>) with conical bottoms and central drains. Set up 100-µm mesh outlet screens.
- Wash the tanks with chlorine (sodium hypochlorite), then rinse with freshwater.
- Fill the tanks with 1-µm filtered and UV-sterilised seawater.
- Ensure the water temperature is within the range of 26 to 30°C, and salinity is between 32 and 36 ppt.
- Install two central air diffusers in each tank to insure medium aeration and gentle water circulation. Use two air diffusers as a precaution against failure.
- Immerse aquarium heaters with thermostats to maintain a constant water temperature in the larval tanks. Set up lids or covers for the night to retain the heat, if necessary.
- Maintain a minimum of 12 hours continuous artificial illumination per 24 hours, with 1–2 fluorescent tubes (400 lux) per tank. Alternatively, expose larval tanks to natural photoperiod and daylight.
- Pour eggs carefully into the larval tanks, using buckets or beakers, to achieve a density of 0.3 to 1 egg per ml.

### Avoid

- Large differences in temperature (over 1–2°C) and salinity of water between buckets and larval tanks as this can affect survival of eggs.
- High levels of aeration during transfer as this can trap the eggs and larvae in a strong current, which can throw them against the tank walls, damaging or killing them.
- High initial egg density. Discard excess eggs. Lower egg densities are preferable because they reduce the risk of total mortality of larvae during the first days.

## Life cycle of sandfish

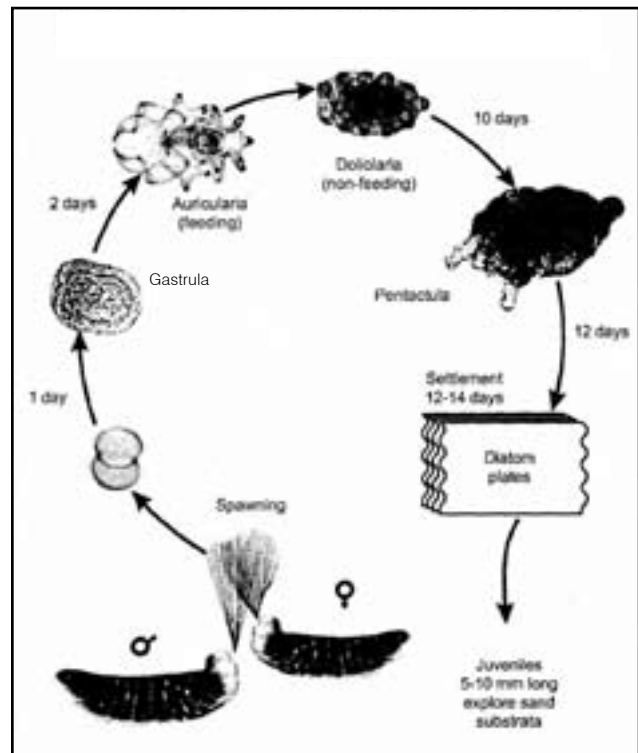


Figure 26. Life cycle of cultured sandfish.

The larval development of sandfish consists of the auricularia (feeding stage) larvae transforming into non-feeding doliolaria larvae before settling as pentactula larvae. Figure 26 illustrates the life cycle of cultured sandfish from spawning to post-settlement.



Figure 27. Gastrula stage.

Stage	Time after fertilisation
Fertilised egg	0
Blastula	40 min to 3 hours
Gastrula	24 hours
Auricularia larvae	
> early	2 days
> mid	4 days
> late	5–6 days
Doliolaria larvae	10 days
Pentactula larvae	12–13 days
Juvenile	15 days

### *Auricularia larvae*

Main features:

- > Transparent slipper-shaped larvae with ciliated bands (for locomotion)
- > A single pre-oral anterior lobe and anal posterior lobe
- > Digestive tract complete: mouth, oesophagus and stomach
- > Slow moving – continuous activity
- > Pelagic and actively feeding on microalgae

Duration of this stage: 8 days

Size range: 700–750  $\mu\text{m}$



Figure 29. Mid auricularia larva.

Size range: 430–563  $\mu\text{m}$

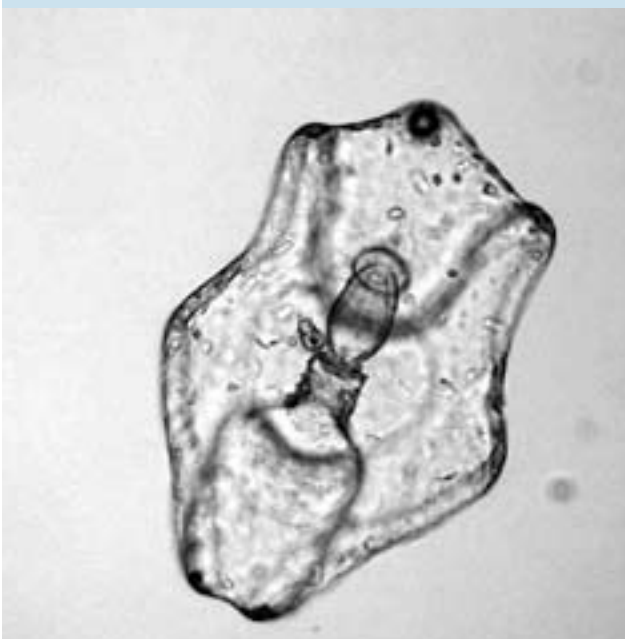


Figure 28. Early auricularia larva.

Size range: 853  $\mu\text{m}$ –1.1 mm

Diameter of hyaline spheres: 50–70  $\mu\text{m}$

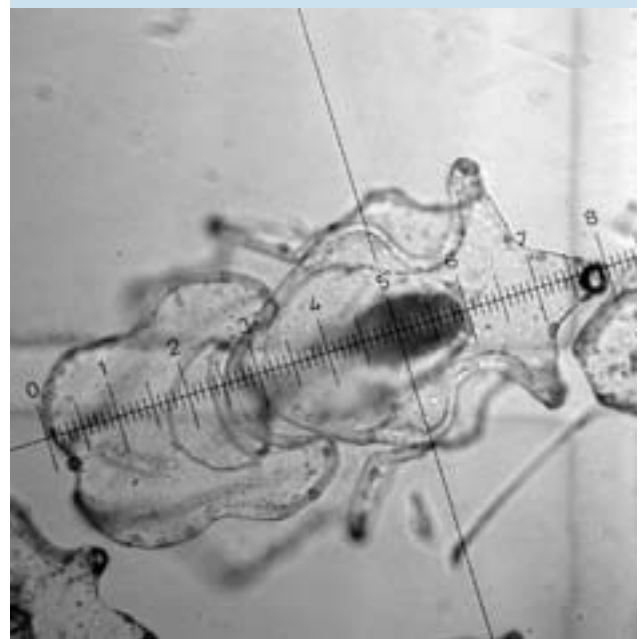
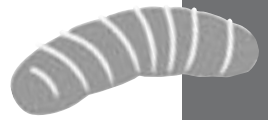


Figure 30. Late auricularia larva.



### *Doliolaria* larvae

Main features:

- Dark-brown, barrel-shaped larvae with 5 ciliated bands around body
- Rapid changes occur inside the body and all adult features begin to form
- Larvae with 5 hyaline spheres on each side
- Diameter of hyaline spheres: 60–80  $\mu\text{m}$
- Short transitional phase with decreasing size before metamorphosis and settlement
- Fast moving

Duration of this stage: 2–3 days



Figure 31. *Doliolaria* larva.

### *Pentactula* larvae

Main features:

- Dark tubular-shaped larvae with 5 tentacles at the anterior end and a single posterior foot (for locomotion)
- Rapid and differential growth
- Moving and crawling over the edge and bottom of tank, and settlement surfaces (e.g. diatom plates)

Duration of this stage: variable

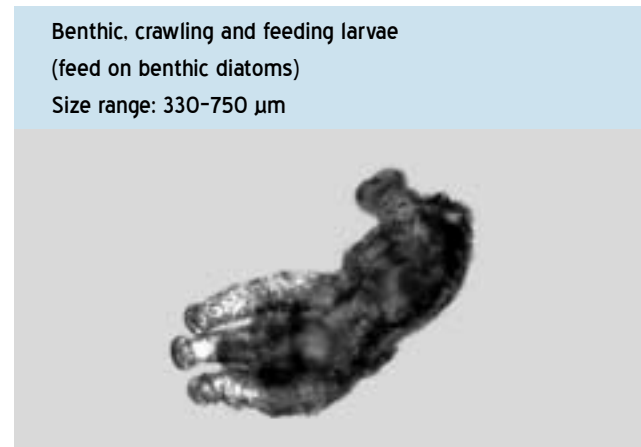


Figure 32. *Pentactula* larva.

### Juveniles

Main features:

- Same shape as adult but with two long tube feet at the posterior end for early juveniles
- Slow moving and strongly attached to settlement substrata
- Growth to 4–5 mm in 1 week

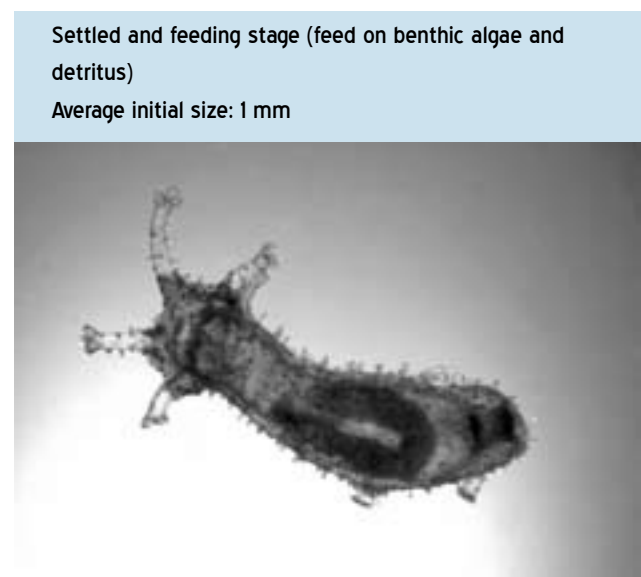


Figure 33. Juvenile.

## How should larvae be reared?



Figure 34. Seawater filtration system.

### Seawater

Seawater should be sand filtered, then passed through 1- $\mu\text{m}$  filter bags or cartridges and finally sterilised by UV.

- Seawater parameters should be maintained as follows:

Temperature	26–30°C
Oxygen (DO)	5–6 ppm
Salinity	27–35 ppt
pH	6–9
Ammonia	70–430 mg/m <sup>3</sup>

### Cleaning tanks

- Siphon the tank base and yellow patches of dead larvae daily for the first four days of larval rearing. Healthy larvae stay in the water column, whereas deformed or dead larvae are found in the lower water column or settled on the bottom.
- Siphon any pink patches resulting from the development of bacteria. Dead larvae, faeces from larvae and overfeeding produce bacteria during the advanced stages of larval rearing.

### Illumination

- Place a lid or a cover on top of the larval tanks for the first two days to keep eggs and early larvae in darkness.





### Water change

- Add ethylenediaminetetraacetic acid (EDTA) ( $5 \text{ g/m}^3$ ) when larval tanks are first filled. EDTA is a chemical used to bind with heavy metals naturally present in seawater; high concentrations of heavy metals can be harmful for larvae. EDTA renders heavy metals harmless.
- Do not change the water in the larval tanks until day 2 (i.e. two days after fertilisation). There are three protocols for changing water:

#### Protocol 1: Partial water change

From Day 2 to pentactula stage	Change 30% of the water using a $100\text{-}\mu\text{m}$ mesh outlet screen inside the larval tanks each day. Use a gentle water flow (maximum 2 L/min). Add EDTA after each water change at a rate of $5 \text{ g/m}^3$ of the added water until pentactula stage.
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#### Protocol 2: Complete water change

Day 2 Day 4 Day 6	Complete water change (100%) every second day until late auricularia stage. Drain the tank completely through a $100\text{-}\mu\text{m}$ sieve immersed in a bowl, at a maximum flow rate of 5 L/min. Transfer larvae periodically from sieve to aerated containers, using beakers. Clean the empty tank. Fill it with $1\text{-}\mu\text{m}$ filtered and UV-sterilised seawater. Add EDTA at a rate of $5 \text{ g/m}^3$ . Transfer and stock larvae at a density of 0.1–0.5 larvae/ml.
During and after late auricularia stage	Change water daily using a flow-through system at a flow rate of 200 ml/min with a $100\text{-}\mu\text{m}$ mesh outlet screen inside the larval tanks.

#### Protocol 3: Partial water change with the antibiotic Erythromycin

Day 2 Day 4 Day 6 Day 8	Change 30% of the water using a $100\text{-}\mu\text{m}$ mesh outlet screen inside the larval tanks. Use a gentle water flow (maximum 2 L/min). Add EDTA after each water change at a rate of $5 \text{ g/m}^3$ of the added water until pentactula stage. Add Erythromycin* at a rate of $2 \text{ g/m}^3$ after each water change.
From Day 10	Carry out a daily water change (30%).

\* Handle the antibiotic carefully. Use protection for the hands and face (no direct inhalation). Erythromycin is used to prevent bacterial infection but is not always successful.

#### Avoid

- Introducing undesirable organisms, such as copepods and ciliates, during water changes, and from algal cultures. Practise water change with  $1\text{-}\mu\text{m}$  filtered and UV-sterilised seawater. Use healthy algal cultures without ciliates. Copepods can be removed by chemical treatment with the insecticide Dipterex (common name, Trichlorfon) at 1–3 ppm for 1–3 hours followed by rapid dilution (50 to 100% water change); it is effective in killing the swimming stages of copepods but not the eggs.
- Contamination from one tank to another by thoroughly rinsing all materials with freshwater before and after use and storing them in containers with chlorinated water.



Figure 35. Feeding auricularia larvae.

- Start feeding at day 2.
- Increase the quantity of microalgae gradually from 20 000 to 40 000 cells/ml. Continue feeding as long as auricularia larvae are still present in the water column.
- Examine the gut content of larvae under a microscope and estimate the residual algae in the water to adjust the amount of food. Well-fed larvae have guts that are brown or golden in colour.
- Provide food twice a day after the water change.

For auricularia larvae, the main microalgae used are: *Chaetoceros muelleri*, *C. calcitrans*, *Isochrysis aff. galbana*, *Rhodomonas salina* and *Tetraselmis* sp. Availability of algal species may vary slightly between locations.

A mixture of algae is better than using a single species for rearing larvae. *C. muelleri* and *R. salina*, given in equal parts, are optimal for larval culture of sandfish. Also, *Isochrysis aff. galbana* given for the first days and then mixed with *Chaetoceros* sp. four or five days later is an adequate diet.

Feeding rates for microalgae commonly used for larval rearing:

Hatching day*	Larval stage	Feeding rate (cells/ml)
2	Early auricularia	20 000
4	Mid auricularia	20 000–25 000
6	Mid and late auricularia	25 000–30 000
8	Late auricularia	30 000–40 000

\*Day 0 is fertilisation.

**Avoid**

- High algal concentration (>40 000 cells/ml). This can inhibit growth and development of larvae and decrease the survival rate. In cases of overfeeding or algal blooms in high light areas, reduce the amount of feed given, and use a high rate of water change.



## The first doliolaria larvae appear! What do they need to settle?

Doliolaria larvae look for a favourable substratum to settle on and metamorphose into pentactula larvae. If suitable conditions are not found, doliolaria larvae continue swimming for a long time, delaying settlement. Suitable settlement surfaces must be provided. Covering larval tanks helps achieve settlement because doliolaria larvae are attracted to light and will otherwise aggregate near the surface.

Methods used to induce settlement of doliolaria larvae include the addition of:

- Algamac 2000 at a concentration of 0.25–0.5 g/m<sup>3</sup>/day.
- Seagrass leaves, which promote a distinctive biofouling layer.

Suitable settlement surfaces include: plastic sheets (PVC, polythene or polypropylene), fibreglass plates, mesh screens, and rough surface tiles suspended in the water. There are four ways to prepare the settlement surfaces:

- Immerse them in cultures of diatoms (*Nitzschia* sp., *Navicula* sp., or *Platymonas* sp.) for a few days.
- Add extracts of filtered seaweed *Sargassum* sp. or seagrass (*Thalassia hemprichii*, *Enhalus acoroides*) over a period of 4–5 days to form a fine coating on the settlement surfaces.
- Paint the surfaces with *Spirulina* (1–2 g dry powder/m<sup>2</sup>), and then leave them to air dry before use.
- Immerse the surfaces for 4–10 days in outdoor tanks under partial shade (50–75%) in running 1-µm filtered seawater to promote natural conditioning with diatoms.

Settlement surfaces should be transferred into the larval tanks when the first doliolaria larva is observed.

### Possible problems

- The biofilm on settlement surfaces comes off easily after 4–5 days in the larval tanks, in shady conditions.
- Settlement surfaces conditioned in unfiltered seawater can introduce predators to the larval tanks. Avoid contaminating larval tanks with unwanted organisms, such as copepods and protozoa.

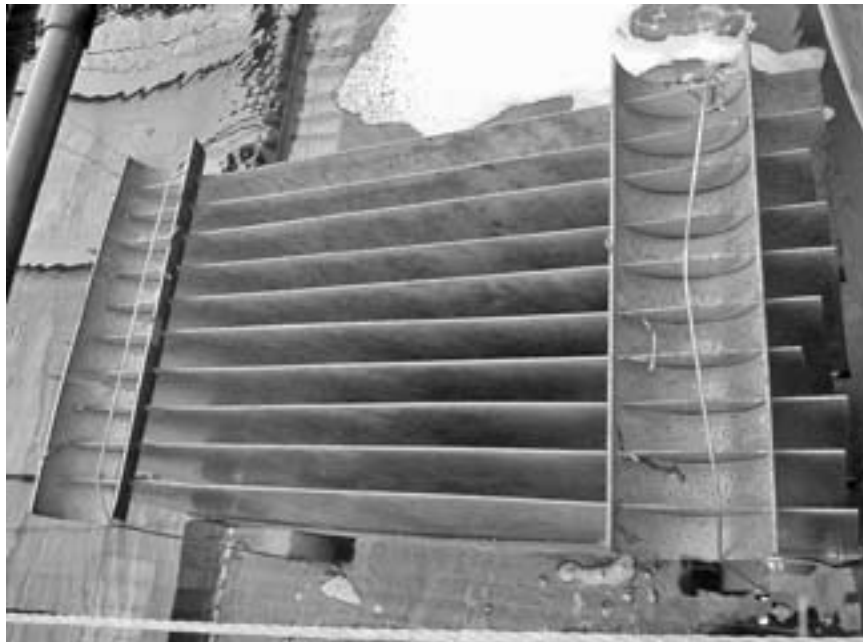


Figure 36. Settlement surface structure conditioned with cultured diatoms.

## Feeding pentactula larvae and juveniles

When conditions are suitable for settlement, doliolaria larvae usually disappear from the water column in around three days. At this stage, they are settled and begin to metamorphose into pentactula larvae. Pentactula larvae need food: fresh and dried algae are an important source of food for pentactula larvae, and for juveniles up to at least 50 mm in length.

- Feed with cultured diatoms (*Nitzschia* sp., *Navicula* sp.) daily from the doliolaria stage in larval tanks, and for the first month in nursery tanks. Add sodium metasilicate (5 g/m<sup>3</sup>) and fertiliser (7 g/m<sup>3</sup>) once a week to promote growth of diatoms in the tanks.
- Supplement feeding with commercial dried algae (Algamac 2000, *Spirulina*). Daily feeding rates are summarised in the table below.

Hatching day	Stage	Algamac 2000	<i>Spirulina</i>
10	<i>Doliolaria</i>	0.25 g/m <sup>3</sup>	0.25 g/m <sup>3</sup>
12	<i>Pentactula</i>		
After 12	<i>Pentactula</i> and juveniles		
After 20	<i>Juveniles</i>	0.5 g/m <sup>3</sup>	
After 30	<i>Juveniles</i>	Up to 1 g/m <sup>3</sup>	Up to 1 g/m <sup>3</sup>

Other possible feeds:

- A fine paste of sieved (40–80 µm) seaweeds (*Sargassum* sp., *Halimeda* sp.) and seagrass (e.g. *Syngodium isoetifolium*).
- A fine-grade shrimp starter pellet at a daily rate of 1–1.5 g/m<sup>3</sup> in the nursery tanks.



Figure 37. Early juveniles in larval tank.





## Review of daily tasks during larval rearing

- Record temperature, oxygen and salinity twice a day, in the morning and afternoon.
- For the first 3–4 days, purge the tank bottom through the central drain pipe.
- Turn off the aeration for a few minutes. Wash the air diffusers with freshwater.
- Gently siphon the bottom of tanks, including any yellow or pink patches, into a 100- $\mu\text{m}$  sieve. Examine the residual dirt under a microscope to check for dead larvae.
- Turn the aeration back on.
- Estimate larval density in the tanks:
  - Take a sample of the water column with a 250 ml beaker. Pipette a 1 ml aliquot and place on a counting chamber (e.g. Sedgewick-Rafter chamber). Count the larvae in three samples of 1 ml,
  - Alternatively, take a sample of the water column in a test tube and count the larvae. Do this three times. Estimate average larval density per ml.
- Observe larval development: take a sample of larvae from the water column by gently submersing a small 100- $\mu\text{m}$  sieve. Pipette a few of them and transfer into a small beaker. Transfer 1 ml of the sample to a counting chamber. Examine the larvae under a microscope. Formalin is usually added to fix the larvae.
- Record the lengths of 10 larvae per tank and the proportion of normal and abnormal larvae.
- Apply the water change protocol. On days when the water is changed, place an air diffuser below the 100- $\mu\text{m}$  immersed sieve to avoid aggregation of larvae on the sieve.
- When the water change is finished, take a sample of the water column in a small beaker or test tube. Count the concentration of algae under a microscope. Add microalgae to the tanks to achieve the desired feeding rate.
- Remove the sieve at the outlet. Rinse it with chlorinated freshwater.



Figure 38. Recording water parameters

## Summary

- Maintain constant water temperature and salinity between buckets and larval tanks when transferring eggs.
- Early sandfish larvae, called auricularia larvae, are motile and planktonic. After 10–12 rearing days, auricularia larvae metamorphose into active swimming doliolaria larvae, which settle on the walls and floor of the tank and on other settlement surfaces. Doliolaria larvae metamorphose into benthic, slow-moving pentactula larvae. Juvenile sea cucumbers, shaped like adults, appear from day 15.
- Larval tanks should be cylindrical (up to 2 m<sup>3</sup>) with conical bottoms and supplied with moderate aeration. Seawater must be 1- $\mu\text{m}$  filtered and UV-sterilised. Larval tanks should be illuminated for 12 hours a day, or have natural photoperiod. Lids or covers are used for the first two rearing days and during the doliolaria stage.
- Water change and feeding start at day 2. The water change can be partial or complete.
- Cultured microalgae are added at the rate of 20 000 cells/ml, increasing to 40 000 cells/ml as long as auricularia larvae are still present in the water column.
- After 10–12 rearing days, doliolaria larvae appear and look for suitable settlement surfaces before metamorphosing into benthic pentactula larvae. Stimulants and conditioned settlement surfaces encourage settlement.
- Pentactula larvae and juveniles feed mainly on diatoms (*Nitzschia* sp., *Navicula* sp.) and commercial dried algae (Algamac 2000, *Spirulina*).





**Figure 39. Preparation of nursery tank includes inoculation with diatoms and insertion of settlement surfaces (plates).**



# NURSERY

## *Culture of early juveniles*

At 25–35 days old, juveniles are transferred from larval tanks into nursery tanks. Nursery tanks are raceways, bigger pools or large tanks of 6 to 10 m<sup>3</sup>, usually made of fibreglass, flexible PVC-cloth liner or concrete. The water in the nursery tanks should be at least 60 cm deep (maximum 1 m). Nursery tanks need to be conditioned prior to the transfer to ensure food is available for the juveniles.

### *Procedure for conditioning nursery tanks:*

- Clean all tank surfaces. Install the aeration system and bare, clean settlement surfaces.
- Fill the tank with 1- $\mu$ m filtered and UV-sterilised seawater, fully immersing the settlement surfaces (i.e. a depth of 60–70 cm).

- Inoculate the water with fresh diatom cultures at a rate of 6–7% of the total volume of water in the tank. Add sodium metasilicate (5 g/m<sup>3</sup>) and a general fertiliser (7 g/m<sup>3</sup>). Switch on the light.
- Turn off the water flow for the first 3–4 days to allow a diatom coating to develop on the plates or other settlement surfaces and tank walls. Maintain moderate aeration and mix the water daily.
- Keep the water temperature constant and warm (26–28°C).

After conditioning, the nursery tanks are ready to receive pentactula larvae and early juveniles.



Figure 40. Nursery tanks.

## Detaching and counting juveniles before transfer to nursery tanks

Pentactula larvae and early juveniles are characterised by a very wide size range and are difficult to detach. The transfer procedure involves detaching juveniles from the larval tanks, estimating their abundance, and then putting them into the nursery tanks.



Figure 41. Detachment by draining.



Figure 42. Detachment by siphoning.

- First, remove the settlement surfaces and attached animals from the larval tanks while holding a plastic tray underneath to catch any juveniles that fall off the plates. Transfer the plates and any loose juveniles to the nursery tanks.
- Second, remove the juveniles from the walls of the larval tanks. There are two methods for detaching juveniles from tank walls.
  - 1) Drain the larval tank completely through the bottom pipe and by siphoning the floor on to mesh sieves (0.3 to 1 mm) placed in bowls. Use a gentle jet of seawater to detach any remaining animals.
  - 2) Add potassium chloride (KCl) to the water using a 1% concentration. Leave for 10 minutes and then replace with normal seawater. The juveniles will detach rapidly when the new seawater is added. Drain the tank onto mesh sieves placed in bowls. Use a spray of 1% KCl to detach any remaining animals.
- Estimate abundance by direct counts of juveniles on plates and sieves.
  - 1) For plates, count all juveniles on several individual plates, selected randomly.
  - 2) For sieves, count only a half or quarter of the total surface where there are high densities of juveniles.
- Use abundance estimates to calculate the survival from the egg to pentactula stage for each larval tank. Survival is usually variable and rarely higher than 1–2%.
- Use abundance estimates to calculate the initial density of juveniles placed in nursery tanks.

### Possible problem

- *The wide variation in the size distribution of juveniles can lead to biased estimates of abundance; thousands of pentactula larvae are too small to count without harming them. Estimates of abundance become more reliable as the juveniles grow larger.*



## The two stages of the nursery phase



Figure 43. Nursery tank with settlement surfaces.

### *First nursery phase (first month)*

The early juvenile stage (<5 mm length) is a vulnerable and critical phase. Substantial mortality can be expected during the week following the transfer of juveniles, due to handling and high density.

Juveniles are maintained in bare tanks until they reach a size of about 1 g. Juveniles should grow to 10–20 mm (0.3–1g) in 30 days. However, high densities can affect growth and survival. To avoid such problems, initial density should be between 500 and 700 juveniles per m<sup>2</sup> of tank floor. At a density of 500 juveniles per m<sup>2</sup>, the survival rate can be up to 50%, and the growth rate can be 0.2–0.8 mm/day after one month.

### *Second nursery phase*

At a size of 20 mm or 1 g, juveniles are transferred to grow on sand because they can now ingest large amounts of sediment. A thin layer of sand (3–5 mm) is evenly distributed on the bottom of nursery tanks. The sand should be cleaned and then enriched with mud or food (e.g. dried algae).

After 1–2 months of grow-out on sand, juveniles need to be reared at optimum densities of 100–300 juveniles/m<sup>2</sup>. Removal of large juveniles will increase the growth of smaller individuals.

After 2 months, the growth rate is 0.5 mm/day, but will slow down if densities reach 225 g/m<sup>2</sup>. The survival rate of juveniles usually exceeds 50% when they are >20 mm.



## Review of tasks during the nursery phase

### Daily tasks

- Record temperature, oxygen and salinity twice a day, in the morning and afternoon.
- Wash the screen placed at the tank outlet.
- Change the water, using filtered seawater (1 µm for the first two months, 10–25 µm later), with constant flow-through of 6 L/min (100–200%). In case of restricted seawater supply, flow-through can be reduced to 3 L/min (40–50%) every night. High flow rates are generally better than low rates.
- Stop the water flow for a few hours for feeding. Feed with diatom cultures, dried algae, seaweed paste and/or fine-grade starter shrimp pellet.

### Weekly tasks

- For the first weeks, leave animals in shade; they avoid bright conditions and prefer shady sides of settlement surfaces.
- Add diatom cultures during the first month.
- Add sodium metasilicate and general fertiliser. Mix the water.
- Invert all settlement surfaces. Diatoms grow in abundance on the upper side but not on the lower side. Pentactula larvae and juveniles stay at the bottom.
- Take a sample of 30 juveniles per nursery tank to estimate the growth rate. Measure total and individual weights and calculate the average. Examine the physical condition of a few animals under a microscope.

### Monthly tasks

- Estimate the number of juveniles per nursery tank to grade densities. Sample over 5% of the tank area, including the settlement surfaces, using small quadrats (20 cm x 20 cm). In cases of high density, collect juveniles with a siphon and hand nets. Distribute surplus juveniles to a new nursery tank.

#### Possible problem

- Unfiltered seawater can result in better growth than filtered seawater, but predators such as copepods can quickly contaminate the tank. Copepods compete for food with the juveniles.

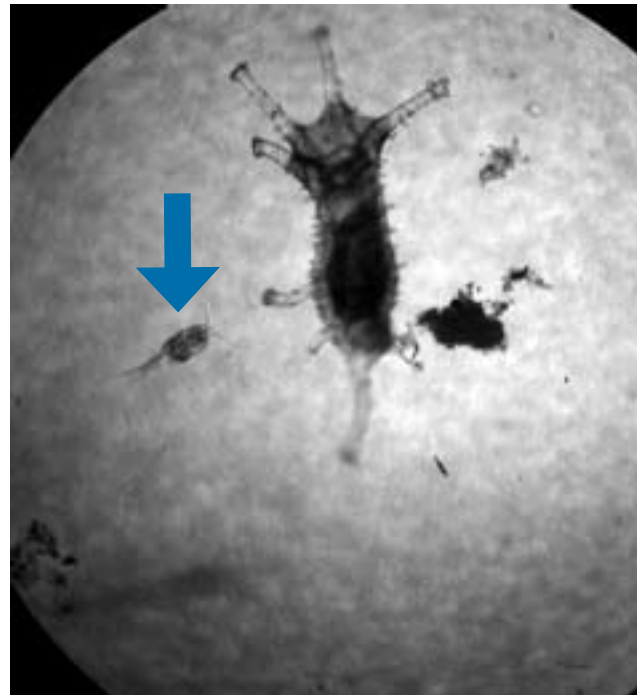


Figure 44. Copepod contamination.

### Summary

- Juveniles can be transferred to nursery tanks after 25–35 days.
- Nursery tanks are raceways, pools or tanks of 6 to 10 m<sup>3</sup>. They must be conditioned before transferring the animals. Conditioning consists of developing a diatom coating on the tank walls and settlement surfaces.
- Water exchange is flow-through or partial, with filtered seawater.
- Transfer of juveniles from larval tanks to nursery tanks requires detaching, counting and grading the animals. Spraying the walls of the tank with seawater after complete draining by siphoning, or chemical detachment using potassium chloride, are two methods of detachment.
- There are two nursery phases. During the first phase, the juveniles are held in bare tanks. After about one month, when they are 20 mm or 1 g, they are transferred to tanks with sand substrata (second nursery).
- Grading consists of reducing the density of juveniles over time. Initial densities range from 500 to 700 juveniles per m<sup>2</sup> in bare tanks; optimum densities vary from 100 to 300 juveniles per m<sup>2</sup> in tanks with sand (second nursery).





## GROW-OUT OF JUVENILES

The following results are from experiments in Vietnam and New Caledonia. Results can be expected to vary from place to place.



Figure 45. Hapas (1 m<sup>2</sup>).



Figure 46. Bag nets (4 m<sup>2</sup>).

### In net pens in ponds

In New Caledonia, to overcome space limitations in nursery tanks, juveniles 15–25 mm long (0.5–1 g), but as small as 5 mm were transferred to ‘hapas’ (1 m<sup>2</sup> net pens with fine mesh) in earthen ponds at densities of 150 juveniles/m<sup>2</sup>. Survival averaged 97% over 23 days and growth rate was higher (0.1 g/day) in hapas with artificial seagrass.

Larger juveniles (1–2 g) can be placed in ‘bag nets’ (4 m<sup>2</sup> net pens with coarse mesh) in earthen ponds at densities of 150 juveniles/m<sup>2</sup>. Feeding was not necessary in ponds with good natural productivity. When productivity was low, good growth was obtained by adding ground shrimp pellets (chicken manure or *Sargassum* sp. can also be used, but growth is not as good). Addition of sandy-mud substrate to the bag nets did not improve growth or survival. Growth rate averaged 0.08–0.1 g/day over 3 weeks.

Weight range	Net pens	Net mesh
0.5–1 g	Hapa 1 m <sup>2</sup> , 1 m deep	660–670 μm
1–2 g	Bag net 1–4 m <sup>2</sup> , 1 m deep	1 mm

#### Possible problems

- To reduce risks of low oxygen levels, high temperature and anaerobic sediment, raise the floor of the hapas and bag nets off the substrata. Measure the temperature, oxygen and salinity of the pond water regularly.
- Some netting materials are too abrasive for sandfish juveniles, or too fragile to be durable. Tentex is a suitable net material.
- Nets become dirty after several weeks in ponds. Brush nets regularly to allow free water flow.



Figure 47. Sandfish juveniles grown in an earthen pond.

### In ponds

In Vietnam, large sandfish (50–500 g) stocked in ponds had growth rates of 2.2–3.2 g/day. These growth rates varied inversely with stocking density in the range 106–170 g/m<sup>2</sup>. Survival was high (88–97%) until the start of the wet season when massive mortality occurred due to stratification and lethally low salinity.

In New Caledonia, juveniles of 1 g have been reared in earthen ponds at a density of 1.4 juveniles/m<sup>2</sup>. The growth rate observed over 1 year averaged 0.8 g/day (24 g/month).

#### Possible problems

- Quality and structure of sediment in ponds, and seawater supply, will affect growth and survival. Poor environmental conditions can lead to excessive growth of filamentous algae and anaerobic sediment.
- Stratification of water from heavy rain leads to low oxygen levels, high temperatures and inadequate salinity (below 20 ppt).
- Poor environmental conditions in ponds can lead to development of skin lesions.

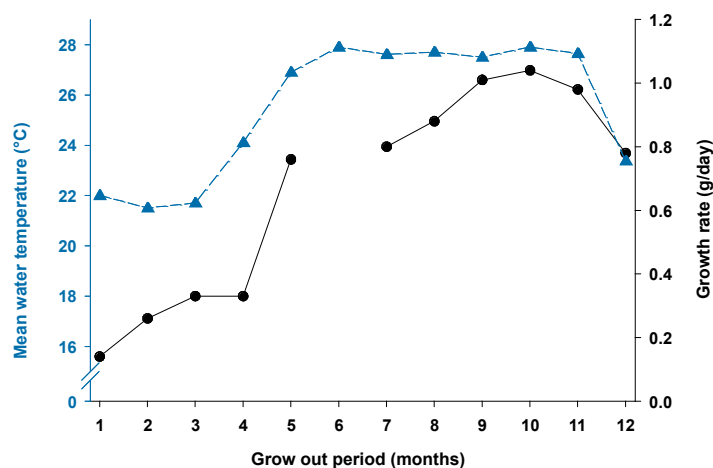


Figure 48. Growth rate of juveniles of 1 g reared in earthen ponds in New Caledonia, and mean temperature of pond water.



## In sea pens



Figure 49. Sea pen of 500 m<sup>2</sup>.

Various sea pens and cages have been used to grow out sandfish. These include 25 m<sup>2</sup> pens made from bamboo screens or palmirah rafters erected in shallow water with 4 mm mesh; 0.6 m<sup>2</sup> rectangular iron cages with 2 mm mesh; 2 m<sup>2</sup> velon screen cages with 7 mm mesh; and 2 m<sup>2</sup> netlon cages with 5 mm mesh. In general, growth and survival of sandfish in sea pens is much lower than in earthen ponds.

In Vietnam, juveniles stocked at high density (500 g/m<sup>2</sup>) in sea pens survived well (98%) but did not grow. Reducing the density to 390 g/m<sup>2</sup>

resulted in high survival (90%) and growth of 1.7 g/day. Juveniles with an average weight of 84 g stocked at 0.73 juveniles/m<sup>2</sup> grew at a rate of 1.05 g/day over 5 months when reared in a 2000 m<sup>2</sup> sea pen. The sea pen had 8 mm mesh and was placed in water 1.5–2.5 m deep with a silty-sand substratum and broken coral.

In New Caledonia, survival 1–20 g juveniles placed in 500 m<sup>2</sup> sea pens in shallow seagrass beds at densities of 4 juveniles/m<sup>2</sup> was 5–9 % after 18 months.



Figure 50. Juvenile sandfish (2.5 to 12 g).

### Summary

- Juveniles as small as 5 mm can be transferred to hapas (net pens with fine mesh) in earthen ponds.
- At 1 g, juveniles can be reared in large bag nets with coarse mesh in earthen ponds.
- Hapas and bag nets should be kept off the bottom of the pond to reduce risks of unfavourable environmental conditions.
- Large juveniles (> 1 g) can be released directly into earthen ponds.
- Juveniles can be stocked in sea pens. Less management is required, but growth and survival are far lower than in ponds.

## PROBLEMS AND POSSIBLE SOLUTIONS

- **Obtaining eggs:** In many countries, eggs can only be obtained during a relatively short spawning season. This limits the scope for farming and sea ranching sandfish. Methods need to be developed to extend the spawning season by a few months.
- **Size of rearing tanks:** Small larval rearing tanks require considerable labour and are prone to variation in temperature. Use of larger larval tanks that require only partial water changes each day will improve the efficiency of larval rearing and lead to higher larval survival through reduced temperature-induced stress. Use of daylight instead of artificial light is also an advantage.
- **Production of large volumes of algae:** Algal production can be increased by using large outdoor cultures. However, keeping larger cultures free of contamination by copepods requires systems for adequate filtration, UV treatment and sterilisation by chlorination-dechlorination.
- **Limited space for nursery tanks:** As larger nursery tanks are required for the first nursery stage, this can often result in substantial costs. However, space can be saved by using additional conditioned plates and by grading to remove larger animals for the second nursery phase. For the second nursery, bag nets in ponds or seabed cages can be used to replace nursery tanks.



Figure 51. Earthen pond suitable for installation of hapas and bag nets.





## PROMISING APPLICATIONS FOR HATCHERY-REARED SANDFISH

Provided methods can be developed for releasing cultured sandfish in the wild that ensure a high proportion of them survive, hatchery-reared juveniles could be used for restocking, stock enhancement and sea ranching programmes.

- Restocking involves releasing cultured juveniles to restore the spawning biomass of a severely depleted fishery to a level where it can once again provide regular, substantial yields.
- Stock enhancement is designed to increase the productivity of a fishery that is still operating reasonably well by overcoming shortfalls in the natural supply of juveniles.
- Sea ranching differs from restocking and stock enhancement in that there is no intention of allowing the released animals to augment spawning biomass or particular age-classes within the population. Instead, animals are placed in the wild to be gathered at a larger size in 'put and take' operations.

The availability of cultured juvenile sandfish also provides potential for farming this species in earthen ponds or sea pens. However, sandfish should not be reared in ponds together with shrimp because shrimp prey on sandfish.

The following questions need to be answered to fully assess the potential for pond farming of sandfish:

- What is the optimal density for stocking sandfish into ponds including those previously used to farm shrimp?
- Do sandfish have a beneficial effect on the sediments of recently harvested and filled shrimp ponds?
- Do sandfish need to be thinned out, or does food have to be added, later in the production cycle to maintain good growth rates?
- If enrichment of sediments is needed, what is the best method?
- Are some sediments better than others for rearing sandfish?



Figure 52. Hatchery-reared sandfish.



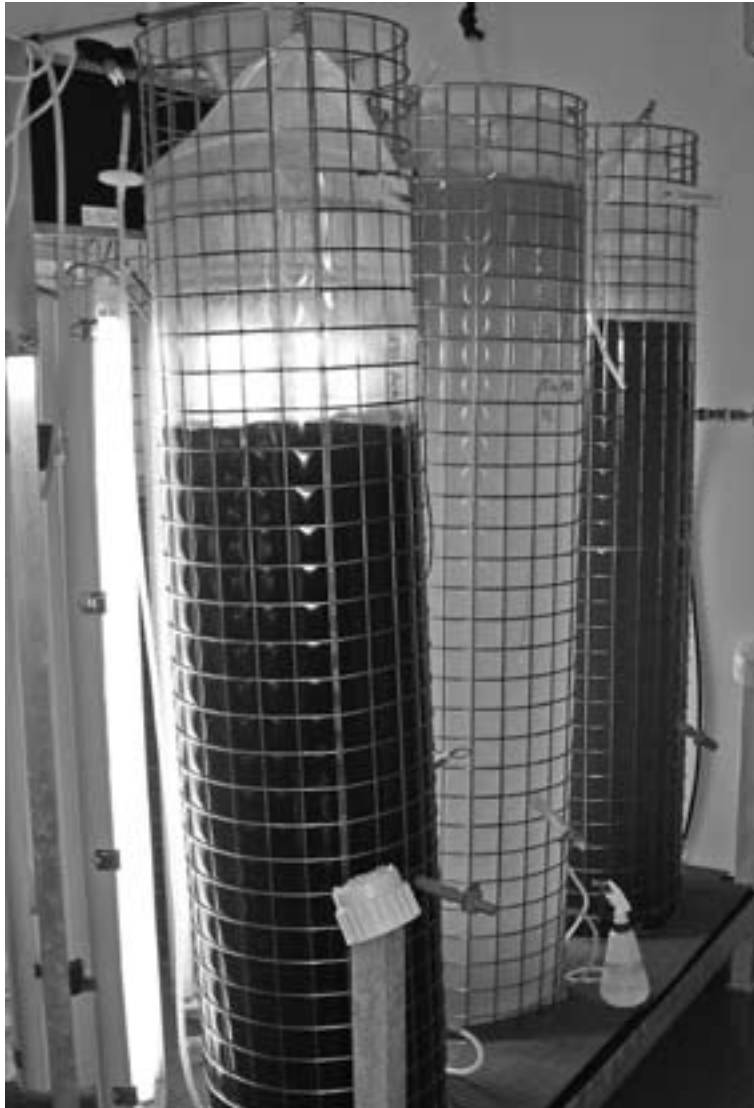


Figure 53. Large volumes of cultured microalgae in 150 L bags in an indoor culture room with artificial light.



# ANNEX 1: ALGAL CULTURE

## Maintenance of algal cultures

### Purchase of stock algal cultures

High quality algae to start new cultures are available from the following organisations:

- CSIRO Marine and Atmospheric Research, GPO Box 1538, Hobart, Tasmania, 7001 Australia
- Culture Collection of Algae and Protozoa, SAMS Research Services Ltd, Dunstaffnage Marine Laboratory, Oban, Argyll PA37 1QA, Scotland
- Plymouth Culture Collection, Marine Biological Association, Citadel Hill, Plymouth, PL 2PB, U.K.

### Maintenance of stock cultures

- Store the stock cultures in Erlenmeyer flasks (250 ml) in sterile and static conditions, close to artificial light in an air-conditioned room (20–24°C).
- Keep two series of stock cultures of each species to reduce the risks of contamination and complete loss of algae.
- Swirl the stock cultures by hand every day to maintain the algae in suspension.
- Sub-culture the stock cultures once a week (or no longer than 2 weeks).

### Conditions required for the algal room (volumes of algae <500 L)

- Avoid contamination by cultures of other organisms, i.e., have a room exclusively for algal culture.
- Maintain air temperature between 20 and 24°C by air conditioning.
- Provide light by fluorescent tubes ('cool white' or 'daylight') 14 hours per day.
- Provide aeration for large flasks, carboys and bags.
- Use a laminar flow or simple glass cabinet for the sterile transfer of algae, if possible.

### Conditions required for large volumes of algae (500 L)

- For indoor cultures, provide artificial light and continuous mixing of water.
- For outdoor cultures with natural daylight, provide continuous mixing of water and protection against rain.

### Possible problem

- *Ciliates could be introduced through the air line due to high levels of moisture. To avoid this contamination, place a filter of 0.2–1 µm in the air line before it enters the culture.*

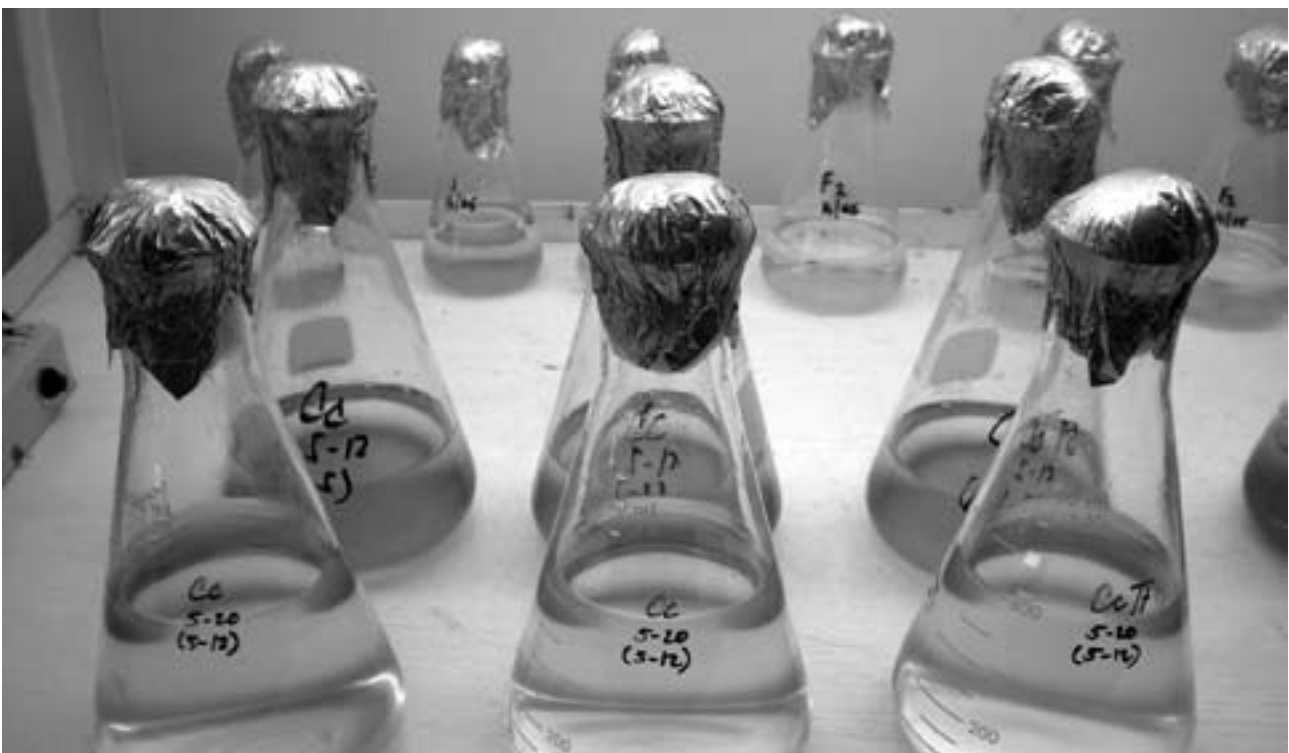


Figure 54. Stock cultures of algae in Erlenmeyer flasks.

## Preparation and inoculation of algal cultures

### Preparation or purchase of culture media

The most common medium used in the culture of microscopic algae is Guillard's *f/2* medium. However, when using this medium the quantity of all ingredients should be doubled; *f/2* medium becomes *f* medium. This *f* medium is more appropriate for culturing *Rhodomonas salina*.

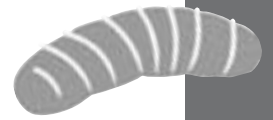
- Prepare the *f/2* (Guillard's) medium (nitrate, phosphate, trace metal mix, ferric citrate, vitamins). A solution of sodium metasilicate is added only for cultures of diatoms. Use the *f* medium for *Rhodomonas salina* culture.
- Alternatively, the media can be purchased from: Culture Collection of Algae and Protozoa, SAMS Research Services Ltd, Dunstaffnage Marine Laboratory, Oban, Argyll PA37 1QA, Scotland.
- Store the media in a dark, clean place in a fridge.

### Preparation of flasks and 10-L carboys

- Wash the culture vessels with detergent, rinse and leave to dry.
- Fill flasks and carboys with 0.2–1 µm filtered and UV-sterilised seawater at low salinity (25–30 ppt).
- Add the culture medium.
- Add the glass tubes (aeration line), cotton and foil caps.
- Autoclave at 121°C for 20 minutes.
- Store flasks and carboys for at least 24 hours before use to overcome the temporary rise in pH.
- Inoculate with algal cultures.



Figure 55. Carboys (20 L).



#### Preparation of 20-L carboys, bags and tanks

- Wash with diluted chlorine (10%), rinse and leave to dry.
- Fill with 1- $\mu$ m filtered and UV-sterilised seawater.
- Add chlorine (12.5% active) at a concentration of 0.2 ml/L for a minimum of 4 hours, or overnight for up to 24 hours without aeration.
- Turn on the aeration for 10–15 minutes to remove residual chlorine. Add sodium thiosulphate (stock solution at 250 g/L) at a rate of 0.2 ml/L for 1 hour to neutralise residual chlorine. Conduct a chlorine test.
- After several minutes, add agricultural fertiliser such as Aquasol (2.5 g/100 L). For diatom cultures, also add sodium metasilicate (3.75 g/100 L).
- Inoculate with algal cultures.

*Sodium metasilicate is added for diatom cultures such as Chaetoceros sp., Navicula sp. and Nitzschia sp.; diatoms use the silicate for production of an external shell.*

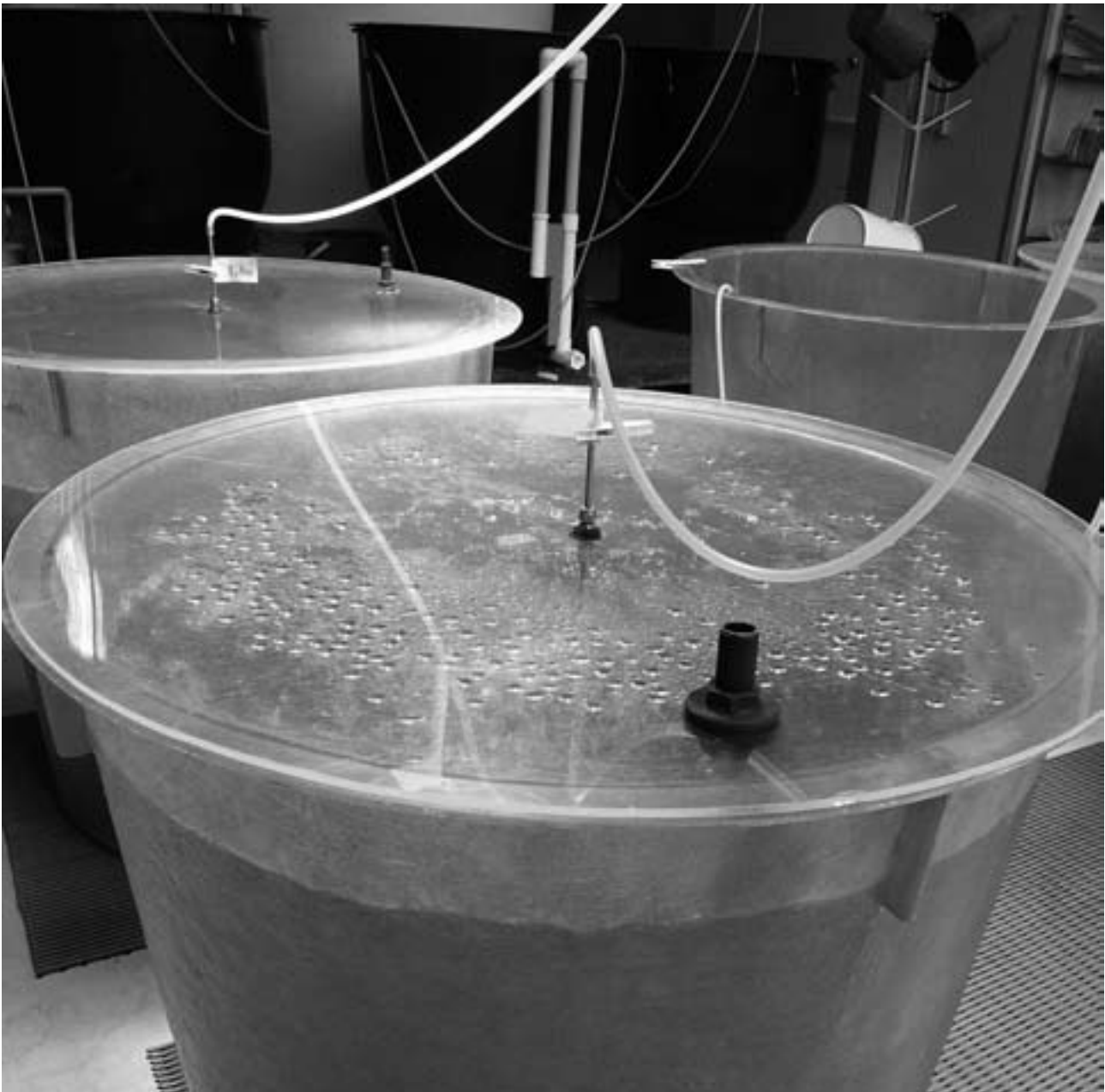


Figure 56. Algal culture in a 500 L tank.

### *Inoculation method for flasks and carboys*

- Carry out inoculations in a laminar flow or simple glass cabinet.
- Swab the cabinet work area and hands with 70% alcohol.
- Place the culture volumes required for transfer in the cabinet close to the flame of a Bunsen burner.
- Flame the mouths of the flasks before and after transfer.
- Working close to the flame, remove the cap of the new flask and quickly add nutrients and algal inoculum.
- Replace the cap immediately.
- Label the flask with the date and the name of the algal species.

<i>Culture volumes</i>	<i>Turnover time</i>
<i>250 ml Erlenmeyer flasks</i>	<i>4–7 days</i>
<i>2 L flasks</i>	<i>3–7 days</i>
<i>10–20 L carboys</i>	<i>4–7 days</i>
<i>150 L bags</i>	<i>7–14 days</i>
<i>500 L tanks</i>	<i>4–7 days</i>

### *Summary*

- *Keep two series of stock cultures for each species of algae in Erlenmeyer flasks.*
- *Subculture once a week (or no longer than 2 weeks).*
- *Maintain the cultures in flasks, carboys and bags in an air conditioned room (20–24°C) with lighting 14 hours per day. Swirl the stock cultures by hand daily and provide regular mixing for large volumes of cultures.*
- *Place a filter in the air line before it enters the culture to avoid contamination by ciliates.*
- *Preparation of an algal culture, and the sterilisation method used, depend on the culture volume.*
- *Sodium metasilicate should be added to the media for the culture of diatoms.*
- *Inoculation and transfer of algal cultures require a clean and sterilised environment.*
- *For outdoor cultures, water temperature must not exceed 32°C.*





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