A hatchery operations manual for rearing sandfish, *Holothuria scabra*, in Tarawa, Republic of Kiribati

SPC
Secretariat
of the Pacific
Community

CPS
Secrétariat général
de la Communauté
du Pacifique

Original text: English

Secretariat of the Pacific Community Cataloguing-in-publication data

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A hatchery operations manual for rearing sandfish, Holothuria scabra, in Tarawa, Republic of Kiribati / Secretariat of the Pacific Community

1. Sea cucumbers — Kiribati.
2. Trepang fisheries — Kiribati.
3. Hatchery fishes — Kiribati.
4. Holothurian populations — Kiribati.

I. Title II. Secretariat of the Pacific Community

593.96099681 AACR2

ISBN: 978-982-00-0867-0

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Prepared for publication at
Secretariat of the Pacific Community headquarters
Noumea, New Caledonia, 2015

Content

Acknowledgments

This manual has been produced by Mr Masahiro Ito, SPC external consultant.

All photographs appearing in this manual were taken by Masahiro Ito, excluding photo page4, by Ruth Garcia.

Figures 19, 21, 23, 25, 26 and 28 were produced by Masahiro Ito and originally appeared in the FAO manual *Technical guidance on pearl hatchery development in the kingdom of Tonga*, and have been reproduced in this manual with the permission of FAO.

This manual is fully funded by SPC.

Overview

Presently, the sea cucumber industry in Kiribati consists of sporadic and intense harvesting at a given island lagoon until local stocks are wiped out. Businessmen and harvesters sometimes then move on to another location where stocks are abundant.

The purpose of this mission was to demonstrate hatchery production techniques for rearing sandfish, *Holothuria scabra* (which does not occur naturally in this country), including broodstock holding and conditioning strategies, and spawning, larval and post-larval rearing techniques. This mission would contribute to, through hatchery technology, supplying the needed biomass for a sustainable sea cucumber industry in the future. If the hatchery technology proves successful in achieving production objectives, additional activities relating to stock management programmes could then be developed in collaboration with coastal communities and other stakeholders, such as private entrepreneurs, public institutions, governmental agencies and non-governmental organisations.

In February 2013, 120 sandfish broodstock, all juveniles averaging 90 g in wet weight, were imported from Fiji. The sandfish were quarantined for three months, and during this period no artificial food was provided (only natural plankton). After the quarantine period, the sandfish were divided into two groups of 60 animals each. One group was placed in a fish pond (Fig. 1) near the airport and the other group was placed in a concrete tank at Tanaea Hatchery in north Tarawa. The concrete tank measured 4 m x 1.5 m x 0.4 m (2,400 L of water) and the stocking density was approximately 10 sandfish m^{-2}. The 60 sandfish that were in the fish pond died before the hatchery demonstration commenced. Therefore, only the 60 sandfish in the concrete tank were available for spawning trials. These broodstock were fed approximately 1% of their wet body weight (BW) per day per sandfish (30 g of fishmeal and 30 g Algamac in total). According to Kiribati aquaculture officers under the Ministry of Fisheries and Marine Resources Development (MFMRD) in charge of the public hatchery, the average BW on 1 August was 65 g,

Figure 1. *Fish pond near Tarawa Airport.*

Figure 2. *Habitat simulator holding tank system for the sandfish broodstock.*

and was 94 g on 1 September. Although the water flow rate was set to more than 400% per 24 hours, it was apparent that water quality was deteriorating because there was no refreshening of the tank's substrate (muddy sand with left-over fishmeal and Algamac). In order to speed up broodstock fattening and to feed more food in higher stocking densities, a habitat simulator holding tank system (Fig. 2 a) and b), which simulates the seagrass bed environment of the Tanaea channel, was built in the hatchery's under-cover area.

Spawning induction and larval rearing protocols for sandfish have been described by Agudo (2007) based on hatchery work in New Caledonia. Although the basic technique used in Kiribati is the same as Agudo's, the hatchery protocols for Tanaea Hatchery were greatly modified to suit the available equipment, tools and materials at the hatchery. Tanks included 500-L rectangular or round flat-bottom tanks for spawning, a 2,500-L raceway tank for larval rearing, and another 2,500-L raceway tank for settlement (the first nursery culture).

The spawning induction technique uses a dry treatment and thermal shock method at 31–33°C after a period of three to four hours, followed by a cold treatment at around 18–21°C, combined with an Algamac (artificial dried algae) bath. Basic methods for microalgal culture, preparing nutrient media, computing culture density, and feeding amounts are provided in this manual. Tanaea Hatchery technicians maintain three microalgae species: *Chaetoceros mulleri*, *Isochrysis* sp. (T-ISO), and *Nannochloropsis oculata* (Fig. 3). In this manual only two species, *C. mulleri* and T-ISO, are suggested for use as food for auricularia larvae. It should be noted that all microalgae are assumed to be cultured under synchronous culture conditions (i.e. cultured under a controlled light–dark cycle), and feeding protocols are based on this. Unfortunately, the light–dark period has not been controlled at Tanaea Hatchery, so the feeding protocols and the effect of the feeding during the first spawning and larval run do not reflect those in this manual. Preparation, conditioning and maintenance of settlement tanks are based on the use of corrugated plastic plates as settlement substrates, so that epiphytes will grow on the tank walls and plates where pentactula and juveniles settle directly on such substrates. The settlement tank with conditioned plates is used for rearing juveniles for about three to four weeks (the first nursery culture) followed by the detachment of juveniles for transfer to grow-out in hapas (cages) or habitat simulators (the second nursery culture).

Figure 3. *Microalgae stocks at Tanaea Hatchery.*

While the ultimate goal is to develop a sustainable sandfish industry in Kiribati, the industry itself cannot get underway until hatchery technology is understood and transferred to the Kiribati people, which then proves it is feasible and self-sustainable. To be successful in transferring sandfish hatchery skills immediately to the Kiribati Fisheries Department personnel and trainees, it is recommended that hatchery trainees be recruited to create a group of core technicians who can then train the next generation of technicians.

Background information on sandfish and conditions at Tanaea Hatchery

Sea cucumbers have been used for food, medicine and other products by the Chinese and Koreans, and the demand is expected to continue unabated into the future. Processed and dried sea cucumber, or beche-de-mer, is a valuable source of income for communities in the Asia-Pacific region because sea cucumbers are easy to collect and do not require any sophisticated processing techniques. Sea cucumbers can be processed locally and stored in such a way that they are preserved until they are shipped out. Sea cucumbers and related products are sold to consumers overseas, making them some of the few exportable products from Kiribati. At present, there is a strong demand from China and other Asian countries for beche-de-mer. This demand has already increased the price of one of the most favoured sea cucumber species in the tropical region, the sandfish *Holothuria scabra*. Among the tropical holothurians, sandfish is considered to be one of the best aquaculture candidates. Over the past few decades, efforts to develop hatchery techniques for *H. scabra* have increased in the Asia-Pacific and Indian Ocean regions: Australia (Morgan 2000, 2001; Giraspy and Ivy 2005; Ivy and Giraspy 2006), Fiji (Hair 2011), India (James et al. 1995), Madagascar (Jangoux et al. 2001), Micronesia (Ito 2010; Ito and Hasurmai 2011; Ito et al. 2010), Philippines (Gamboa et al. 2011), Solomon Islands (Battaglene et al. 1998; Mercier et al. 2000) and Viet Nam (Pitt and Duy 2004; Duy 2011). Hatchery and grow-out trials have also been undertaken by international agencies such as the Secretariat of the Pacific Community and the WorldFish Center (Battaglene and Seymour 1998; Purcell 2005; Purcell and Eeckhaut 2005). The WorldFish Center has developed mass production methods and a manual for sandfish hatchery techniques (Agudo 2007) and has worked on developing optimal releasing strategies for hatchery-produced sandfish juveniles in New Caledonia (Purcell 2005; Purcell and Kirby 2006). Other commercially high-value holothurians such as white teatfish (*Holothuria fuscogilva*) were successfully produced from the hatchery in Kiribati in 1997 (Mr Sato, Japan Overseas Fishery Cooperation Foundation, pers. comm.) and subsequently restocked in the wild for grow-out (Friedman and Tekanene 2005; Purcell and Tekanene 2006).

Establishing and maintaining a sandfish hatchery involves basic equipment and some specialised equipment and materials but not necessarily high-tech ones (Table 1). Sandfish is a deposit (detritus) feeder of organic matter in sand and mud. A sandfish hatchery requires two units: a plant unit to produce the algal diet, and an animal unit to conduct spawning and rearing of larvae and juveniles. Microalgal culture is usually carried out in a temperature-and-light cycle controlled indoor system for stock, and high density starter cultures under synchronous monospecific culture conditions. If necessary, large-scale outdoor cultures are involved. Spawning and larval and juvenile rearing can be done either indoors or in semi-outdoor (undercover) conditions. Spawners (broodstock) are best kept at an ocean grow-out farm or in a habitat simulator tank system at the hatchery. A hatchery technician is needed to handle all aspects of algal culture, spawning, larval and post-larval rearing as well as broodstock conditioning and juvenile culture at the ocean grow-out farm.

For a low-cost hatchery operation, the site should:

- have access to clean seawater, unpolluted without industrial and domestic heavy discharge, good water exchange by tidal movement;
- be easily accessible from both the land and the sea;
- have a safe building foundation;
- have access to electricity (public electricity supply) and freshwater (public or rain water supply);
- be within proximity to a township; and
- be within minimum transportation range between the hatchery and ocean grow-out farm and the broodstock holding system.

Table 1. *Summary of basic equipment and materials used for rearing sandfish at Tanaea Hatchery:*

Microalgae room	3 m x 4 m built-in algal culture room.
Spawning tanks	Two 500-L rectangular or round flat-bottom tanks and/or one 2,500-L raceway.
Incubator	One 500-L or one 1,000-L or one 2,000-L flat-bottom or conical bottom tank.
Larval rearing tanks	Two 2,500-L raceway tanks and four 1,000-L round flat-bottom tanks.
Major equipment and tools	Two air-conditioners (min. 5,200 BTU), one autoclave (46-L top loading chamber), one generator (5 kVA for emergencies), one air pump (124 W for emergencies), 20 fluorescent tubes (20 W), one inline UV steriliser (40 W), 10" cartridge filters and housing (four 1 µm for seawater and two for rainwater), one filter for algae room (0.2 µm or 0.5 µm), 30 inline air filters (0.2 µm), three timer switches for 14 L–10 D synchronous algal culture, one laminar flow chamber with propane gas and Bunsen burner, PVC pipes, elbows, tees, joiners and valves (1/2", 3/4", 1"), PVC hoses (3/16",1/2", 3/4", 1", 1–1/4",1–1/2"), plankton screen for sieves (one each of: 50 µm, 80 µm and 100 µm), settlement plates (min. x 345) and plate holder (min. x 23).

Clean seawater does not necessarily mean crystal clear lagoon water; it may contain a little silt but not industrial waste. Unless the staff lives in close proximity to the hatchery, or the hatchery has staff housing, a short travel distance to the hatchery should be taken into account for a long-term project. It is essential not to design a hatchery that can only be operational by using a large capacity generator for its electricity. If public electricity and freshwater supplies are available, such consumptions must be minimal. A well planned and designed hatchery project can lead to its long-term success in terms of economical sustainability. A microalgae room requires a small space (e.g. 3 m x 6 m), accommodating a secondary seawater filter (1 µm and 0.2–0.5 µm) and inline UV steriliser (40 W), a bench for chemical preparation and microscope work, a laminar flow chamber for algae inoculation, and three-stage culture benches for stock culture, high-density starter culture and mass culture. Room temperature and lighting need to be maintained at 20–22°C by one or two small air conditioners (5,200 BTU each) and a light–dark cycle of 14 L–10 D (14 hours light and 16 hours dark periods) for synchronous culture. The culture benches accommodate polycarbonate tanks (max. of 8 at 30 L and 4 at 100 L); 16, 3–5-L flasks; 36, 250-mL flasks; and 30, 20 W fluorescent lighting tubes. Other equipment includes an air pump (linear diaphragm, 124 W), binocular microscopes and an electronic balance. A top-loading type autoclave (46-L chamber, 30 cm diameter x 65 cm deep) and a small refrigerator (108 L) are placed outside the algae room.

In order to maintain proper hygiene for hatchery work, all equipment and tools must be washed thoroughly; first by using chlorinated water and detergent, and then rinsing off the detergent and placing the equipment in chlorine baths, and finally by rinsing with filtered rainwater and drying on a dust-free bench. Some equipment and tools are then stored overnight, or dipped in the chlorine bath and rinsed again with filtered rainwater just before use. These basic rules ared summarize below in table 2.

Table 2. *Summary of precautions before, during and after working at hatchery:*

Make sure your hands are clean; wash them with soap, paying particular attention to dirty finger nails.
Soak your feet in a chlorine bath before entering the larval and/or microalgae room.
Do not work in your own shoes. Always wear designated rubber boots or work barefoot.
Always rinse cleaned and dried equipment with filtered rainwater (1 µm) before using.
Wash used equipment and tools with chlorinated water to wash away excess waste and dirt.
Conduct a second wash using detergent, washing off any dirt thoroughly with a soft sponge or brush.
Rinse with 1 µm-filtered rainwater and completely wash out any residual soap or detergent.
Soak equipment overnight in a chlorine bath (diluted sodium hypochloride, NaHClO). Do not mix with soap as this may release Cl_2 (chloride gas).
Rinse completely with 1 µm-filtered rainwater. Make sure there is no residual chlorine.
Always hang and dry equipment after it has been cleaned. Do not leave on the floor or on a dirty bench.
If necessary, use ethanol (ethyl alcohol) or an isopropyl alcohol spray (75–80% solution) on equipment and wait for it dry. Note: The use of methanol (methyl alcohol) is a health hazard in a small algae culture room.
Do not touch the inside of the cleaned surface of any equipment and tools such as a bucket, container, tank, tub or flask.
Wash filter cartridges and housings after every use. Wash out any dirt with pressurised freshwater (1 µm-filtered rainwater), soak in a chlorine bath, rinse with 1 µm-filtered rainwater, and dry on a designated bench. Use a 0.2–0.5 µm filter for microalgal culture. Keep it in a sealable plastic bag with ethanol-sprayed inside. For the filter housing, spray ethanol inside.
Always clean the floor; wash with freshwater (chlorinated town water or rainwater). It is the best if the floor is dry before you begin working the following morning.
Do not disturb the animals (larvae and juveniles) and minimise unnecessary shock and stress. Avoid unnecessary entry to the microalgae culture room and larval rearing unit.

Broodstock management techniques

Broodstock conditioning is usually conducted in earthen ponds, ocean enclosures, or in a tank. All 120 sandfish broodstock imported from Fiji were juveniles whose average wet body weight (BW) was 90 g. Of these, 60 died in a fish pond, and 1 of the remaining 60 died in a concrete tank at Tanaea Hatchery. Average BW in early August 2013 was 65 g, and was 94 g one month later in September. The concrete tank measured 4.0 m long x 1.5 m wide x 0.5 m deep (with 2,400 L water volume), and the stocking density was approximately 10 sandfish m^{-2} or 650–940 g m^{-2} between August and September 2013. Up until mid-September, the broodstock had been fed approximately 1% of their BW daily with fishmeal (30 g) and Algamac (30 g) in the concrete tank (Fig. 4 a and b) with a water flow rate of more than 400% per day. Water quality deteriorated in the tank's bottom substrate (muddy sand with left-over fishmeal and Algamac), which had not been refreshed and the tank's water had not been changed.

On 24 September, these 59 dying sandfish were transferred to a 2,500-L raceway tank (Fig. 5a) that measured 4.8 m long x 1.0 m x by 0.45 m deep. About 0.5–1.0 inch of muddy sand was placed in the tank, which was well aerated and had a water flow rate of 100% per hour. This raceway was used temporarily until a habitat simulator holding tank system (high density conditioning tank system) became available for accommodating all the broodstock. This was necessary because the feeding rate needed to be increased to at least 1.5–2% BW daily in order to speed up broodstock fattening.

Figure 4. *Concrete tank for broodstock holding and fattening (a and b); Sandfish in concrete tank (c).*

Feeding amounts were recalculated after measuring Body Weight (BW) on 2 October, prior to spawning induction work. A new feeding regime for the broodstock in the raceway tank was set to 1.5% BW daily: the feed mix consisted of 40 g of fishmeal, 40 g of seagrass (species such as *Sargassum* spp.), and 20 g of Algamac (Fig. 5b). The average BW was 109.4 g for 59 broodstock; the minimum BW was 46 g and the maximum BW was 232 g. Thus, an average of 1.7 g of feed mix was given daily to each individual.

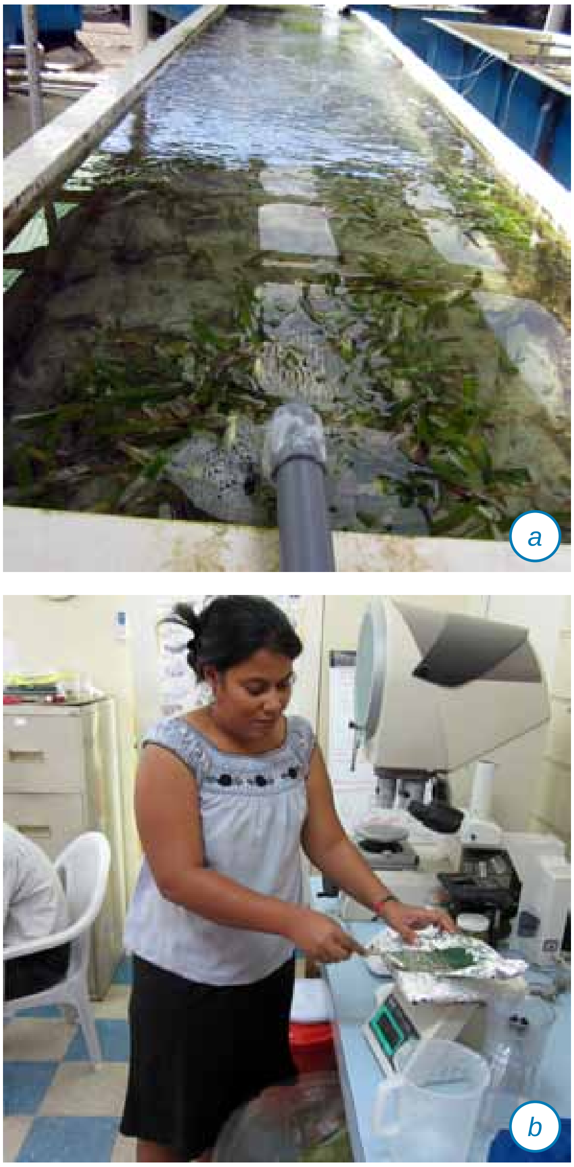


Figure 5. *Raceway tank for temporarily holding sandfish (a), and preparing Algamac (b).*

According to Kiribati's aquaculture officers, there is no seagrass bed in the lagoon close to the hatchery. As shown in Figure 6, however, seagrass beds do exist in the channel right next to the hatchery, and the seagrass is likely turtle grass, or *Thallasira* sp., which is similar to seagrasses found in sandfish habitats in the Federated States of Micronesia (Pohnpei), New Caledonia and Australia. This seagrass has been used in Pohnpei as additional feed for juveniles and broodstock.

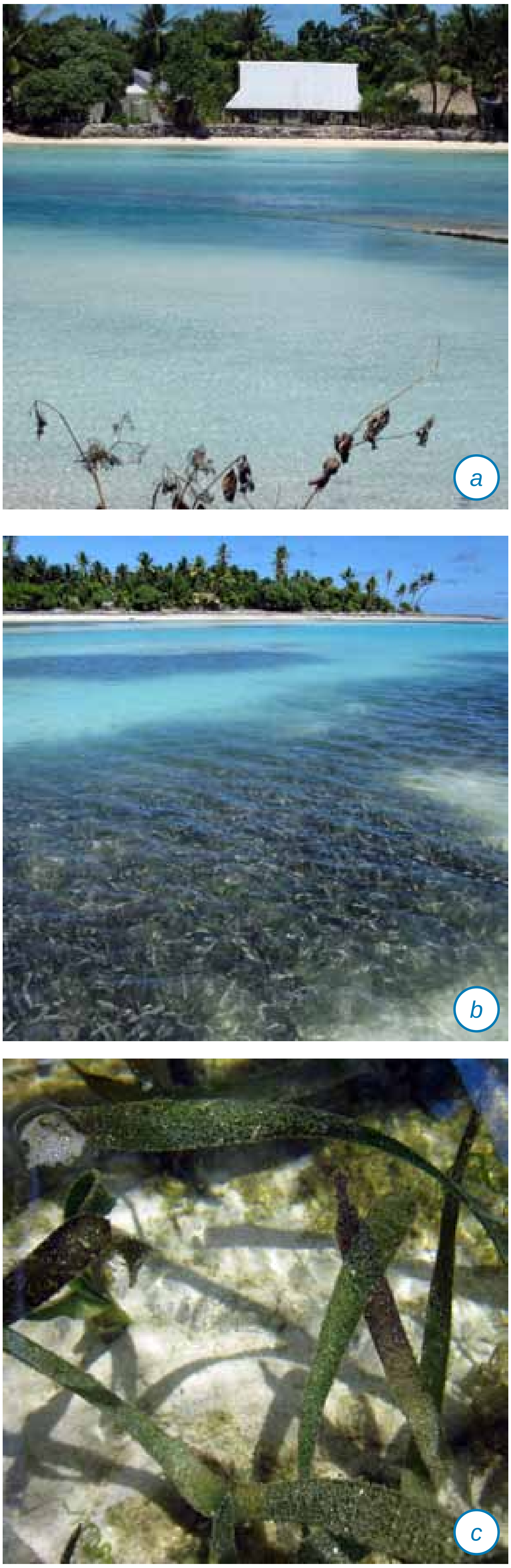


Figure 6. *Seagrass beds (a and b) and* Thallasira *sp. (c) found near Tanaea Hatchery.*

Building a habitat simulator for broodstock fattening and juvenile grow-out

The previous broodstock holding system needed to be improved from being a simple tank to a more complicated but self-sustainable and low-maintenance system. This would enable hatchery technicians to have more time for other duties instead of labour-intensive broodstock maintenance work. Thus, it was thought that a habitat simulator holding tank system would work best for broodstock fattening and juvenile grow-out, and this system has been used successfully by the College of Micronesia in Pohnpei (Federated States of Micronesia). A fisheries officer was assigned to help with the daily preparation of materials and tools as well as hands-on training of constructing the habitat simulator tank system (Fig. 7 a–f). This way, he will be able to make more habitat simulators in the future for juvenile grow-out and broodstock conditioning.

Figure 7. *Constructing a habitat simulator tank. Perforated piping and airlift stand pipeset on the tank floor (a); a bottom layer of coral stones (b); coral gravel for the second layer (c); fine sand as a top layer (d).*

Figure 7. *Tank is ready for seagrass to be planted (e); and conditioning the tank and seagrass using a recirculating seawater system (f).*

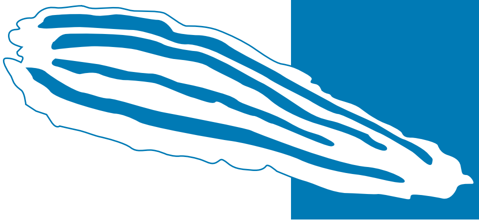
After the second spawning induction work on 15 October, all of the sandfish, including 31 spawners of 100 g BW or heavier, and 28 smaller animals were transferred to the habitat simulator (Fig. 8) for long-term, high-density fattening work.

The habitat simulator used a 2,500-L raceway tank (4.5 m x 0.8 m x 0.7 m); the surface area of the top layer of the sandy substrate was approximately 3.6 m^2 and the water volume was approximately 2,000 L. The water inlet (exchange) rate was 5–10% per day in order to stabilise fluctuations of pH and salinity from evaporation, rainfall or plants releasing carbon dioxide at night or oxygen at daytime into the system.

The top layer, which consisted of fine sand and mud sieved through a 1-mm mesh screen, was 10–15 cm deep so that sandfish broodstock were able to completely bury themselves. Water movement within the habitat simulator system consisted of a gentle downweller that recirculated water through the sand, coral gravel, coral stones and perforated PVC floor piping by airlift pumps. Onset of the stocking density of the sandfish here on 2 October was 16.4 animals (1,793 g) per m^2 (Fig. 9 a and b). The feeding regime was set to 1.5% BW per day per sandfish, the same as a temporary holding raceway tank.

Figure 8. *Stocking broodstock in the habitat simulator.*

Figure 9. *Sandfish broodstock in the habitat simulator. Sandfish on the top layer (a) and buried in the sand (b).*



Spawning strategies

Surprisingly, almost all of the knowledge and skills of the fisheries technicians regarding sea cucumber hatchery work (with white teatfish) conducted in 1996–2005 had been lost, together with a white teatfish hatchery manual. No one among the current fisheries staff knew anything about: a) the basic preparations and methods for spawning induction, b) sampling and counting eggs and larvae, c) feeding protocols, d) equipment and tool preparations, e) handling broodstock, or f) maintaining the hygiene of hatchery operations, particularly microalgal culture. In other words, all of the important aspects of operating a sea cucumber hatchery operation did not exist. In addition, for this first spawning attempt it was necessary to use whatever materials were available, some of which are not typically used for spawning induction work. Therefore, the strategies described here are applicable only to the Tanaea facility and its logistics, or to a similar situation elsewhere. The author accepted the current conditions for conducting this demonstration and did little to modify the hatchery facility in order to keep costs down. The spawning induction methods described here are basically the same as those used by the College of Micronesia Land Grant Program, which uses thermal shock (from cold to warm water conditions), followed by a treatment of dried microalgae (Algamac). All spawners are disinfected using iodine (100 ppm iodine bath for 1 minute) prior to transferring into a spawning tank. The seawater was filtered to 1 µm with a bag filter.

Preparation

No complicated equipment preparation is needed for spawning induction: water (which should be filtered through a 1-µm inline UV steriliser, if available); simple air filters, such as cotton-stuffed pipe and/or disposable syringe; thermometer; 500–1,000-L tanks for spawning and incubating eggs; 20–30-L plastic buckets for collecting eggs and making overflow buckets; 50 µm, 80 µm and 100 µm mesh sieves (plankton screen) for collecting and cleaning eggs (50–80 µm) and collecting larvae (80–100 µm); a small handmade plunger; micro-pipette (0.2–5.0 mL); rafter counting chamber; hand counter; Pasteur pipette for sampling and counting eggs; glass tubing; binocular microscope; and other basic laboratory equipment.

For spawning induction work, about 100 broodstock (spawners) are usually collected from the wild or from a pond or ocean enclosure, and fresh broodstock are collected for each spawning attempt. Sandfish do not occur in Tarawa except for the 59 specimens (imported from Fiji) kept alive at Tanaea Hatchery. Unless additional specimens are imported, these 59 are the only broodstock in Kiribati. Because there are so few of them, mortality is not allowed during and after induction treatments. Spawning methods should consider this special circumstance in Tarawa. Prior to spawning induction work, spawners should be removed from the broodstock holding or conditioning tank and kept out of the water during the cleaning process using a soft sponge. Furthermore, you should measure wet BW and then rinse spawners with 1 µm-filtered seawater and quickly transfer the spawners to a cold water tank in the algae room (at 18–21°C) or with ice packs. This cleaning and measuring process may take 30 minutes, and forms a part of the stimulation process (dry exposure and stress by handling). Hot seawater can be prepared by simply boiling seawater. Two, 10-L deep pans are enough to make 250–500 L of warm water at 32–34°C.

Males usually spawn before females, and the response time is usually 30–60 minutes. If many males respond and release sperm simultaneously, the spawning males should be removed from the tank before the water becomes too cloudy to see the females spawning. Avoid a polyspermic fertilisation (caused by too many sperm suspended around the eggs), although plural spermatozoa, or “sperm”, attachment improves the fertilisation rate. There is a marked variation in the number of spermatozoa to fertilise eggs successfully. The number of spermatozoa necessary to initiate the fertilisation impulse in an egg presumably correlates to the degree of maturity of particular eggs (Wada 1963b). A fully mature egg may be fertilised by a single spermatozoan, while an under-ripe egg probably requires more than one spermatozoa to become fertilised. Also, some spermatozoa make a functional attachment to an egg prior to fertilising one and these non-fertilising spermatozoa stimulate the egg (Wada 1963a, b). It is always a good idea to record information about the spawned individuals — noting the sex, size, date and time of spawning — for the next spawning programme or broodstock conditioning.

For spawning induction at Tanaea Hatchery, a variety of tanks were available, such as a 2,500-L raceway tank, a 500-L rectangular or round polyethylene tank, a 1,000-L polycarbonate tank, and either 1,000-L or 2,000-L round conical bottom, fiberglass tanks. A small 500-L rectangular tank was considered to be the best for this demonstration purpose (Fig. 10 a–c). This is because all of the broodstock were very small, and the majority were just over one year old, and even if they responded to the induction work, they may not have produced many eggs or may have been forced to release premature ova.
A very small-scale operation with simple materials, tools and strategies were thought to be sufficient for the current situation at Tanaea Hatchery, which can still conduct spawning runs without making modifications to the facility or without having to purchase large tools and equipment. Actual preparations and the making of tools and equipment necessary for conducting spawning-runs began about one week prior to spawning induction work. This also coincided with other necessary tasks (e.g. microalgae mass culture and settlement plate-tank conditioning).
Seawater was supplied from the existing system used for rearing giant clams, and was filtered to 1 µ by using filter bags. There were no appropriate sizes of PVC pipes, fittings and valves available, so only the materials on hand were used to roughly regulate the water flow, exchange rates and air supply. Also, no screen was available for making 50 µm, 80 µm and 100 µm sieves. Therefore, small 15 cm diameter and 10 cm deep sieves (measuring 60 µm and 100 µm) were made by the hatchery's technicians (Fig. 11) prior to spawning work.

Figure 10. *A 500-L rectangular tank used for spawning induction, incubating eggs and larval rearing. The tank on the left has a crack and leak on the bottom (a); one of the tanks is used as a tank base (c); and one tank is used as an egg incubator and for larval rearing (c).*

It should be noted that the above described materials and tools were used because none other were available. Sieves measuring 20–30 cm in diameter and 20–30 cm in depth are normally used to collect eggs, particularly fragile larvae, in order to not damage them as they pass through the sieve.

Figure 11. *Sieves on the overflow bucket for collecting eggs and larvae.*

Spawning induction methods

Spawning induction strategies in Tarawa are basically the same as those used elsewhere, and include the use of a thermal shock system combined with Algamac bath methods. The procedures of the first spawning trial (on 2 October), which used 30 spawners (average BW 140 g, minimum BW 100 g) and the second spawning trial (on 15 October), which used 31 spawners (average BW 130 g, minimum BW 100 g) are summarised below.

1. Boil seawater in a 5-L flask using an autoclave or an immersion heater, or in a large deep pan where the water is heated by a fire.
2. Measure BW and select spawners using at least those that are 100 g in the first trial. Return any that are less than 100 g to the holding tank.
3. Transfer spawners to a 100-L cold tank (21°C for trial #1 and 18°C for trial #2) and keep for three to four hours. Prepare cold seawater (1 µm filtered) beforehand the preceding day and place in the algae room (Fig. 12 a) and b).
4. Transfer spawners to an iodine bath for 1 minute at 100 ppm (3 g Betadine per 30 L freshwater). Betadine contains 1% weight/volume iodine.
5. Begin the spawning induction immediately after the iodine bath by transferring spawners into the spawning tank (500-L tank with 250 L of water) at 31–33°C.
6. Wait for males and females to spawn at least one hour, and continue to clean droppings on the tank floor by siphoning (Fig. 13 a and b).
7. If they do not respond, transfer the spawners to an Algamac bath (6 g/30 L filtered seawater) for 30 minutes (Fig. 14).
8. Quickly rinse off the Algamac, and return the spawners to the spawning tank.
9. Wait for males and females to spawn; males usually spawn before females release their eggs (Fig. 15 a) and b).
10. If female(s) release eggs, scoop the eggs up with a beaker or bucket; begin draining the tank and collecting eggs with a combination of 60-µ and 100-µ mesh sieves (Fig. 16 a) and b). If no female responds after two hours, return all spawners to the broodstock holding tank.
11. After washing and rinsing off the sperm for 10–20 minutes, transfer the eggs to a 20-L bucket (to make 15 L volume of 1-µm filtered seawater volume).
12. Take four samples of 1 mL volume (or two samples of 2 mL volume) using a pipette while plunging the sampling bucket.
13. Count the eggs under a microscope using a Rafter Counting Chamber, and compute the estimated total number of eggs; check the fertilisation by confirming the 1st polar body or more advance embryonic development such as 2-cell stage or 4-cell stage (Fig. 17 a–c).
14. Place the eggs in a 500-L incubator tank (with 250 L of 1-µm filtered seawater) (Fig. 18), and provide gentle aeration. The maximum stocking density of eggs can be up to 10–15 eggs mL-1.

It should be noted that it is better not to leave the spawners for more than four hours in the warm water from the onset to the termination of induction work described above. Terminate the spawning trial before killing the animals. If animals are left for more than four hours at 32–34°C, post-spawning mortality could be high. If the animals have not spawned within two or three hours, return them to ponds at the farm or to holding tanks.

Figure 12. *A 100-L tank in the algae culture room (a); and cold water temperature treatment (b).*

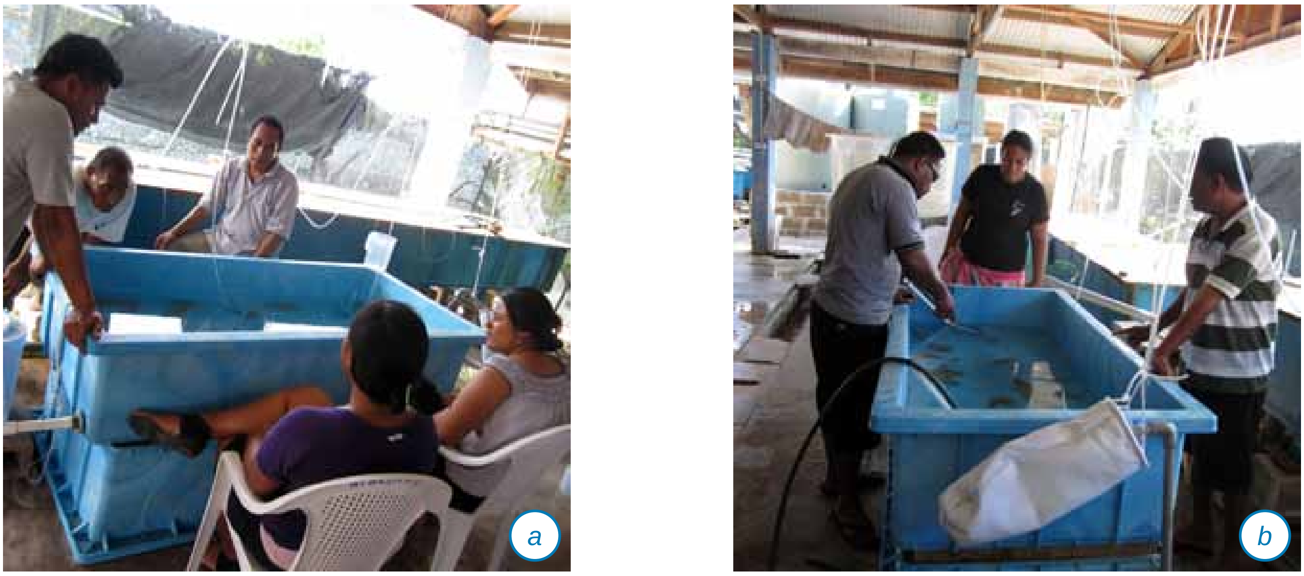


Figure 13. *Waiting for spawning (a) and keeping the tank floor clean (b).*

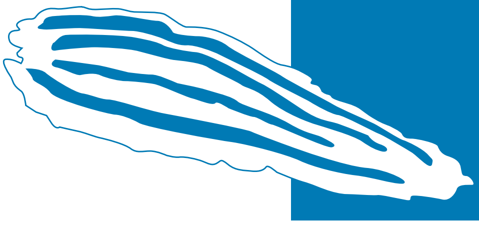
Figure 14. *Algamac bath treatment.*

Figure 15. *Spawning males (a) and females (b).*

Figure 16. *Collecting eggs by draining the spawning tank (a); the eggs scooped into a 2-L beaker (b).*

Figure 17. *Counting and observing eggs (a); 2-cell stage (b); 4-cell stage (c).*

Figure 18. *Incubator (i.e. a 500-L rectangular tank).*



Collecting, counting, measuring and incubating eggs

Spawning normally begins with the males releasing sperm. Immediately afterward (or within 30 minutes), the females release their eggs. Those males that have released their sperm should be removed from the induction tank before the tank becomes too cloudy to recognise others that are spawning. The males that are removed should be kept in a separate bucket or smaller tank in order to collect any excess sperm. Sperm samples should be taken to confirm that the sperm are active (motile) and viable. Before a female releases her eggs she stands up, and although nothing comes out from the hind end, the gonopore does become swollen. The female releases a spurt of eggs, which can be collected in a beaker or bucket. A female usually releases eggs only once, but some release eggs between two and four times in a given spawning session. If it was not possible to scoop up the eggs, the spawning tank can be slowly drained so that the eggs can be collected from the bottom of the tank, noting that sandfish eggs do not float but instead sink to the bottom. After determining whether the eggs have been fertilised, sieve the eggs with 100-µm mesh screen to remove debris, and then collect the eggs with 50-µm screen. Rinse the eggs with filtered seawater to wash out excess sperm. Transfer the eggs to 20-L bucket (with 15 L of water) so that samples can be taken to estimate the total number of eggs. The protocol for sampling, counting and computing eggs are as follows:

- Take 2-mL samples while gently plunging the bucket containing the eggs.
- Prepare four samples for counting.
- Use a Pasteur pipette and Rafter counting chamber for counting eggs.
- Estimate the total number of eggs. In formula for this, using a 15-L bucket, as an example is:

$$A/4 \times 15{,}000$$

$$\text{where } A = (A_1 + \text{……..} + Ai)$$

A = average of counted eggs in 2 mL samples
i = number of samples (here i = 4)

After counting, transfer the eggs into the incubator (500–1,000 L). The stocking density of fertilised eggs should be less than 15 eggs mL^{-1}. About 15–20 minutes after fertilisation, the first polar body can be seen under the microscope; followed by the second polar body in about 40 minutes; and 2-cell stage in 60 minutes; morula stage in 1–3 hours; and blastula and early auricularia in 16 hours. See Table 11 for a summary of the embryonic, larval and post-larval development of sandfish.

Microalgal culture

The basic design of a microalgae room should include a secondary seawater filter (1 µm and 0.2 µm) and an inline UV steriliser (40 W); a bench for chemical preparation, microscope work and a laminar flow chamber for inoculating algae; and three-stage culture benches for stock culture, high-density starter culture and mass culture. Room temperature and lighting should be maintained at 20–22°C by two small air conditioners (5,200 BTU each) and light–dark cycle of 14 L–10 D (14 hours light, 16 hours dark) for synchronous culture. The culture benches used in Kiribati accommodate a maximum of 16, 30-L polycarbonate tanks; 15, 3-L or 4-L flasks, 36, 250-mL flasks, and fitted with 39, 20-W fluorescent lighting tubes. Other equipment included: air pump (linear diaphragm, 124 W) and laboratory equipment such as a binocular microscope and electronic balance. An autoclave (46-L chamber, 30 cm diameter x 65 cm depth) and a small refrigerator (108 L) were placed outside the microalgae room.
A combination of two species of algae, *Cheatoceros mulleri* and TISO, are used at Tanaea Hatchery. The following microalgae (unicellular marine phytoplankton) are widely used for hatchery operations in tropical and subtropical environments: *C. mulleri*, *C. simplex*, *Pavlova lutheri*, *P. salina*, *Rhodomonas maculata*, *Tetraselmis suecica* and *Nannochloropsis oculata*. These master stock cultures were obtained from the Commonwealth Scientific and Industrial Research Organisation (CSIRO) in Australia, which

keeps microalgae species for aquaculture purpose under pure and bacteria-free conditions (see http://www.csiro.au/en/Organisation-Structure/National-Facilities/Australian-National-Algae-Culture-Collection.aspx.

Preparing and processing the culture was simplified but followed basic rules of hygiene that resulted in high-quality feeds by minimising time spent on producing microalgae. Hatchery technicians should follow these preparations and processes, count culture density daily and compute feeding amounts. The aim of this section is to offer practical knowledge on microalgae culture techniques used in sea cucumber hatchery operations in Kiribati. Some of the complicated biological and chemical procedures were skipped so that the fisheries technicians who do not have comprehensive knowledge of microalgae and microbiology could still conduct larval rearing work. Although only two species (*C. mulleri* and T-ISO) are kept and used in the algal room at Tanaea Hatchery, additional species are discussed in the following section.

Microalgae species and their selection for feeding

The nutritional aspects of microalgae in the mariculture of bivalves (mollusks) have been well documented (e.g. see Brown et al. 1989 for a literature review). Culturing microalgae to feed larval and post-larval stages of sandfish is the most important aspect of hatchery operations. Although many algal species have been used as food for aquaculture, they are not equally successful in supporting the growth of particular target species and stages of development. This relates to differences in size (cell volume), digestibility and nutritional value of microalgae. Nutritional value depends primarily on the biochemical composition of the algae and the specific nutritional requirements of the animal. The following live microalgal species are commonly used for rearing pearl oysters, finfish, shrimp and sea cucumber larvae and post-larvae:

Chaetoceros mulleri* and *C. simplex: Bacillariophyceae (diatom/brown algae) *C. mulleri* was formerly called as *C. gracilis*. *C. mulleri* and *C. calcitrans* have good nutritional value (Volkman et al. 1986 and have been used widely for shellfish hatcheries. Although they are not thermally tolerant, they grow without being adversely affected at around 25–28°C. These diatoms are always favoured as food sources in tropical and subtropical hatchery operations. If an air conditioned facility is available with room temperature ranging from 20°C to 24°C, *C. mulleri* and *C. calcitrans*, together with *Pavlova lutheri*, will be an ideal food source. For the hatchery in Pohnpei, Federated States of Micronesia, *C. simplex* has also been used. *C. simplex* was originally isolated from Okinawa in southern Japan (Ito, unpublished data) and Mie in central Japan. This species has been used as an alternative to *C. mulleri* because of its thermal tolerance and because of its good growth rate at temperatures of 28–30°C. However, the results of larval rearing were not as good as for *C. mulleri*. A further study could clarify this issue. *C. mulleri* has four spines and is immobile.

Pavlova lutheri* and *P. salina: Prymnesiophyceae (golden-brown algae) *P. lutheri* is a cold water species that was originally isolated from Finland. It has excellent nutritional composition, particularly Omega-3 (w3) HUFA (highly unsaturated fatty acids), that is, EPA-eicosapentaic acid (20:5w3) and DHA-docosahexaic acid (22:6w3). *P. lutheri* was formerly called as *Monochrysis lutheri* and has been used for pearl oyster hatchery and grow-out work in Japan since the 1950s. In the 1990s, *P. salina* was introduced to tropical and subtropical hatchery trials. It was originally isolated from the Sargasso Sea. Nutritionally, *P. salina* is similar to *P. lutheri*. According to E. Ponis and G. Parisi (2006), *P. salina* is a thermal tolerant tropical species and is expected to be a good food source for tropical hatchery operations, being an alternative to *P. lutheri*. Because *P. lutheri* was originally isolated from cold water (Finland), it requires an air conditioner in culture conditions. On the other hand, *P. salina* can be cultured outdoors or semi-outdoors of tropical conditions at around 28°C. However, care should be taken to culture *P. salina* because of its very slow initial growth (log phase) around 7–10 days to attain 3–6 x 10^6 cells mL^{-1}, with a short peak period after reaching stationary phase and 3–4 days to attain 6–8 x 10^6 cells mL^{-1} (Ito, unpublished data). *P. salina* is also less tolerant than *P. lutheri* to bacterial contamination. In other words, slower growth means that it will have more chances of contamination during the culture. It is also known that the dead cells of *Pavlova* release a toxin from which a high mortality of aquatic organisms' larvae was suspected in some cases using an excess of *P. lutheri* as a single food source (Brown et al. 1989).

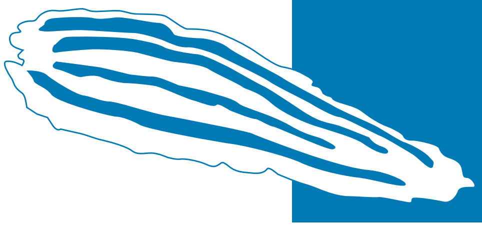
P. lutheri has three flagella ("tails") and rotates and swims in spiral movements. Therefore, it is necessary to kill or immobilise it with formalin (5–10% seawater formalin) in order to count the cells with the aid of a hemocytometer.

T-ISO (a Tahitian clone of *Isochrysis* sp.): The Tahitian clone of *Isochrysis* sp. (T-ISO), which was first isolated and identified in 1971 in Tahiti in French Polynesia, has been one of the best known microalga for use in tropical and subtropical aquaculture. T-ISO shows fast growth, 3–5 x 10^6 cells mL^{-1} after 3–5 days of inoculation from starter culture, and survives for long periods while maintaining high density during the stationary phase, 6–9 x 10^6 cells mL^{-1} for 7–10 days (Ito, unpublished data) under a relatively low light intensity, indicating that this species is tolerant of bacterial contamination. It is regarded as a suitable food for long-term larval rearing. Nutritionally it is good but lacks one of the w3-HUFA, particularly lacks 22:6w3 known as DHA, suggesting that it may be wise to avoid using this species as a single food source during larval rearing. Combinations of T-ISO and *Chaetoceros* spp. or *Pavlova* spp. have also been used successfully for pearl oyster hatchery operations in Australia, Indonesia and Japan. T-ISO also has three flagella and swims; therefore, it is necessary to kill or immobilise it with formalin (5–10% seawater formalin) in order to count the cells with the aid of a hemocytometer.

Rhodomonas maculata: Rhodophyceae (red algae) *R. maculata*, which was formerly called *Cryptomonas maculata*, has relatively high protein and lipid contents. This species swims actively with flagella. Because of the large cell size, which is 50–100 times larger than *P. lutheri*, the author includes *R. maculata* to the feeding schedule in pearl hatchery work when larvae become large enough (e.g. the eyed umbo veliger of pearl oyster). Elsewhere, *R. salina* is used in hatchery work with other animals, such as sea cucumbers. Similar to other flagellates, it swims rapidly and so it is necessary to kill or immobilise it with formalin (5–10% seawater formalin) in order to count the cells with the aid of a hemocytometer.

Nannochloropsis oculata: Formerly called "marine type *Chlorella*", this microalga was isolated from brackish river water in Japan. After numerous trials during the 1960s, this species was found to be the best food for shrimp and sea bream farming, mainly for growing rotifer, *Brachionus plicatilis*, which has been used widely in Japan since the 1960s. The rotifer was used for the primary larval diet for shrimp and marine finfish. *N. oculata* is high in w-3 HUFA and is highly tolerant to fluctuations of the culture environment — such as water temperature, salinity and light — and is the smaller than other dietary microalgae. Its cell wall, however, is harder than *Chaetoceros*, *Pavlova*, *Rhodomonas* or *Tetraselmis*, and the author avoids using it as a main food source for larval stages of pearl oysters and sea cucumbers.

Tetraselmis suesica: Prasinophyceae (green flagellate) such as *T. suesica* and *T. chiuii* are known to have a wide tolerance to temperature fluctuations and salinity ranges. It is believed to be nutritionally excellent, particularly for post-larval (spat) and broodstock culture. Because of the larger cell size compared with *Pavlova* spp. and *C. mulleri* (being 100–200 times larger than *P. lutheri*), *T. suesica* may not be suitable for feeding smaller larvae of pearl oysters. *T. suecica* has four (tetra) flagella but is immobile and does not swim actively; therefore, it is not necessary to use formalin to count them under microscope.



Culture techniques

The mono-specific culture (pure culture) of microalgae is a requirement for providing high quality food for larvae and post-larvae. Failure of the microalgal culture is mainly caused by human errors such as a lack of hygiene, careless handling and inappropriate equipment. A good sense of hygiene is of utmost importance to successful hatchery operations. The author usually uses combinations of four species for pearl oysters and three species (*C. mulleri*, *P. lutheri* and *R. maculata*) for sea cucumbers. The master stock cultures are usually obtained from the CSIRO (Table 3).

Table 3. *Master stock culture of microalgae.*

Scientific name	CSIRO*culture code	Source	Culture medium
(Bacillariophyceae)	CS-176	Hawaii, USA	f/2**
Chatoceros mulleri (Lemmermann)	CS-251	Mie Prefecture, Japan	f/2
Chaetoceros simplex (Ostenfeld)			
(Cryptophyceae)	CS-85	Dee Why, NSW, AUST	fE**
Rhodomonas maculata Butcher			
(Prasinophyceae)	CS-187	Brest, France	f/2*
Tetraselmis suesica (Kylin) Butcher			
(Prymnesiophyceae)	CS-177	Tahiti, French Polynesia	f/2
Isochrysis sp. (TISO)			
Pavlova salina (Droop) Green	CS-49	Finland	f/2
Pavlova lutheri (Droop) Green	CS-182	Sargasso Sea	f/2

**Commonwealth Scientific and Industrial Research Organisation, Australia.*
***See Guillard and Ryther (1962) for Medium f (f/2 and fE).*

The culture process follows basic rules of hygiene and feed production. During the hatchery run, all hatchery technicians are expected to perform these culture preparations and processes, count culture density daily, and compute feeding amounts. A schematic diagram of algal culture bench setting and a typical flow-chart of microalgal culture are shown in Figure 19.

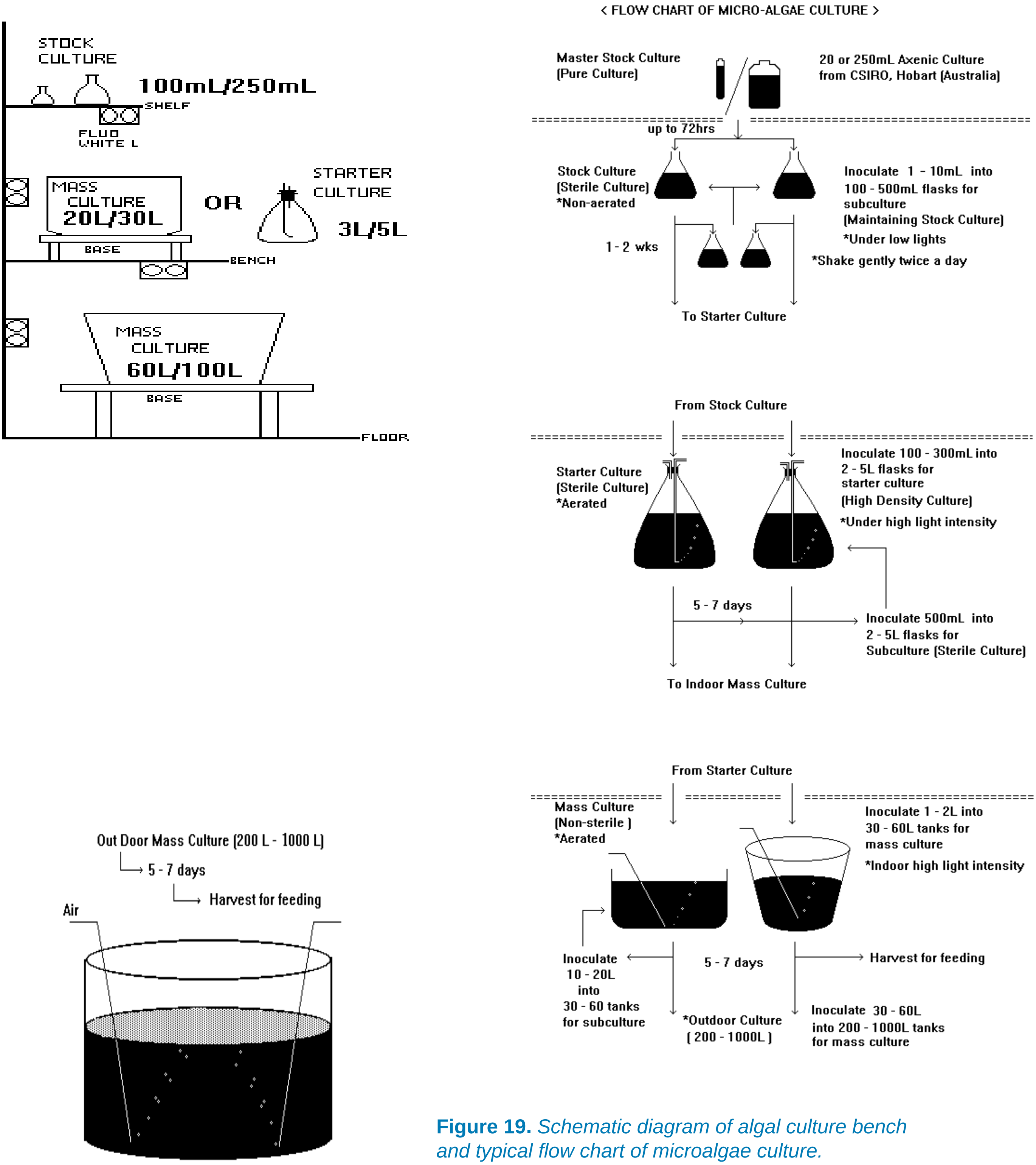


Figure 19. *Schematic diagram of algal culture bench and typical flow chart of microalgae culture.*

Stock culture (axenic non-aerated culture): 250-mL flasks are usually used for stock cultures (Fig. 20). To simplify the preparation, the flasks with nutrient media are autoclaved altogether as one unit for 15 minutes at 121°C. The autoclave sterilises the media and equipment at a high temperature (105–121°C) and under high pressure (~1.2 kg/cm^{2}G). Heated steam from the water reserve inside the autoclave's chamber kills germs. The nutrient medium in the flask should not be boiled because this will alter the chemical property and the nutritional value of the medium. This is why the chamber pressure needs to be higher than normal (one atmospheric) pressure so that the water does not boil, even at 100°C. The purpose of this procedure is to minimise the risk of contamination by potential pathogens and other algal species or other microorganisms in the flask before commencing the mono-specific cultures.

Figure 20. *Microalgae stock culture at Tanaea Hatchery.*

The subculture of stock culture is one of the most important procedures to keep algal species alive for long periods and, when required, it must be ready to commence a "starter" high density culture. Bacterial contamination often occurs during this stock culture procedure, causing heavy contamination in subsequent starter and mass cultures, which ruins the whole hatchery operation. Therefore, the inoculation for subculture should be conducted in a laminar flow chamber (Fig. 21) to minimise contamination by airborne pollutants. If this equipment is not available, inoculations can still be done in a simple box-shaped chamber with an open ceiling or a partitioned work bench using a Bunsen (gas) burner or an alcohol burner. Meticulous care is required for performing subcultures: surface-to-surface contact should be avoided when attempting the transfer of microalgae aseptically from one flask to another; touching sterile surfaces of a culture medium and equipment will lead to bacterial contamination.

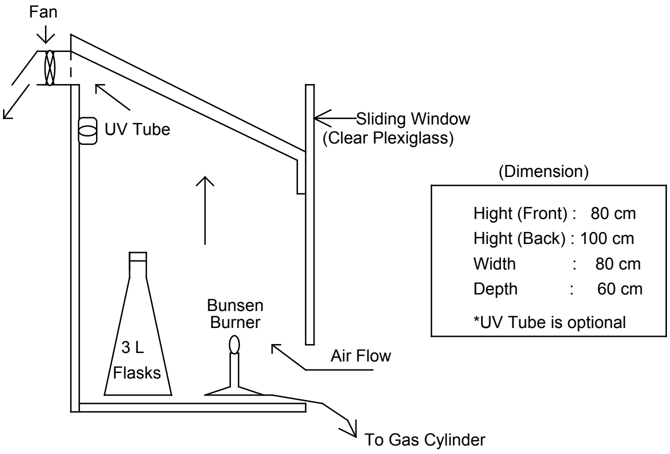


Figure 21. *Laminar flow chamber for inoculating stock culture and high-density culture.*

Stock cultures may be kept for two to four weeks, depending on the culture temperature and the algal species, under low light conditions (more specifically, about 50 µEm^{-2}S^{-1} or 500– 1,000 lux, and about 1 m from a single 40-W white-light fluorescent tube). They are then further inoculated (5–10 mL) into new culture media. All stock cultures are kept under non-aerated static conditions, although a gentle shaking is necessary once or twice a day to help the cells in suspension. It is essential to prepare at least one duplicate stock culture to avoid a shortage of algal stocks.

Starter culture (axenic aerated culture): After 1–2 weeks of culture, about 200 mL of the stock culture can be inoculated into the starter (high density) culture, normally 2.5 L or 3.5 L volume (Figs. 22 and 23). The flasks with nutrient media are autoclaved together as one unit for 45–60 minutes at 121°C. The aim is to obtain a high-density culture (e.g. 8–15 x 10^{6} cells mL^{-1}) under sterile (axenic) aerated conditions, and at higher irradiation (100–250 µEm^{-2}S^{-1} or more than 4,000 lux), and in front of two 40-W white-light fluorescent tubes.

Figure 22. *Starter culture at Tanaea Hatchery.*

A subculture for making an extra starter culture and/or a mass culture is preferred during the exponential phase, which may occur four to seven days after inoculation, because the young and viable algal cells are the ones most desirable for further cultures. The flasks with nutrient medium and all fittings should be sterilised by autoclaving before use.

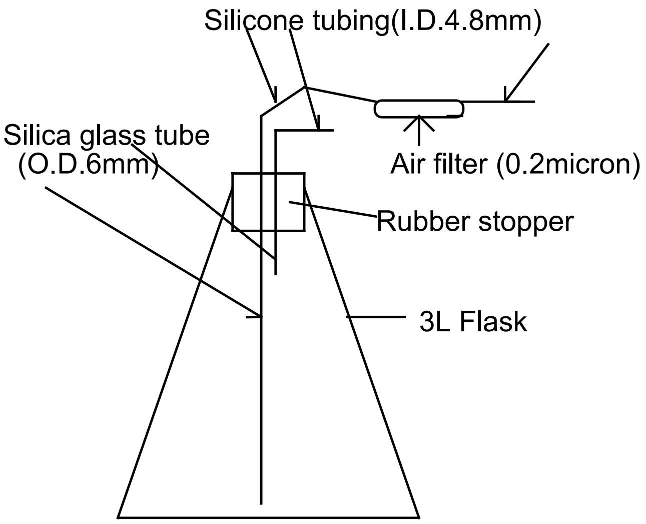


Figure 23. *Typical setting of flask (3–5 L) for high-density axenic culture.*

Mass culture (non-axenic aerated culture): Once a starter culture reaches an exponential phase of high density (6–10 x 10^6 cells mL^{-1}), it can be either subcultured to begin another starter culture or inoculated (1–2 L) into a new 20–30-L mass culture (Fig. 24). When the mass culture reaches a density greater than 2 x 10^6 cells mL^{-1}, it can be either harvested for feeding larvae or subcultured and/or inoculated into a large-scale mass culture (100–1,000 L). Attention should focus on minimising contamination although it is inevitable that bacterial contamination will occur during mass culture. When foam appears on the surface and/or the algae clumps and the water becomes cloudy, this indicates heavy contamination and the culture should be discarded. All the culture tanks, lids and aeration tubing must be washed with chlorine, rinsed thoroughly with freshwater (or rain water), and then sprayed with alcohol (75–80%) before use.

With mass culture tanks that are 300 L or larger, fresh water is used for the final rinsing after washing with chlorine. It is impossible to sterilise mass culture media by using an autoclave, and so sterilisation needs to be done by using chemicals (i.e. 15 ppm sodium hypochloride) followed by neutralisation with 45 ppm sodium thiosulfate (see Tables 4 and 5). If a 1-µm (nominal) filter is available, it is advisable to filter rainwater before use.

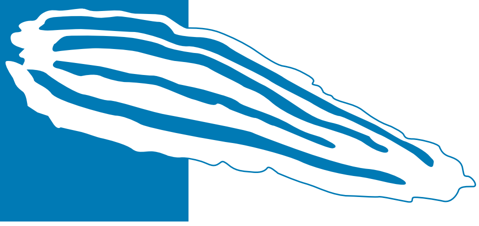
Figure 24. *Mass culture at Tanaea Hatchery.*

Table 4. *Concentration of sodium hypochloride for sterilising culture media.*

Concentration of NaHClO	Volume (mL) of 15 ppm sodium hypo chlorite (NaHClO) solution for culture tank									
(% w/v)	250 mL	1 L	2 L	3 L	10 L	30 L	100 L	200 L	500 L	1,000 L
2.5	0.15	0.6	1.2	1.8	6	18	60	120	300	600
3	0.13	0.5	1	1.5	5	15	50	100	250	500
3.3	0.11	0.45	0.91	1.36	4.55	13.64	45.45	90.91	227.27	454.55
3.5	0.11	0.43	0.86	1.29	4.29	12.86	42.86	85.71	214.29	428.57
4	0.09	0.38	0.75	1.13	3.75	11.25	37.5	75	187.5	375
4.5	0.08	0.33	0.67	1	3.33	10	33.33	66.67	166.67	333.33
5	0.08	0.3	0.6	0.9	3	9	30	60	150	300
5.25	0.07	0.29	0.57	0.86	2.86	8.57	28.57	57.14	142.86	285.71
5.5	0.07	0.27	0.55	0.82	2.73	8.18	27.27	54.55	136.36	272.73
6	0.06	0.25	0.5	0.75	2.5	7.5	25	50	125	250

Table 5. *Neutralisation of culture medium with 45 ppm sodium thiosulfate ($Na_2S_2O_3$-$5H_2O$).*
Prepare 45 gL^{-1} $Na_2S_2O_3$-$5H_2O$ working solution (= 45 ppm)

Culture volume 1000 mL	250 mL	1 L	2 L	3 L	10 L	30 L	100 L	200 L	500 L
Volume of working solution 1,000 (mL)	0.25	1	2	3	10	30	100	200	500



Nutrient media preparation

Guillard's F/2 medium[1] (Guillard and Ryther 1962) and modified-F medium are the most commonly used media for microalgal cultures in Oceania. However, these nutrient media involve some complicated preparation and storage, particularly the preparation of trace metal solutions and working solutions. A ready-to-use nutrient media and/or trace metal mix in powder or liquid form can be obtained commercially, most of which can be stored at room temperature for about a year, and have been used for aquaculture elsewhere. There are some problems, however, with their short shelf life (only one year), and so it is necessary to order and purchase them in small batches. This is, however, too expensive for use as a sustainable and long-term project in Kiribati, which lacks a proper industrial infrastructure, is geographically remote from industrial nations, and has a hot and humid climate. On the other hand, by purchasing original pure chemicals, you can make sufficient nutrient mixes without difficulty. Therefore, preparing a nutrient media from pure chemicals is an essential skill. Culture media are a limiting factor in a hatchery operation. At Tanaea Hatchery, a particular culture media has been in use since 1997, probably originating from the Japanese Overseas Fishery Cooperation Foundation white teatfish project (see Table 6). Hatchery staff can continue to use such media because stocks of pure chemicals are abundant. In order to reduce the nutrient preparation time, other culture media are listed in Tables 7 and 8 (i.e. MI-1 Medium and MI-G1 Medium with modified PII-metals, modifying from those well-known media such as F or PII-metals). Working solutions are made up in simplified ways in order to minimise handling mistakes by hatchery technicians.

Table 6. *Culture media currently being used at the hatchery in Tarawa.*

	Stock culture (mL) 200 mL	Starter culture (L) 1 L	Mass culture (L) 5 L	Mass culture (L) 30 L
Filtered seawater	160 mL	800 mL	4.5 L	
Working solution A	2 mL	10 mL	50 mL	
Working solution C	0.2 mL	1 mL	5 mL	100 mL
Working solution D	0.4 mL	2 mL	10 mL	100 mL
Working solution F*				100 mL

** F/2 medium is a half-strengh of F medium*

1. *Working solution A (main nutrients and trace metals):*

KNO3	14.4 g
KH2PO4	0.9 g
Na2-beta-glycerophosphate	2.1 g
Fe-EDTA	0.2 g
MnCl2	0.2 g
Tris	100 g
Clewat-32 (3)**	2 g

Add rainwater to make up a 1-L solution and adjust pH with H_2SO_4 (pH 8.0–8.2) and store in the refrigerator.

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[1] F/2 medium is a half-strength of F medium.

2. *Working solution C (vitamin mix):*

Vitamin B_1	0.2 g
Vitamin B_{12}	4 mg (take 4 mL from 0.1 g/100 mL original solution)
D-biotin	2 mg (take 2 mL from 0.1 g/100 mL original solution)

Add rainwater to make up a 1 L solution and store in the refrigerator.

3. *Working solution D (for diatom):*

2 mg (take 2 mL from 0.1 g/100 mL original solution)	30 g
L-cystine	1 g

Add rainwater to make up a 1 L solution and store in the refrigerator.

4. *Working solution F*

KNO_3	100 g
KH_2PO_4	15 g
Clewat-32 (2) *	20 g
Fe-EDTA	1 g

Add rainwater to make up a 1 L solution and store in the refrigerator.

* *Clewat-32 (2)^{TM} is the powdered trace metal mix and the commercial product of Teikoku Chemical Co. Ltd., Japan. The trace metal contents (per 1 kg) of Clewat-32 are as follows:*

3.8 g of $FeCl_3$-$6H_2O$	as Fe
7.7 g of $MnCl_2$-$4H_2O$	as Mn
1.6 g of $ZnCl_2$	as Zn
0.07 g of $CuSO_4$-$5H_2O$	as Cu
6.3 g of $(NH_4)_6Mo_7$	as Mo
24.7 g of H_3BO_3	as B
0.23 g of $CoCl_2$-$6H_2O$	as Co

***Clewat-32 (3)^{TM} is the powdered trace metal mix and the commercial product of Teikoku Chemical Co. Ltd., Japan. The trace metal contents (per 1 kg) of Clewat-32 are as follows:*

3.8 g of $FeCl_3$-$6H_2O$	as Fe
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1.6 g of $ZnCl_2$	as Zn
0.07 g of $CuSO_4$-$5H_2O$	as Cu
6.3 g of $(NH_4)_6Mo_7$	as Mo

Table 7. *Nutrient medium (MI-1) for microalgal culture.*

	Concentration (mg/L)	
$NaNO_3$	Stock/Starter culture (200-250 mL/2-4 L)	Mass culture (30-100 L/500-2,000 L)
Na_2HPO_4-$12H_2O$	100 mg/L	50 ml/L
$NaHCO_3$	14 mg/L	7 mg/L
Na_2-EDTA	12.6 mg/L	6.3 mg/L
Mod-PII metal mix*	18.1 mg/L	9.05 mg/L
(Clewat-32)	1mL/L	0.5 mg/L
Vitamin B_1	(500 mg/L)	(250–50 mg/L)
Vitamin B_{12}	0.1 mg/L (100 ug)	0.05–0.01 mg/L
D-biotin	0.0002 mg/L (0.2 ug)	0.0001–0.00002 mg/L
Na_2SiO_3-$9H_2O$**	15 mg/L	7.5 mg/L

** Mod-PII metal mix is an original trace metal mix developed by M. Ito, and modified from PII metals (Loeblich and Smith 1968).*

*** Sodium metasilicate ($Na_2SiO_3 9H_2O$) is required for diatom culture (e.g.* Chaetoceros *spp.).*

For making modified PII metals take 10mL each of original stock of:

- $FeCl_3$, $MnCl_2$, $ZnCl_2$, $CoCl_2$ and Na_2MoO_4,
- 1 mL of original stock of $CuSO_4$
- 30 g of H_3BO_3
- 11.9 g of Na_2EDTA
- Add distilled water to make 1 L working solution; store in the refrigerator

Table 8. *Medium MI-G1* for green algae (e.g. Nannochloropsis oculata)*

	Concentration (mg/1L)
$(NH4)_2\text{-}SO_4$	100 mg
$CaHPO_4$	15 mg
$(NH_2)_2CO$	10 mg
Mod-PII metals	0.06 mL**

**Green algae medium (MI-G1) is an original nutrient medium developed by M. Ito, modified from Hayashi & Seko (1986) where the trace metals were replaced with Mod-PII metals instead of using Clewat-32.*

***0.06 mL of Mod-PII metals contains:*

$FeCl_3\text{-}6H_2O$ – 15.6 µg;
$MnCl_2\text{-}4H_2O$ – 10.8 µg;
$ZnCl_2$ – 2.4 µg;
$CoCl_2\text{-}6H_2O$ – 1.2 µg;
$CuSO_4\text{-}5H_2O$ – 2.4 µg;
$Na_2MoO_4\text{-}2H_2O$ – 3.0 µg;
H_3BO_3 – 360 µg;
$Na_2\text{-}EDTA$ – 714 µg

Estimating culture density

For taking a microalgal sample, a Pasteur pipette (which must be sterilised by spraying alcohol on it before being placing it inside the culture tank) is used. Use a separate pipette for each species and tank. The sample can be kept in a small vial and then collected from the vial and placed on the counting chamber, or hemocytometer. Centre the cover-slip over the counting chamber. Place the tip of the pipette in one of the two grooves and release the sample from the pipette into the groove. It is important that no air bubbles form, otherwise the estimated count will be incorrect. Keep releasing the sample until both grooves have been filled and there are no air bubbles. Also be sure that there is not too much of the sample between the cover-slip and the counting chamber. The cover-slip should not be able to slide around. If the microscope does not have holding arms for the cover-slip, use your finger to apply pressure to the cover-slip while releasing the sample.

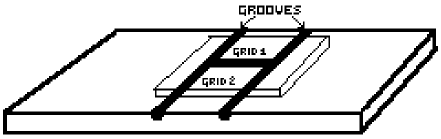


Figure 25. *A hemocytometer.*

As shown in Figure 25, the counting chamber consists of two grids on which the microalgae are to be counted. Use a low-magnification (40x) on the microscope to first find one of the grids. Then, use a higher magnification (100x) to focus on the center of the grid that has the highest number of boxes (i.e. 25 boxes, each one 0.2 mm x 0.2 mm, consisting of 16 small boxes each one 0.05 mm x 0.05 mm). Using a hand-held counter, count the number of microalgae within the center grid; that is, the large area (1) = 1 mm x 1 m, consisting of 400 small boxes (Fig. 26).

The gap between the cover-slip and the counting chamber is 0.1 mm (0.01 cm). Thus, the volume of three dimensional space of this large area (1) is: 1 mm x 1 mm x 0.1 mm = 0.1 mm^3 = 0.000 1cm^3 = 10^{-4} mL^{-1}. If a total of *A* cells are counted in the space over this area (1), the estimated number of cells per mL in the sample is *A* x 10^4. In order to obtain the algal density in the culture, get average counts from four replicates of grids (samples): (*A*1 + *A*2 + *A*3 + *A*4) x ¼ x 10^4 cells per mL. It is best to count in a pattern, either going up and down or across and back. If the microalgae are touching the border line of the box, it is to be included in a count. If the size of a cell body is very small such as green-algae (e.g. *Nannochloropsis oculata*) and it is only touching the outside of the border line, do not include in a count (Figs. 27 and 28).

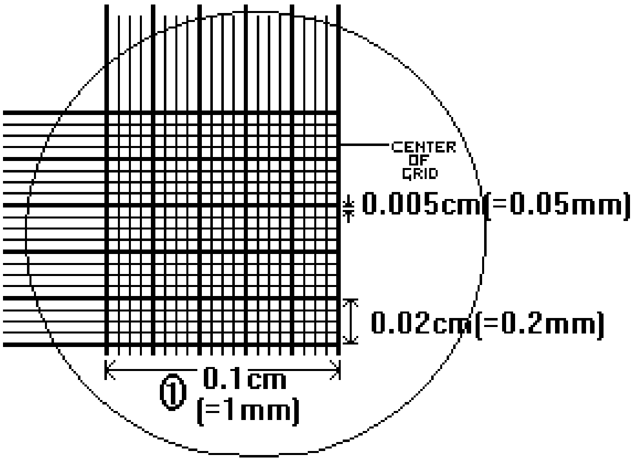


Figure 26. *Grid on a hemocytometer.*

Figure 27. *Looking for the grids on the hemocytometer.*

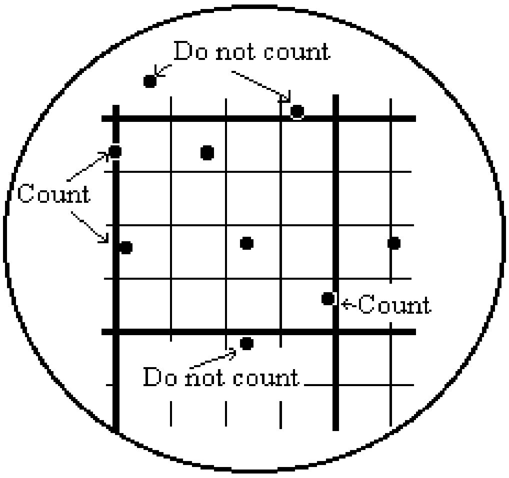
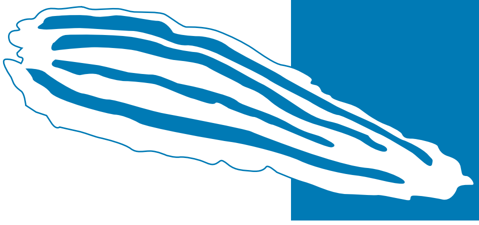


Figure 28. *A grid illustrating which cell bodies to count and not count.*



Calculating the amount of feed for larvae

The amount of feed (number of microalgal cells) is based on the feeding strategy of the sandfish larvae, which was provided in the feeding schedule (see Table 10) to maintain algal suspension (converted to *C. mulleri* cells per mL). For example, starting with 500 cells mL^{-1} on day 1, increasing to 5,000 cells mL^{-1} on day 2, and gradually increasing to 10,000 cells mL^{-1} on day 9, and then gradually decreasing to 500 cells mL^{-1} on day 14. The ratio of *C. mulleri* vs TISO is approximately 1:1.5. These feeding ratios are determined primarily by differences in cell volumes as well as considering their nutritional differences. The total daily feeding amount is divided into three; one for the morning feed, and two for the evening. To determine the daily feeding amount of each microalga (divided into morning and evening feeding), simply input the culture density of *C. mulleri* (e.g. 2.15 million cells mL^{-1}) and TISO, so that computer (MS Excel spreadsheet) calculates the amount (Figs. 29 a and b). It is also necessary to input some data such as the initial and ongoing number of larvae in each rearing tank.

It should be noted that all microalgal cultures must be done by "synchronous culture", a light–dark cycle-controlled algae culture where cell division occurs simultaneously. Therefore, the cells become a uniform size for each species (e.g. 12 L–12 D, 14 L–10 D or 15 L–9 D). It is very easy to make this synchronous condition by installing timer switches in fluorescent tubes in the microalgae culture room.

Formula:
{daily individual total feeding amount of *C. mulleri* (mL/larva) = the {algal suspension in the tank (cells/mL) / culture density of *C. mulleri* (cells/mL)} x {rearing tank water volume (mL)} / {number of larvae}.

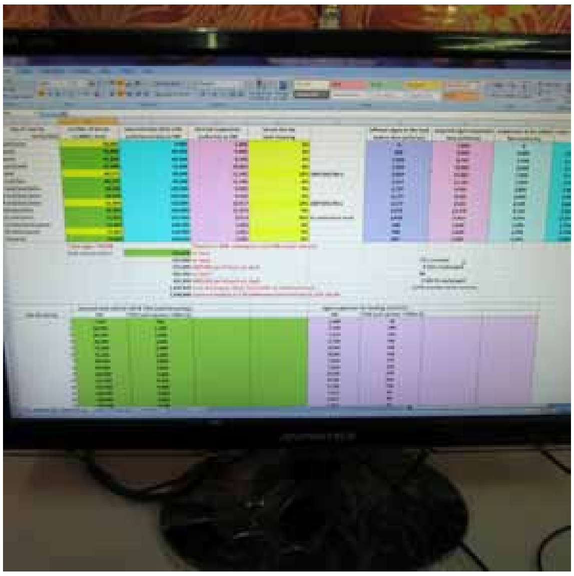


Figure 29. *Calculation of daily feeding amount using MS Excel spreadsheet.*

Maintaining microalgal cultures

Below are the basic procedures that are usually practiced at a commercial hatchery for preparing equipment and flasks, and culturing microalgae in aseptic conditions. Not all the materials and equipment listed are available at Tanaea Hatchery, such as alcohol and a UV steriliser. However, hatchery technicians must understand and practice these to maintain hygiene as much as possible for successful work.

Basic preparations and precautions for hatchery work.

1. Soak in freshwater and wash with detergent flasks to be used for microalgae culture, brushing off any excess dirt or other material. While it is not always necessary, hydrochloric acid (5–10% HCl solution) can be used to clean the bottom of flasks by soaking them in the solution when dirt is difficult to remove. Collect the used hydrochloric acid in a glass bottle for re-use.

2. Rinse with freshwater 5–10 times, completely washing off any residue from detergent or chemicals.

3. Dry flasks upside down so that airborne dust and dirt do not collect inside the flask.

4. Spray alcohol (ethanol or isopropyl-alcohol, 75–80% solution) on flasks and then rinse with distilled or filtered rainwater, and dry flasks upside down.

5. Put the lid (aluminum foil) on the flasks or place them in a dust-free cabinet for storage.

6. Rinse flasks with 0.2 µm (or 1 µm) filtered seawater and UV sterilised seawater before use.

7. Do not touch clean flasks or other equipment.

8. Wash your hands, paying particular attention to dirty finger nails and oily fingers, with soap and rinse off any soap or chemical residue. Spray hands with alcohol before commencing work.

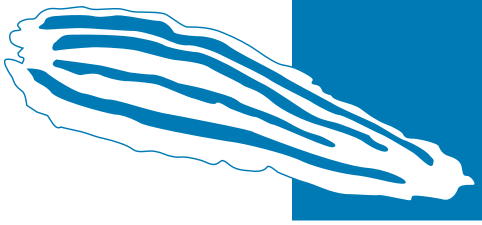
9. Spray alcohol on the surface of culture flasks, containers, fittings and work bench when entering the room.

10. Keep the floor and bench clean and dry and, if necessary, clean the floor with chlorinated freshwater.

11. Soak your feet in a chlorine bath before stepping into the room.

12. Periodically check and clean air filters and air outlets of air pump, air conditioner and ventilator.

13. Always keep the room's door and windows closed, and avoid unnecessary entry into the room.



Rearing larval sandfish

The success of the hatchery run depends on broodstock condition, equipment preparation for spawning induction, larval rearing, settlement and juvenile grow-out as well as microalgal culture for feeding larvae. Therefore, hatchery technicians must spend time learning basic laboratory practices, including wet-lab work and making equipment and tools for larval rearing. Most importantly, rigid protocols must be followed while working at the hatchery (see Tables 2 and 9). For example, 1,000–2,000-L polycarbonate, polyethylene or fiberglass tanks are commonly used for rearing larvae. Some basic rules for using those tanks include: 1) seawater temperatures must be in the range of 27–29°C and salinity should be 34–36 ppt; 2) seawater must be filtered to at least 1 µm and, if available (although not necessary), a UV steriliser (inline type) should be installed; 3) if tanks are place directly on the floor, the floor needs to have a 60-cm-deep drain groove or gutter for sieving and siphoning; 4) the ceiling of the larval rearing area should be covered to protect against contaminants such as falling dust; 5) all air tubing must be fed into the tanks from above to avoid direct contact with the floor; 6) equipment and tools should not be left on the floor; and 6) a sieve (plankton screen) is one of the most important items used during hatchery runs, so it must always be checked for any cuts, scratches or pin-holes.

Feed for sandfish larvae and juveniles combines microalgae and naturally occurring epiphytes. In Kiribati, a mixture of two to four microalgae species were used, with *C. mulleri* being the main diet, supplemented with two other diets of *P. lutheri* and *R. maculata, or T. suesica*. Average algal mix suspension was maintained, starting at 500 cell mL^{-1} on day 1, 5,000 on day 2, gradually increasing to 10,000 cell mL^{-1} on or around day 9, and then gradually reducing back to 500 on day 14. Subsequent to the first nursery culture, homogenised decaying seagrass (turtle grass) or seaweed (brown algae) are added weekly in amounts of 125–250 g per 1,000 L water. At the onset, the stocking density of the larvae is usually kept between 0.1 and 0.3 larvae mL^{-1}. Microalgae are only fed for the first two weeks and are then switched to naturally occurring epiphytes, seagrass extract, Algamac and/or fishmeal. Algamac is an artificial diet for larvae, juveniles and broodstock, and was developed by a US manufacturer (Aquafauna Bio-Marine, Inc.) for abalone hatchery production.

Larval rearing methods

After 6–14 hours of incubation, the larvae reach the gastrula stage of embryonic development in the incubator tank. The tank may then be drained to collect the gastrula, or the gastrula may be kept in the incubator until they develop into early auricularia larvae at round 16–20 hours after fertilisation. The gastrula and/or early auricularia larvae are collected with a combination of 80 µm and 100 µm mesh sieves. They can initially be stocked at 0.1–0.3 larvae mL^{-1}. The larval rearing method described here is based on a combination of “batch culture” (static or stagnant water in the rearing tank with occasional tank changes) and a partial or full-time flow-through (where tank water flows in and out continuously from the tank).

According to Ito (1995), growth (in terms of the length and width of larval stomachs) is an important indicator of larval quality. Ito found that a concentration of microalgae at a density of 5,000 cells mL^{-1} was adequate for the early larval development of *Stichopus japonicus*, and feeding was increased to around 30,000 cells mL^{-1} during the late larval stages. In the case of *Holothuria scabra*, the author usually increases the algal suspension density of microalgae to around 10,000 cells mL^{-1} during the late auricularia stage. During the batch culture — from day 1 to day 11 — larvae are fed a combination of *C. mulleri* and TISO twice daily, starting with 500 cells mL^{-1} on day 1, 5,000 mL^{-1} on day 2, and increasing gradually to 10,000 mL^{-1} on days 9 and 10, and then decreasing to 5,000 mL^{-1} and finally down to 500 cells mL^{-1}. Hatchery protocols, including a work plan for spawning, larval and post-larval rearing, and juvenile rearing (Fig. 30) are summarised in Table 10.

Table 10. *Work plan of hatchery operations in Tarawa, including water exchange rate, sieve mesh sizes, and algal suspension (cells mL^{-1}).*

Water exchange rate (%); sieve mesh size; development stage	Day of rearing	Algal cellsuspension density
Spawning: collect and wash eggs (50/80 µm); incubate for 14–18 hours	0	0
Begin larval rearing; gastrula and early auricularia	1	500
No water exchange	2	5,000
Tank bottom cleaning (or increase amount of water in tank by 30%); mid-auricularia stage	3	6,000
No water exchange	4	6,000
Tank water changed and drained (or water exchange 100% every four hours); (80/100 µm); mid- and late auricularia	5	7,000
No water exchange	6	7,000
Tank bottom cleaning (or increase water volume by 30%)	7	8,000
No water exchange	8	8,000
Tank change and draining (or water exchange 100% every four hours); (80/100 µm); late auricularia and doliolaria	9	10,000
No water exchange	10	10,000
Tank draining and collecting auricularia, doliolaria and pentactula (80/100 µm); transfer to settlement tank for grow-out (1st nursery culture)	11	5,000
No water exchange	12	5,000
Increase water volume by 30% (or start flow-through water exchange 100% every 24 hours); (80/100 µm)	13	500
End of feeding with microalgae	14	500
Start full-time flow-through 100% every 24 hours; (100 µm); if needed, give additional food (seaweed or seagrass extract 125 g + Algamac 0.5 g)	15	
	...	
Remove sieve	20	
	21	
Give additional food (seaweed or seagrass extract 250 g + Algamac 1 g) if epiphytes are not growing well.	22	
	...	
Give additional food (seaweed or seagrass extract 250 g + Algamac 1 g), if epiphytes are not growing well.	29	
Detach and collect juveniles (5–10 mm, 0.3 g); transfer juveniles to hapa or habitat simulator for grow-out (2nd nursery culture)	30	

Seawater was filtered to 1 µm for larval rearing. Although the combination of sieves used is usually 50 µm, 80 µm and 100 µm, in this case, only 60-µm and 100-µm sieves were used in trial #1 (from 2 October) and a 90-µm sieve was used in trial #2 (from 15 October). On October 2, one female spawned only about 50,000 eggs and, therefore, only a small rectangular tank (500 L) was used for larval rearing followed and egg incubation. The eggs were collected with a 60-µm mesh sieve, while larvae were collected using either 90-µm or 100-µm sieve. Seawater was filtered to 1 µm through a bag filter and gentle aeration was given throughout larval rearing. On day 1 of larval rearing, the initial water volume

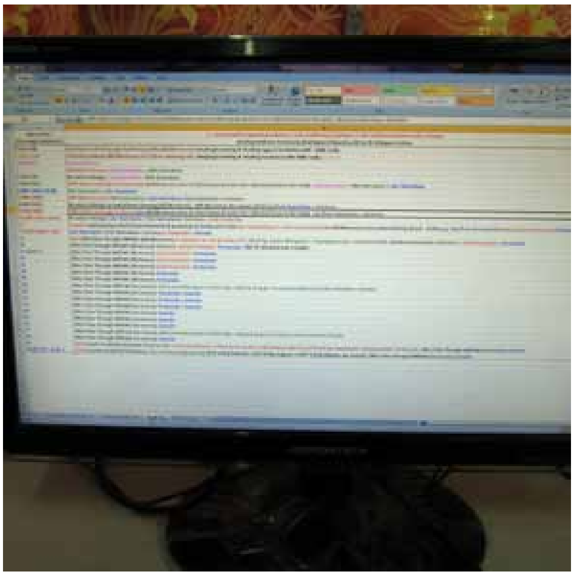


Figure 30. *A work plan (using a MS Excel spreadsheet) that covers spawning induction to larval and post-larval rearing and juvenile culture up to 30 daysafter spawning.*

was set at 250 L and was increased approximated 30% every two days except for on days 5 and 9, when 100% water exchange was conducted for four hours. On day 11, the tank was drained, and all swimming stages and settled pentactula were collected (Fig. 31 a and b) so as to commence settlement (the first nursery culture of juveniles). On October 15, the second spawning induction was conducted successfully; one male and two females spawned and approximately 225,000 eggs were obtained. One 2,500 L raceway (Fig. 32) was used for larval rearing from 16 October on day 1. Larval rearing protocols were simplified in order to suit the logistics of Tanaea Hatchery: tank draining and tank change did not take place (because only one 500 L rectangular tank was available) on days 5 and 9; no tank floor cleaning by siphoning occurred because only a few hundred or thousand larvae were estimated to be in the tank; the estimated number of larvae on day 5 was 3,000, and by day 11 there were only a few hundred to nil, no animals was found in the 15 L sampling bucket. On 16 October (day 1 of trial #2), the estimated number of gastrula were 45,000 (estimated by counting 18 animals per 4 mL sample from a 10-L bucket).

Feeding done twice daily: once in the morning when the hatchery officer arrived between 08:00 and 09:00, and again in the late afternoon before the hatchery personnel left at 16:15. Three microalgal species are available in stock culture at Tanaea Hatchery: *Chaetoceros mulleri*, TISO and *Nannochloropsis oculata*. Two of these species, *C. mulleri* and TISO, were used for feeding larvae. The proportion of *C.mulleri* and TISO was 0.8 vs 0.2 as cell volume. A simplified daily feeding table (Fig. 33 a) and daily work schedule for larval and juvenile rearing (Fig. 33 b) were given to hatchery staff to manage the daily microalgae culture density for each species. Feeding amounts were low, between approximately 5,000 cells mL^{-1} and 10,000 cells mL^{-1}, which was converted and expressed as *C. mulleri* cells. Hatchery staff took algal samples and counted the culture density, and then entered this information into the computer, which automatically calculated the daily (morning and afternoon) feed amount for each species. For example, {daily individual total feeding amount of TISO (mL/larva) x culture density of TISO (cells/mL)} = {required algae suspension in the rearing tank (cells/mL) x rearing tank water volume (mL)}/ number of larvae.

Figure 31. *Draining the larval rearing tank and collecting swimming and settled stages of larvae.*

Figure 32. *Larval rearing tank (2,500-L raceway).*

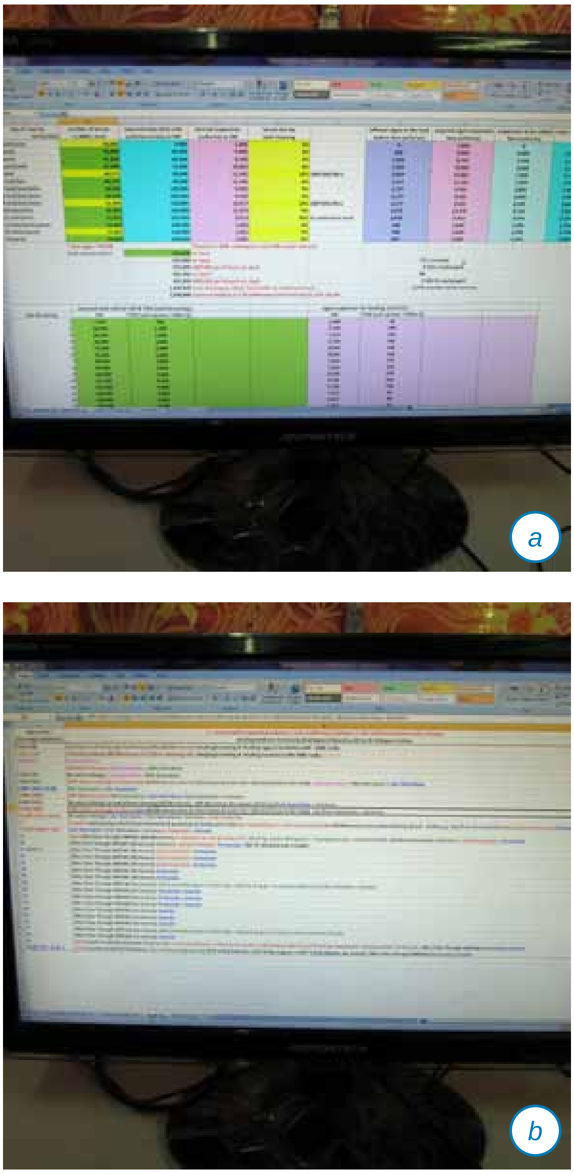


Figure 33. *Feeding table for sandfish larvae (a) and daily work plan for spawning, larval rearing and juvenile grow-out (b).*

Collecting, counting, measuring and stocking larvae

The collection and selection of larvae are done by using a combination of 80-µm and 100-µm sieves. Methods for sampling, and estimating and measuring the number of eggs or larvae were basically the same: take four 2-mL samples from a 15-L bucket with a micro-pipettor (increments of 20 µm up to 5 mL), counting under an adjustable microscope using a Sedgwick Rafter Graticules Counting Chamber. An ocular micrometer of the microscope must be calibrated with a stage micrometer before first use in hatchery work. An eye piece unit (EPU) of the ocular micrometer does not correspond to actual length under a particular microscope. An ocular micrometer is calibrated by a stage micrometer. If 1 EPU (1/100th of total unit length) under a magnification of 100 is 9.96, the 1 EPU is 9.96 µm. Measurements of larvae include body length the longest antero-posterior length, and body width the widest from right to left. When conducting measurements, measure at least 30 specimens to carry out statistical analyses later.

Larval development

On day 5 of larval rearing during trial #1, mid- and late-stage auricularia larvae were found: mid-auricularia were 600 µm and late auricularia were 700 µm in body length (Fig. 34 a and b). On day 7, the late auricularia stage (with more hyaline spheres) was found in the sample, and on day 11, late stage metamorphosing (auricularia folding the appendages) was observed. The developmental stages of sandfish larvae were taught to hatchery staff by using live and preserved specimens or from previous publications by others. The estimated number of swimming stages — including larvae and post-larvae — were less than a couple of hundred. Embryonic, larval and post-larval development of sandfish — including size ranges and durations — are summarised in Table 11 (note that most of the data were obtained by the author during his work in Pohnpei, Federated States of Micronesia).

Figure 34. *Mid-auricularia stage on day 5 (7 October 2013) (a), and late auricularia stage, also on day 5 (b).*

Table 11. *Summary of embryonic, larval and post-larval development stages of sandfish.*

Development stage	size (in µm)	first seen – last found
Fertilised egg diameter	150–160	
1st polar body		15–30 minutes
2nd polar body		30–40 minutes
Blastula		3–4 hours
Gastrula	300– 420	6–14 hours
Development stage	size (in µm)	first seen – last found
Auricularia early stage	430–570	16 hours – day 4
Auricularia mid-stage	600–800	day 3–8
Auricularia late stage	700–1,200	day 5–13
Doliolaria	440–670	day 7–14
Pentactula	420–1,000	day 9–15
Juvenile — one month old	3–4 mm	
Juvenile — two months old	10–40 mm	

On day 11 (or day 13), the larval tank is drained in order to collect both swimming stages (late auricularia, doliolaria) and settled stages (pentactula and juveniles). All collected larvae and post-larvae, including juveniles, are transferred to the settlement tank. Sampling and counting are only done for swimming stages. As described in the following section, settlement during the pentactula stage is on a hard substrate (e.g. corrugated plastic plates and raceway tank walls and floor). Therefore, it is essential to provide food on hard substrates. Culturing naturally occurring epiphytes (unknown mixed diatoms and other algae) or attaching/chain diatom (such as *Navicula* sp. and *Skeletonema costatum*) have been conducted elsewhere. A thick solution of Algamac is also used by painting the surface of plastic substrates and keeping them dry overnight, and submerging them into the settlement tank the following day. At Tanaea Hatchery, naturally occurring epiphytes were used as a food for pentactula and juvenile stages.

Conditioning of settlement plates and tank

When the hatchery was initially inspected, many used plastic corrugated plates (used for abalone and trochus settlement) together with the plate holders were found piled outside the raceway tank areas. Because most of the plate holders were heavily damaged and rusted, only 23 holders were recovered. Approximately 400 plates were recovered and washed, disinfected with chlorine, and then dried for several days (Fig. 35 a). One of the raceway tanks (2,500 L) was designated as a settlement tank, so conditioning of the settlement tank and plates started a week prior to the spawning induction trial (Fig. 35 b and c). To help grow naturally occurring epiphytes and diatoms on the plates, seawater was filtered through a 65 µm mesh screen and additional nutrient media (Table 12) were used for boosting the growth of natural epiphytes, particularly naturally occurring diatoms, in the settlement tank.

Figure 35. *Preparation of settlement tank and plates (a); after conditioning for one week (b); and growing naturally occurring epiphytes two weeks after conditioning (c).*

The nutrient media consisted of:

- 30 g sodium meta-silicate
- 100 g potassium nitrate
- 14 g disodium beta-phosphate
- 20 mL vitamin mix
- 0.4 g Fe-EDTA
- 4 g Clewat-32

If epiphytes are not growing well by days 13–15, provide a supplemental feed mix of seaweed extract 125 g and Algamac 0.5 g weekly until around day 30. In situations where no preparation was made to grow epiphytes for the first nursery culture, it is possible to spray or paint a thick solution of Algamac (10–30 g L^{-1}) on settlement plates (both sides) and keep dry overnight before transferring animals for settlement and the first nursery culture.

Table 12. *Nutrient medium used for conditioning settlement plates and tank at Tanaea Hatchery.*
Culture volume = 2,000 L in raceway tank*

KNO_3	100 g
Na_2-beta-glycerophosphate	14 g
Fe-EDTA	0.4 g
Clewat-32	4 g
Vitamin mix Solution C	20 mL
Na_2SiO_3-$9H_2O$	30 g

**Conditioning of the settlement plates and tank should be done one week prior to spawning induction.*

Transferring larvae and post-larvae from larval rearing tank to settlement tank

The majorities of swimming stages are usually occupied by doliolaria and fully developed late auricularia on day 11 (Fig. 36). In order to demonstrate the first nursery culture for pentactula and juveniles, the tank was drained to collect all the animals onto a 100 µm sieve. Collected specimens were scattered throughout the settlement tank in the under-cover area of the hatchery (Fig. 37 a).

Figure 36. *Collecting the swimming stages (late auricularia and doliolaria) and pentactula of sandfish.*

Juvenile rearing

According to Agudo (2007), the nursery phase or juvenile grow-out phase is divided into two phases. In the first phase, juveniles range in size from 5 mm at 25–35 days to 10–20 mm at 55–65 days. In the second nursery culture phase, juveniles grow from 20 mm or 1 g to larger sizes. The pentactula and juveniles that settle into the settlement tank will be cultured (the first nursery culture) without transferring them to other tanks. When transferring to an ocean nursery, juveniles that are around 5 mm (at onset of the first nursery phase) need to be protected by net pens and bag nets. Juveniles larger than 1 g (> 20 mm) can be released directly into the natural environment or earthen ponds. In addition, sea pens with 3–10 mm mesh may be needed to grow juveniles in an ocean enclosure system for larger sizes (e.g. 50 mm).

Rearing pentactula and early juveniles in the settlement tank (first nursery culture)

All of the settlement plates (345 plates in 23 holders) were covered with brown-coloured (natural diatoms) epiphytes (Fig. 37 b). If too much sunlight penetrates into the settlement tank, green seaweed (i.e. *Enteromorpha* sp.) may become dominant before diatoms cover the substrates. Therefore, covering with shade screen is required for cutting out sunlight. Because swimming and feeding stages of auricularia larvae may still be present in the settlement tank, it is necessary to continue feeding with microalgae (*C. mulleri* and TISO) until around day 14. Thus, the larval rearing continues in a static water condition of which the seawater should be filtered to 1 µm (Fig. 37 c). On day 13, water in the settlement tank needs to be increased by at least 30% of the water volume from day 11, and from day 15 the water can continuously flow at a 100% daily water exchange rate. If any swimming stages are present in the settlement tank, it is wise to use a combination of 80-µm and 100-µm sieves to collect the larvae, which should then be returned to the tank. The first nursery culture usually continues for a month or around day 30 when juveniles reach 5–10 mm or 0.3–0.5 g.

Figure 37. *Transferring swimming and settled stages into the settlement tank on day 11 of larval rearing (a); first nursery culture in the settlement tank (b and c).*

Culturing juveniles in the habitat simulator or hapa (second nursery culture)

Around day 30 of the hatchery run, juveniles should be 5–10 mm, or 0.3 g or larger. Therefore, it is wise to transfer them to another culture stage, the second nursery culture. Considerable information is available on juvenile grow-out and the second nursery culture (see the References section of this manual). The author had intended to use a land-based tank culture system (habitat simulator) for the second nursery culture, as well as for broodstock conditioning. Although many other places use hapas, or floating net cages, within ocean enclosures or earthen ponds as a standard method, a habitat simulator has some advantages over hapas, such as easy access to juveniles, faster maintenance work with less logistics, and fewer predators in the tank. Furthermore, about 10 mm (or 1 g) juveniles are usually stocked in a hapa at 400–500 individuals m^{-2}. In a habitat simulator using a 2,500-L raceway tank with floor area of approximately 3.6 m^2 (Fig. 38), onset stocking density of juveniles at the second nursery culture was a couple of thousand up to 10,000 (or 1,000–3,000 juveniles m^{-2}).

During the second nursery culture, it may be necessary to feed juveniles (about 10 mm, or 0.3–1 g) as much as 284–945 g of seaweed and/or seagrass, and 32–105 g Algamac ProteinPlus in a 2,500-L habitat simulator. After five months, these juveniles may reach 50 mm (10 g or larger). If a total of 5,000 juveniles are kept in the habitat simulator towards the fifth month of cultivation, 675 g seaweed and/or seagrass and 75 g Algamac ProteinPlus will be needed daily (or 4,725 g of seagrass and 525 g Algamac ProteinPlus weekly).

- 5,000 juveniles with an average BW of 10 g = total BW of 50,000 g;
- 1.5% BW = 10 x 0.015 x 5,000 = total 750 g of food daily.

At Tanaea Hatchery, feeding of juveniles and/or young adults in the habitat simulator tank consists of a daily feeding at 1.5% BW, which includes fishmeal at 45 g (0.675% BW), plus seagrass or seaweed at 45 g (0.675% BW), plus Algamac ProteinPlus 10 g (0.15% BW). Therefore, 5,000 juveniles with an average BW of 10 g require 337.5 g of fishmeal and 337.5 g of seagrass, plus 75 g of Algamac[2] daily; the weekly amount is 2,362.5 g each of fishmeal and seagrass, plus 525 g of Algamac.

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[2] Algamac™ and Algamac Protein Plus™ are live algae replacement and commercial products of Aquafauna Bio-Marine, Inc., USA.

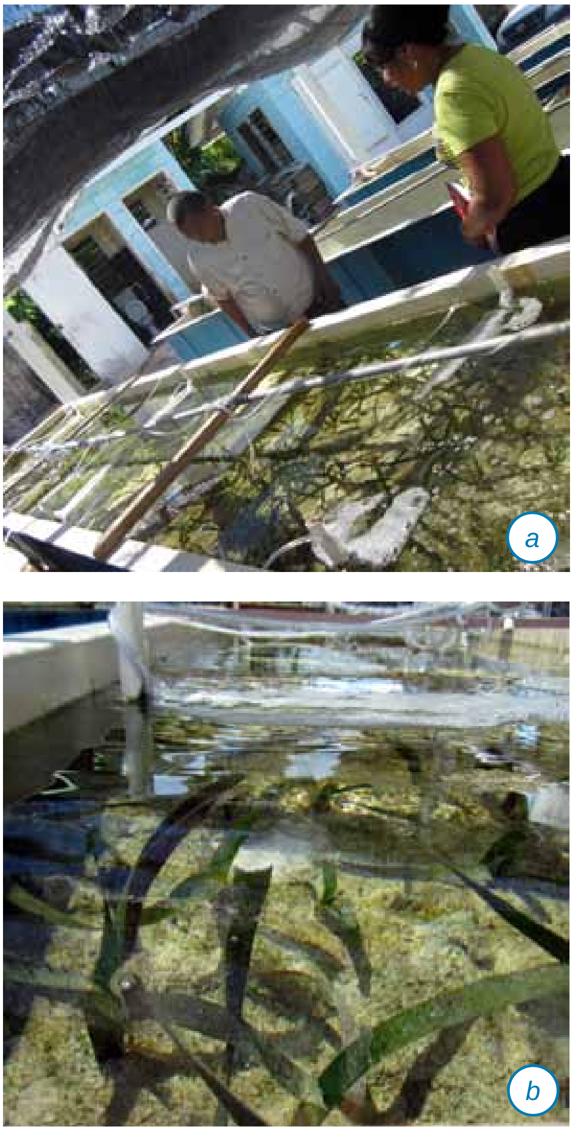
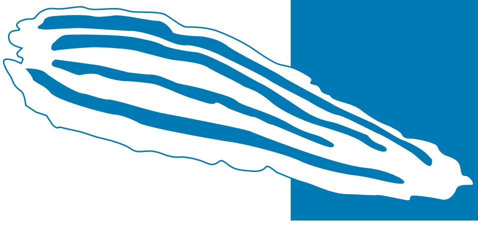


Figure 38. *Habitat simulator tank for the second nursery culture.*

Turtle grass, *Thalassira* sp., is the dominant seagrass in front of Tanaea Hatchery (Fig. 39 a and b). This is very fortunate for the hatchery staff as it can be used in experimental hapas in the Tanaea channel because sandfish in Pohnpei are abundant between the seagrass bed and the mangrove shore in the tidal flat zone. During this mission, the author found many juveniles of different species, probably *Bohadschia* sp., buried in the sandy bottom of the seagrass beds in Tanaea channel. These seagrass beds may be suitable for raising sandfish juveniles in enclosures. Caution must be taken when such enclosures or hapas are built because there are many starfish (brittle starfish) in the same shallow area of the Tanaea channel (Fig. 39 c) that may be one of the major predators, together with crabs, hermit crabs and carnivorous snails.

Figure 39. *Juvenile grow-out site with the seagrass bed for hapa and enclosure (second nursery culture). Site in front of Tanaea Hatchery (a); seagrass* Thalassira *sp. (b); potential predator, brittle starfish (c).*



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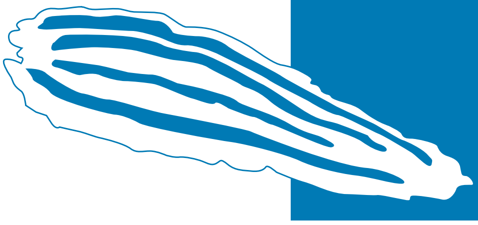

Glossary

APL:	antero-posterior length
aseptic:	clean, without contamination
auricularia:	larval stage of the sea cucumbers (early, mid and late stages, swimming filter feeder)
autoclave:	pressurised high-temperature stream steriliser
axenic:	sterile, bacteria-free, pure culture condition
blastula:	embryonic stage before gastrula stage
broodstock:	parent animals for spawning; spawners
BW:	body weight (wet)
detritus:	small pieces of dead and decomposing plants and animals, detached and broken down fragments of a structure
doliolaria:	transitional stage between auricularia and pentactula (swimming, non-feeding)
ft:	feet, 1 ft = 0.305 m = 30.5 cm
gametes:	eggs (ova) and sperm (spermatozoa)
gastrula:	final embryonic stage of the sea cucumbers (non-feeding, swimming)
hapa:	a cage used to rear juvenile sandfish
hermaphrodite:	both sexes (bisexual), genderless (e.g. the giant clam has both sexes within one clam)
inch:	1 inch = 2.54 cm = 25.4 mm
kg:	kilo gram, 1 kg = 1,000 grams = 2.2 pounds
$kg\ cm^{-2}$ G:	unit of atmospheric pressure
lb(s):	pound(s), 1lb = 453 grams
lux:	old unit of light intensity
µm:	micron meter = 1/1,000,000th of meter
mile:	1 mile = 1.609 km = 1609 m
mL:	milliliter (1/1,000th litre)
morula:	embryonic stage before blastula stage
pentactula:	transitional stage between doliolaria and juvenile (crawling, feeding epiphytes)
ppm:	part per million (1/1,000,000)
ppt:	part per thousand (1/1,000)
sandfish:	*Holothuria scabra*
synchronous culture:	light–dark cycle controls algae culture where the cell division occurs simultaneously
turtle grass:	*Thallasira* spp.
$\mu Em^{-2}S^{-1}$:	micro Einstein per square meter per second; unit of irradiation (light intensity based on the number of photons hitting the given area).