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GIANT CLAM PRODUCTION IN THE REPUBLIC OF THE MARSHALL ISLANDS: A CONDENSED GUIDELINE

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1. Introduction

Many of the Pacific Island countries and territories (PICTs) have cultured giant clams by using varying methods that are particularly designed to suit individual country environmental conditions and to support the operation of hatcheries. This guidebook aims to provide basic information on culture techniques that are essential to giant clam mariculture. It is not the intention of this manual to provide culture methods in detail. It will only provide essential information, since giant clam culture manuals are available through many sources.

In the Republic of the Marshall Islands (RMI), two hatcheries are government operated – namely Arno and Woja hatcheries. These hatcheries have been practicing extensive culture methods. This manual attempts to introduce the semi-intensive method as an alternative in order to improve the production level at all stages of giant clam culture, from hatchery through to the land-based nursery and oceanic nursery.

A popular giant clam species that attracts the attention of hatcheries is the iridescent *Tridacna maxima*, which is cultured for the purpose of supplying the international ornamental trade market. Hatchery managers need to take extra care not to confuse *T. maxima* with *T. noae*, when conducting a hatchery run, since hybrids of these two species are not as viable as pure breeds.

Recommended literature on giant clam culture can be found in manuals such as that of Heslinga et al. (1990), Braley (1992), Calumpong (1992) and ACIAR monograms. Much of the information contained within this manual has been derived from these publications. While this is so, improved protocols from the above authors on giant clam aquaculture are included in the relevant sections of this manual.

2. Giant clams

Giant clam exploitation in PICTs – including RMI – can be traced back many years. Many of the reasons for harvesting giant clams nowadays relate to food security for coastal communities and to supply highly valued Asian markets and the international ornamental trade. All eight species of giant clam, namely *Tridacna gigas*, *T. derasa*, *T. squamosa*, *T. maxima*, *T. crocea*, *Hippopus hippopus* and *H. porcellanus* have been successfully cultured during the past decades.

There are a further five species that have since been recognised, namely *T. rose wateri*, *T. mbalavuana*, *T. squamosina*, T. *noae*, and *T. lorenzi* (Moorhead 2018).

Giant clam culture involves three basic phases: hatchery, land-based nursery and ocean nursery grow-out phases. A diagrammatic view of the stages in the life and culture of giant clams is presented in Figure 1 (Calumpong 1992). Internal organs of giant clams are shown in Figure 2.

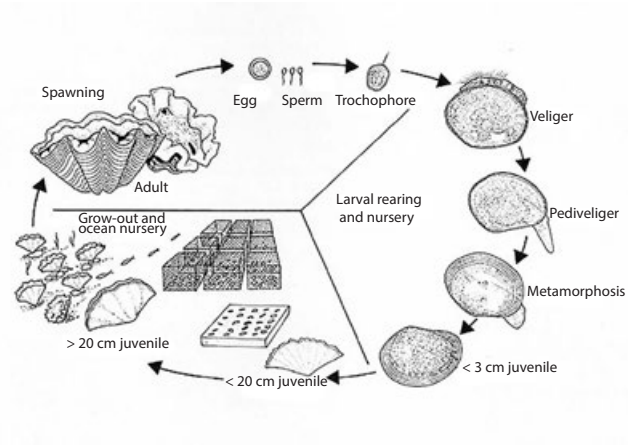

Figure 1. Giant clam life cycle and farming steps

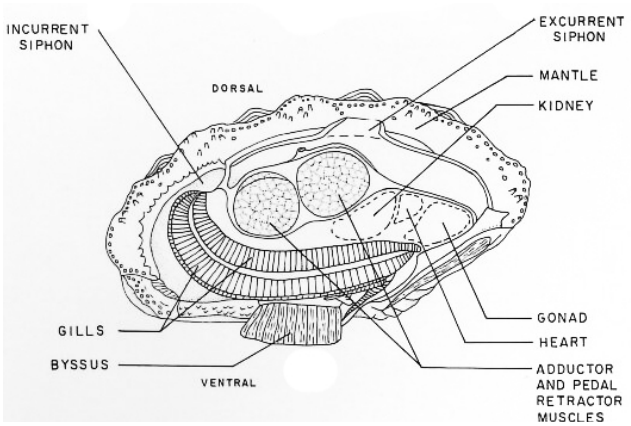

Figure 2. Internal organs of a giant clam

3. Broodstock collection, transportation, management and preparation

It is common knowledge that natural stocks of giant clams in many PICTs are facing extinction. In RMI not all of the thirteen species identified are present. Some of the giant clam species present are: *T. squamosa*, *T. maxima*, *T. noae* and *H. hipoppus*. Since giant clam culture aims to supply the international ornamental trade, broodstock selection is influenced by special attributes, such as mantle colour, mantle pattern, shell shape and health conditions.

3.1 Broodstock collection

In RMI larger species of giant clams like the *T. gigas* and *T. derasa* are rarely found. The smaller species of clams are more abundant and are found on the reef flats. They are collected by using a sharp knife or hammer to carefully cut away the byssal threads that hold on to the substrate. The species with the byssal threads are *T. maxima*, and *T. noae*.

3.2 Broodstock transportation

The best way of transporting giant clam broodstock is using cooler boxes with lids to prevent direct heat from the sun. When transporting giant clams outside the water on an open deck they must be laid on their side to avoid the mantle collapsing on to vital organs like the heart. They must be kept moist throughout the journey.

3.3 Broodstock management and conditioning for spawning

Broodstock should be maintained in a location where they can be monitored for their health and gonad development. Broodstock located on the reef flats must be inspected frequently for health and security reasons. If broodstock clams are maintained in cages or open reef flats, they should be cleaned regularly to prevent being smothered by algae and to maximise water flow over the clams. They should be located at a certain depth so that sun light to reach them. At the land-based nursery tanks, they should be observed daily as part of management and conditioning. The health condition of the giant clam influences gonad development. The provision of plenty of sun light and a good exchange of seawater to bring in nutrients is essential. In the land-based nursery, supplement food and aeration can be also provided.

WORKING PROCEDURE 1

- **Cleave off growth on the shells' chip, scrub shells and chlorinate (see working procedure 2 for preparation of chlorine solution).**
- **Rinse off chlorine solution.**
- **Fill tank with unfiltered seawater covering the shells of the clams.**
- **Monitor temperature (25–32°C), salinity (32–35 ppt[1]) and dissolved oxygen (5.5–7 ppm[2]), twice a day, morning and afternoon.**
- **Add supplementary feed daily, green water or commercially formulated diet. Dissolve 20 g of commercially formulated feed in 3 L of water, transfer the feed in large bin and equally distribute in tank.**
- **Observe for spawning activities in the afternoon.**

[1] Parts per thousand
[2] Parts per million

3.4 Broodstock preparation for spawning

The body of giant clams, especially on the shells, hosts many different biological organisms. Most of these organisms are parasitic in nature. They should be removed prior to spawning. One of the most dangerous parasites is the boring sponge – also known as the orange eye, boring sponge (*Cliona* sp.) – and treatment with a chlorine solution to destroy the sponges should be carried out. The chlorine solution should be diluted with fresh water to a suggested concentration of 10 mL (or 10 g) per L of fresh water. If there is no chlorine solution, common household bleach with 8% sodium hypochlorite as the active ingredient can be applied, as it is described in the "working procedure 2" detailed below. Apply 10 mL of the household bleach per L of fresh water. After cleaning the shell, information on individual clams should be recorded, such as an allocated number for identity purposes and other distinct features (mantle colours) should be noted.

WORKING PROCEDURE 2

- **Remove all growth on the body of the giant clams.**
- **Rinse thoroughly with seawater.**
- **Prepare chlorine solution by (a) 10 g dissolved in 1 L or (b) 10 mL of household bleach (8% sodium hypochlorite) in 1 L.**
- **Turn the clam upside down. Using a painter's hand brush apply chlorine solution on the shell. Care must be taken that chlorine solution does not touch the flesh of clam.**
- **Put clam on side for 10–15 minutes and rinse with seawater. Return clam into the tank.**

3.5 Disinfection and preparation of equipment: semi-intensive method

One day prior to conducting spawning, prepare the equipment that is required for the collection of eggs. Disinfect all hatchery equipment including incubation tanks, larval rearing tanks, aeration stones, tubes and buckets with a chlorine solution. Fill the tanks with seawater filtered through a 1 µm filter. If there is no UV radiation, filtered seawater to 1 µm is sufficient, but care must be taken to maintain the filter at its proper condition in order to avoid bacterial infections.

4. Spawning of giant clams

The giant clams are induced to spawn through application of external stimulus. These methods include the use of thermal stress, gonad stimulation and chemicals by using serotonin. These methods have been proven to be successful in releasing gametes from tropical bivalves, including giant clams.

4.1 Spontaneous spawning

The giant clams can experience enough stress to release gametes when they are transported from the holding areas (or freshly harvested from the reef) to land-based tanks. Spawning may occur within a short period. The majority of spontaneous spawning happens due to clams having a ripe gonad and this is normally witnessed in the afternoon. The evidence of lunar periodicity of spawning in giant clams is well documented. Most of the data indicates that the natural spawning cycle of giant clams occurs for several days after the full moon phase.

WORKING PROCEDURE 3

1. **Scrub clean and chlorinate clams, and all equipment needed for egg collection.**
2. **Monitor temperature, salinity and dissolved oxygen.**
3. **Follow working procedure for egg collection.**

4.2 Gonad extract as a spawning inducer

A mature giant clam is sacrificed to obtain gonad material. The gonad material is extracted from the mature clam (Figure 3) and placed in a blender or macerated in a beaker, and stirred until a consistent smoothness is attained. The mixture is sprayed around the incurrent siphon of the broodstock to stimulate gamete release. The sperm release generally occurs after application of gonad extract. Excess gonadal material can be frozen for future application.

WORKING PROCEDURE 4

1. **Open the giant clam to remove the gonad from the flesh and other organs.**
2. **Blend or macerate the gonad to obtain a consistent smoothness and add 500 mL of filtered 1µm seawater.**
3. **Filter the gonad water through 120 µm sieve screen to filter out the flesh. If there is still flesh in the gonad water, sieve a second time with an 80 µm or 100 µm sieve screen.**
4. **When giant clam opens up, 5–10 mL of gonad solution is slowly squirted into the incurrent siphon of each broodstock clam using a syringe.**
5. **If no spawning activity occurs within 5–10 minutes, additional gonad solution should be squirted into the incurrent siphons of the broodstock clams.**
6. **Store unused gonad solution in freezer for use later when needed.**

Figure 3. Fully matured gonad of *T. squamosa*

4.3 Serotonin as an inducer for spawning

Serotonin (5-hydroxytryptamine creatinine sulphate complex) as an inducer for spawning species of the giant clam is recognised and has been proven to work. The serotonin solution is made from dissolving 0.17 g of crystalline serotonin in 250 mL of filtered (1 µm) seawater. The serotonin solution is light sensitive; therefore, the container will need a cover to protect it from losing strength. Store any unused solution in the refrigerator at 4–8°C.

Applied dosage will vary between species of clams and the age and health of the individual. The smaller species (*T. maxima*) may require a dosage of 0.5–1 mL, while a large specimen of *T. gigas* or *T. derasa* may require a dosage of 3–4 mL. The needle should be cleaned with fresh water and sterilised (ethanol or methylated spirits) after each use. (Injection site is shown in Figure 4.)

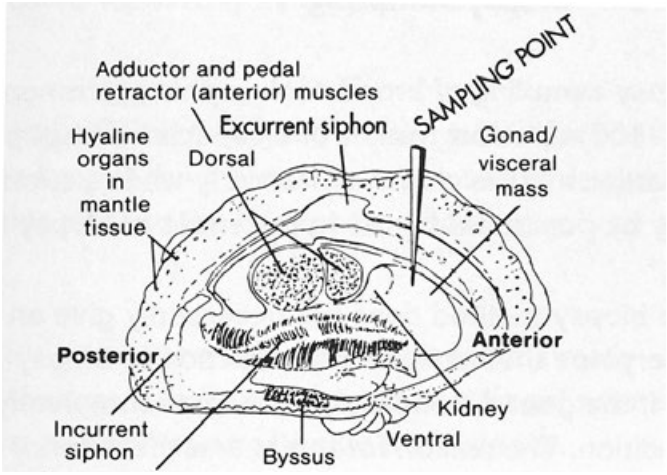

Figure 4. Note the position for the biopsy sampling point, inject clam with needle for serotonin inducement at the same region

WORKING PROCEDURE 5

1. Weigh 0.17 g of crystalline serotonin using electronic balance.
2. Measure 250 mL of filtered 1 µm seawater into conical flask.
3. Dissolve 0.17 g of crystalline serotonin in conical flask in 100 mL of seawater from the flask.
4. Stir until all crystals disappear and pour it back into the flask.
5. Pour equally into several conical flasks and store in refrigerator. Completely wrap the flask or bottle with aluminium foil to prevent disintegration of the chemical by sun light.
6. Using a 150 cm hypodermic needle and a 10 mL or 20 mL plunger syringe, pipette required volume of serotonin solution to induce spawning.
7. Place a needle in the gonad region and then slowly apply pressure to drive the needle in to the mantle cavity. The next pressure point is the wall of the gonad. Slowly apply pressure to puncture the gonad and drive needle 1–3 mm into the gonad. Squirt serotonin and withdraw the needle.
8. Apply serotonin to 3–5 broodstock clams to release gametes to stimulate other clams.
9. Record the identification of the broodstock injected with serotonin.
10. Store the unused solution in refrigerator.

4.4 Temperature shock

Rapid changes in temperature in a giant clam's environment can cause stress that results in release egg. Allow seawater in the tank or bins to be heated by the sun untill there is increase in temperature in the range of 3–5°C from the ambient air temperature. Otherwise, put the broodstock clams directly in the sun on their side. Put the clams back in the tank and observe for spawning. Similarly, water fast flashing and fast refilling of tanks can be applied. If no eggs are released by about 6 pm, all the water in the spawning tank should be drained and replaced with new 1 µm filtered seawater. Repeat the procedure for 3 consecutive days. If there is no success in the spawning attempt, the broodstock can be left in the holding tanks for 7 days to monitor further spawning activity. Continue providing supplement feed to support gonadal development.

5. Fertilisation

5.1 Collection and fertilisation of eggs

When sperm is released from an individual clam, collect it in a separate container. The sperm should remain viable in the collection container for at least 30 minutes and should be replaced occasionally to maintain the freshness of the sperm. When eggs in the sperm mixture are first noticed take a sample and observe under the microscope to confirm. The suspected individual clams should be removed from the broodstock spawning tank or bin immediately when they begin releasing eggs. They should then be rinsed with 1 µm filtered seawater and placed in a new separate container. The temperature in the new spawning container should be checked to ensure that it is the same as the holding tank.

The eggs will be released into the water and fertilisation of the eggs should be conducted directly into the container within 10–15 minutes of egg release. If sperm water is dense, then approximately 30–60 mL is required to fertilise 30 L of concentrated eggs in a container (Braley 1992). An individual clam has the capacity to release millions of eggs during one spawning event. Carefully monitor egg concentrations in spawning containers. If the egg concentration is too dense in the container then divide into a couple of containers. Aerate the containers to avoid low dissolved oxygen levels in the containers that can lead to fatalities in the developing embryos. All containers holding fertilised eggs should be moved into the larval room, have additional filtered water added to the container to decrease temperature fluctuations, egg densities should be diluted if required, and aeration and eggs counts should be performed.

WORKING PROCEDURE 6

- **Take 1 mL sample of sperm water from the sperm container to observe sperm mobility under a microscope.**
- **Perform sperm count to provide estimate of sperm concentration.**
- **Label all sperm water containers with identification of the broodstock.**
- **Take sample of suspected egg release and observe under microscope to confirm presence of eggs.**
- **Isolate the clam in the separate spawning bin. Ensure that the clam is fully submerged.**
- **Check temperature of bin and spawning tank to confirm that the two are in close range around 28–30°C.**
- **Allow clam to release eggs into the bin.**
- **Take sample of eggs to observe egg maturity.**
- **Determine volume of the container holding the egg water.**
- **Pool the sperm into 100 mL beaker or cylinder.**
- **Fertilise eggs by adding 30–60 mL of sperm to 30 L of egg water.**
- **Gently stir the egg suspension to distribute the sperm evenly. Allow to stand for 1–2 hours.**
- **Remove clams from the spawning bin and place eggs collected in a new bin.**
- **Take samples to determine quantity, quality and fertilisation rate of egg.**

5.2 Estimating the number of eggs

After the washing of eggs is completed, eggs are transferred into a container with a known volume. Using a plunger, homogenise egg water in the container by gently pushing it up and down. Use a 1 mL pipette to take samples from the container and place the sample directly into a Sedgewick rafter counting chamber or fabricated counting chamber (Figure 5), and then count under a microscope. At least 6–10 (1 mL) samples should be taken from each container to allow an accurate estimate of egg density. If the eggs are too dense in the container it may be necessary to dilute the 1 mL samples in order to be able to count in the chamber. The usual dilution factors are 1:5 or 1:10.

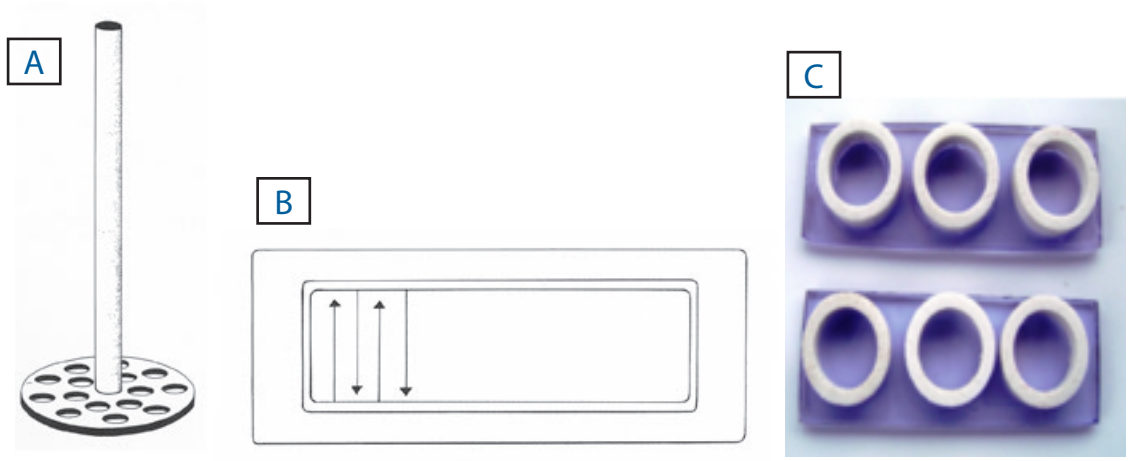

Figure 5. Equipment needed for egg counting

WORKING PROCEDURE 7

- **Homogenise egg water thoroughly with plunger and take 6 x 1 mL samples in Sedgewick rafter counting chamber or fabricated counting chamber.**
- **Observe under microscope and count all eggs in the chamber.**

FOLLOW CALCULATION FOR ESTIMATION OF EGGS:

- **Assume that 6 x 1 mL sample counts were made from 30 L bin totalling to 840 eggs.**
- **Average of counts = (total sum of six counts)/(number of counts) = (480 eggs) / (6 counts) = 140 eggs per mL**

$$\text{Average of counts} = \frac{\text{total sum of six counts}}{\text{number of counts}} = \frac{480 \text{ eggs}}{6 \text{ counts}} = 140 \text{ eggs per mL}$$

In 30 L bin total eggs = (30 L*(1000 mL)*(140 eggs) = 4.2 million eggs

5.3 Fertilisation rates

The fertilisation rate of eggs is determined by taking 6–10 1 mL egg water samples approximately 2 hours post-fertilisation. Counts should be made using a microscope. Separately count the fertilised eggs that show embryonic development (cells being divided) within the samples and count the total number of eggs in the chamber.

The percentage of eggs that are developing as embryos can be estimated. If the fertilisation rate is above 80%, this indicates a good batch of eggs and subsequent larvae. However, if fertilisation rates are lower it is indicative of a poor batch of eggs and only a low number of larvae is expected to survive.

WORKING PROCEDURE 8

CALCULATION TO DETERMINE FERTILISATION RATE:

- **Take 6 x 1 mL samples and count all the eggs in the chamber.**
- **Assume the average count, made by using working procedure 10 above, is 840 eggs.**
- **Out of 840 eggs counted 126 eggs will be unfertilised.**
- **Rate of unfertilised eggs** $= \frac{126 \text{ eggs}}{840 \text{ eggs}} \times 100\% = 15\%$
- **Therefore fertilisation rate** $= 100\% - 15\% = 85\%$

6. Larval culture: Semi-intensive (static method)

The Australian Centre for International Agricultural Research (ACIAR) describes the practice applied in giant clam hatcheries in PICTs in their culture manuals and the Pacific Fisheries Development Foundation (Bradley 1992; Heslinga et al. 1990). Several modified techniques were developed to culture giant clam larvae and they have all successfully produced juvenile clams.

In semi-intensive giant clam culture, the static method is commonly practiced. The hatchery run takes 5 to 8 days, but it can take longer when transferring the larvae to outdoor tanks. Good hygiene practice is important in the semi-intensive hatchery run.

Hatchery equipment, including tanks, air stones and air delivery tubes, buckets and sieves should all be chlorinated after every use. Larval tanks should be stocked to full capacity at a rate of 25–30 eggs per mL on Day 0. Tanks are filled with filtered seawater and aeration is applied and left until drained on every alternating day beginning from Day 2. Normally, antibiotics (Neomycin sulphate and Streptomycin sulphate) are added at a concentration of 10 ppm in the tanks as a prophylactic in order to control bacterial infection.

On Day 1, remove protein scum from the tank top and leave undisturbed. Trochophore and veliger larvae can be viewed in the larval tank with the help of a torch. Take a sample to observe the larval development under the microscope. On Day 2 tanks are fully drained, trochophore and veligers are collected on to an 80 µm sieve and counted to determine stocking densities. The healthy swimming veligers are restocked in the clean larval tanks at a much lower stocking density (15–20 larvae per mL). It is shown that feeding every day improves survival of the larvae. The feeding of larvae can then start. Between Day 2 and Day 5 larvae should develop through to D-veliger and pediveliger. At this stage gut, foot and velar retractor muscle are clearly visible. During Day 4 and Day 5 the foot increases in length as the larvae develops into pediveliger. Any digested food should be visible in the stomach in the form of a brownish colour in the middle of the larvae. Between Day 4 and Day 8 larvae are inoculated with zooxanthellae.

6.1 Stocking rates in the hatching tanks

Stocking densities of fertilised eggs in a larval rearing tank should be in the range of 25–30 eggs per mL. All larval rearing tanks should be supplied with aeration for the whole period of the hatchery run. Moderate aeration is required for the first 24 hours of egg development in the larval tanks to assist in suspending the developing eggs and maintaining oxygen concentrations in the water. After hatching on Day 2, the density of larvae can be reduced to 15–20 larvae per mL. In addition to aeration, physically stirring the water with a plunger will greatly assist the larval development process and keep them suspended in the water column. The aeration can then be increased.

6.2 Extensive culture system

All of the steps described in the semi-intensive method for the giant clam culture can be applied to the extensive culture method. Spawning is conducted as normal, and eggs are collected and counted. All tanks are chlorinated, rinsed with 1μm filtered seawater (samples of sieves are shown in Figure 6) and allowed to air dry. The drain standpipes are covered with an 80 μm screen to prevent eggs or larvae from being accidentally drained. Aeration should be used when needed. On spawning day, fertilised eggs are counted and directly stocked into the larval tanks outdoors. Tanks can be stocked at a very high density with no application of antibiotics. On Day 4, the larvae can be fed with high-density zooxanthellae. Normally, when carrying out the extensive culture method, the larvae are left in the nursery tanks until they become visible to the naked eye in around 6–8 months after stocking.

6.3 Antibiotics

The antibiotics Neomycin sulphate and Streptomycin sulphate are used as a prophylactic in the larval rearing process for the first couple of days to control any increase of opportunistic bacteria in the culture vessels. The antibiotic mixture is made by dissolving antibiotic powder at a concentration of 10 ppm (10 mg per 1 L of filtered seawater) and poured directly in to the larval tank. Combining the two antibiotics provides higher survival rates than when they are used separately.

Figure 6. Example of sieves

6.4 Stocking veligers in culture tanks (indoors)

On Day 2, veliger larvae, once sieved and counted, are stocked into the larval tanks at 15–20 larvae per mL. On each sieving day (Day 2 to Day 8) the larval stocking density is reduced to half. The larval tanks filled with 1 μm filtered seawater must be aerated and tanks are left static.

Daily monitoring of larvae should occur at first thing in the morning. All water quality parameters such as temperature, dissolved oxygen, aeration, pH and salinity should be checked. Larvae are best viewed by shining a torch into the water column. Larvae should be evenly distributed throughout the tank. Feed is applied every day. Placing any type of plastic black disc under the water in the tank can improve the visibility – so that the larvae can be seen in front of the disc – or, siphon and collect larvae on to a sieve screen of appropriately 80 μm and view under the microscope.

7. Larval settlement

Veliger larvae will develop into pediveliger larvae between Day 4 and Day 8, post fertilisation. The larvae will stop swimming and settle to the bottom of the tank. Pediveliger are approximately 170–200 µm in shell length depending on the species and have a foot that allows the clam to move around the bottom of the tank. At about Day 8 the vital organs of the juvenile clams, such as gill racks and statocyst, are visibly distinct.

High mortality of larvae is experienced during this period when pediveliger are metamorphosing into juveniles. Approximately 8 to 10 days are required before metamorphosis is complete and during this stage a whole range of physiological and morphological changes take place.

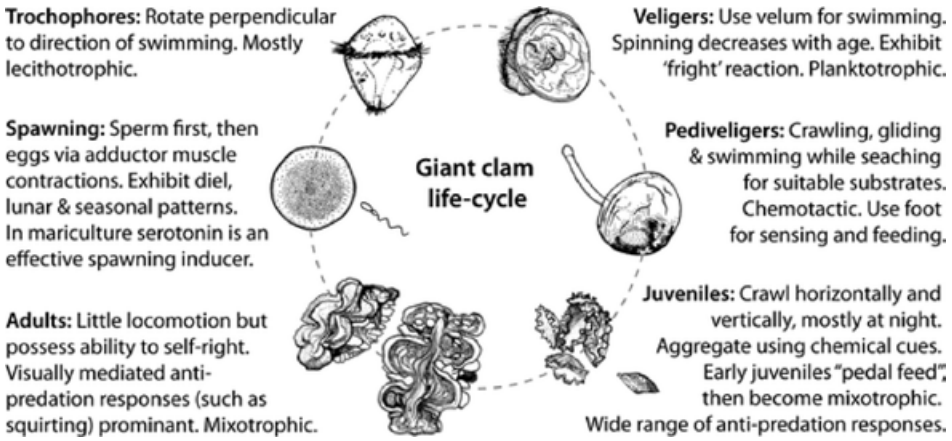

Figure 7. Stages of larval development. A: Day-3 veliger, B: Day-5 pediveliger, C: Day-7 metamorphosed juvenile

7.1 Zooxanthellae

Tridacnidae clams are easily distinguished from other clams by their symbiotic relationship with a unicellular alga, the zooxanthellae.

WORKING PROCEDURE 9

Both the alga and clam benefit from this mutual relationship, which persists for the entire life of a clam. In the natural environment, zooxanthellae are found in tropical coral reefs. In the hatchery, the zooxanthellae are obtained by scraping the mantle of a sacrificed clam or using a blender to pulverise the mantle and then sieve it through a fine mesh to separate the flesh from the zooxanthellae.

1. **Remove the mantle tissue from the giant clam.**
2. **Wash in fresh water, cut into smaller pieces and place into a food blender with filtered seawater.**
3. **Blend the mantle until the texture is consistent.**
4. **Next, pour it through an 80 µm sieve screen, collecting zooxanthellae in a new container.**
5. **Pass the zooxanthellae water for the second time using smaller sieve screen (35 µm or 55 µm).**
6. **If there is no blender, remove all flesh from the mantle and scrape with a clean sharp knife.**
7. **Using the knife, scrape the mantle and collect all the brownish fluid coming off the mantle.**
8. **Pass through an 80 µm sieve screen, collecting zooxanthellae in a new container.**

Zooxanthellae should be added to the larval tanks on the first day that pediveliger are seen and applied three times a day from the second day. Settled larvae and early juvenile clams can be observed under the microscope to determine if zooxanthellae have been taken up by the clam. They will appear as golden brown spheres in the gut or on the developing body of the mantle.

8. Juvenile culture

The giant clam larvae settled in land-based nursery tanks will take approximately 6 months after stocking to be seen with the naked eye.

The seawater coming into the tank should be filtered to 25 μm using filter bags for at least the first 2 months in the nursery tanks. The nursery tanks must be aerated and have at least one water exchange per 24 hours.

Algal growth can be controlled by use of herbivorous fish, gastropods and trochus. The filamentous species of algae are a challenge to control as even the grazers will not graze on them (please see detailed information on section 8.2.).

8.1 Estimating clam numbers in the nursery tanks

Sampling juvenile clams in the nursery tanks provides vital information regarding the survival of the clams, density, health and growth. Sampling can be easily conducted with a loop ring made from plastic coated wire or PVC pipe (Figure 8). The area within the ring should be siphoned into a small 100 μm sieve screen. The juveniles should be washed into a petri dish or beaker and viewed under the microscope.

WORKING PROCEDURE 10

- **Make a ring from plastic coated wire, non-toxic wire or PVC pipe.**
- **Calculate the area of the ring.**
- **Facing away from the tank, throw the ring into the tank.**
- **Take 6 samples by siphoning from the inside of the ring.**
- **Observe under a microscope and count for each ring.**
- **Take average of the 6 samples.**
- **Calculate total number of juveniles in the whole tank.**

CALCULATION:

- **Area of circle ring A = πr^2 where π = 3.14, r = radius of ring**
- **Assume ring diameter = 1 50 cm, therefore A = 3.14*(7.5 cm) 2 = 176.625 cm^2**
- **Convert to m^2 = 176.625 cm^2 /10,000 cm^2 = 0.01766 m^2**
- **Assume average sample = 80 juveniles**
- **Therefore total juveniles = $\frac{80 \text{ juveniles x } 25 \text{ m}^2}{0.01766 \text{ m}^2}$ = 42,468 juvenile clams**

Land-based nursery phase

8.2 Cleaning protocols

The brownish soft textured algae will be the first algae to colonise the tanks. These are good algae and grazers will be able to graze upon it. The second type of algae to colonise tanks are the green and brown filamentous species; these are the problematic algae. A number of methods can be used to control the growth of the fouling algae, including the use of shade cloth over the tanks, manually removing algae and using herbivorous fish, gastropods and snails that will feed on the algae. Brooming or hand wafting can help loosen the algae from the bottom of the tank. Algae and debris can be removed by siphoning through a sieve. The size of sieve or strainer selected will depend on the size of the clams in the tank. Wash the materials collected on the sieve in a bucket to separate the clams. When clams grow larger, tanks can be drained and then refilled. As clams grow larger, small herbivorous fish, gastropods and snails can be introduced for grazing in the tank. Removal of waste produced by the grazers must be done daily since it can encourage growth of filamentous algae.

8.3 Herbivore polyculture

The use of herbivorous animals, both invertebrates and vertebrates, especially some species of gastropods and fish are useful in the removal of the algae. Particular care must be taken when selecting algal grazers, not to select fish of certain sizes that could damage small clams. Large trochus are efficient grazers but are not practical to use with small clams as they will crush and kill them. A number of different grazers can be used together but will require more cleaning time of the tank.

8.4 Flow rates and aeration

The control of seawater coming into the tank is important. The flow rate should be set so that tanks achieve at least one complete turnover every day. This is not to be confused with flow rate when larvae are first stocked in the tanks. High volumes of seawater passing through the tanks will bring high quantities of nutrients for juvenile clams for their growth and zooxanthellae maintenance. Aeration improves the dissolved oxygen level and aids the mixing of seawater to keep the temperature at a constant level.

WORKING PROCEDURE 11

- **To calculate volume of settlement tank:**

  volume = l * w * h; where l = length of race way, w = width of race way, h = height of the drain pipe.

  Assume that l = 15 m, w = 1.5 m and h = 0.30

  Volume of the race way = l * w * h = 15 m * 1.5 m * 0.3m = 6.75 m^3 or 6750 L

  To achieve one complete turnover of water in the race way in 24 hours:

- **Flow rate** $= \frac{\text{volume}}{\text{time}} = \frac{6750\ \text{L}}{24\ \text{hr}} = \frac{281\ \text{L}}{1\ \text{hr}} = \frac{281\ \text{L}}{60\ \text{min}} = 4.7\ \text{L per min}$
- **Take container with known volume and a stopwatch.**
- **Turn the tap on and time the water collected in the container to get 4.7 L in 1 minute.**
  Process might be repeated a number of times to get it exact.
  If two turnovers are required, multiply the flow rate by 2.
- **Repeat the process with all of the race ways or settlement tanks.**

8.5 Harvesting of juvenile clams

The triage of clams through harvesting and restocking into other tanks is necessary if the densities of clams are high in tanks. Frequent thinning will help growth and survival rates. Clam clumps must be harvested and redistributed within the tank.

Juvenile clams attach themselves to the substrate when they are young. When harvesting or thinning, use implements that are flat, flexible and sharp. The clams should not be pulled from the substrate or bottom of the tanks.

Harvest the clams first before completely draining and cleaning the tanks. When draining the seawater, ensure that a sieve is used to collect clams together with algae. Hose off the remaining materials in the tanks to collect the clams. The clams need to be separated from the algae and cleaned. After cleaning, return the clams and refill the tank. The size of the screen will be determined by the size of clams harvested.

8.6 Volumetric and weight count estimates

Juvenile clams are harvested by cutting off their byssal attachments with a sharp harvesting knife. All knives such as a kitchen knife, table knife or painters' knife are suitable implements for the work. Harvested clams are washed, clean counted and sample measured before they are placed back into tanks or transferred to the ocean nursery. Randomly select clams and make about 5–10 groups of 30 clams, and measure them to determine shell length. A small amount of seawater is added into a graduated cylinder and the volume noted. The 30 individuals are then placed into the graduated cylinder. The increase in water volume in the graduated cylinder is then recorded. The process is repeated for the 5–10 groups of samples and their average volume calculated.

WORKING PROCEDURE 12

BY VOLUME

- Randomly select 30 clams per group for 5–10 groups.
- Fill the measuring cylinder with seawater and record the volume (A).
- Place 30 clams in the measuring cylinder and record the volume (B).
- The difference in volume (C) = (B - A), which is the volume displaced by 30 clams.
- Repeat the process for the 5–10 groups of clams.
- Add all the volumes (C) to find the average.
- Repeat the process for all the harvested clams and record volume.
- Assume average volume of displaced seawater by 30 clams is 3 mL.
- Number of clams required for ocean nursery is 2200.

TO CALCULATE THE VOLUME REQUIRED:

- 30 clams = 3 mL
- then 2200 clams = x volume;
- so that x volume = flow rate = $\frac{2200 \text{ clams} * 3 \text{ mL}}{30 \text{ clams}} = 220 \text{ mL}$
- Add 100 mL of seawater into 500 mL measuring cylinder then add clams until a final volume of 320 mL is reached.

TO CALCULATE THE TOTAL HARVEST:

- Assume that total volume of harvest is 2000 mL. What is the total number of clams harvested?

30 clams = 3 mL,

then x number of clams = $\frac{2000 \text{ mL} * 30 \text{ clams}}{3 \text{ mL}} = 20{,}000 \text{ clams}$

WORKING PROCEDURE 13

BY WEIGHT

- Weigh empty container (Wa).
- Separately weigh each group of 30 individual clams in a plastic container, (Wb).
- Weigh the total number of clams (wf).

Assume that the average weight obtained for 30 clams is 15 g.

Number of clams required for ocean nursery is 2200. What weight is required?

30 clams = 15 g then x number of clams = $\frac{15 \text{ g then x number of clams}}{30 \text{ clams}} = 1100 \text{ g}$

To calculate the total harvest:

Assume volume total harvest is 3000 g.

To calculate the total number of clams harvested:

30 clams = 15 g then x number of clams = $\frac{30 \text{ clams} * 3000 \text{ g}}{15 \text{ g}} = 6000 \text{ clams}$

9. Giant clam culture ocean nursery

9.1 The ocean nursery

The ocean nursery culture system is the next phase of giant clam culture from the land-based nursery culture system. In the land-based nursery, the juvenile clams are cultured to grow to sizes ranging from 18–25 mm and transferred to the ocean nursery system. There is no pumping and aeration, or provision of supplementary nutrients. Different species are found to grow well in different environmental areas; *T. gigas* and *H. hippopus* show good growth in intertidal waters, while *T. derasa* together with the other species grow well in subtidal sites.

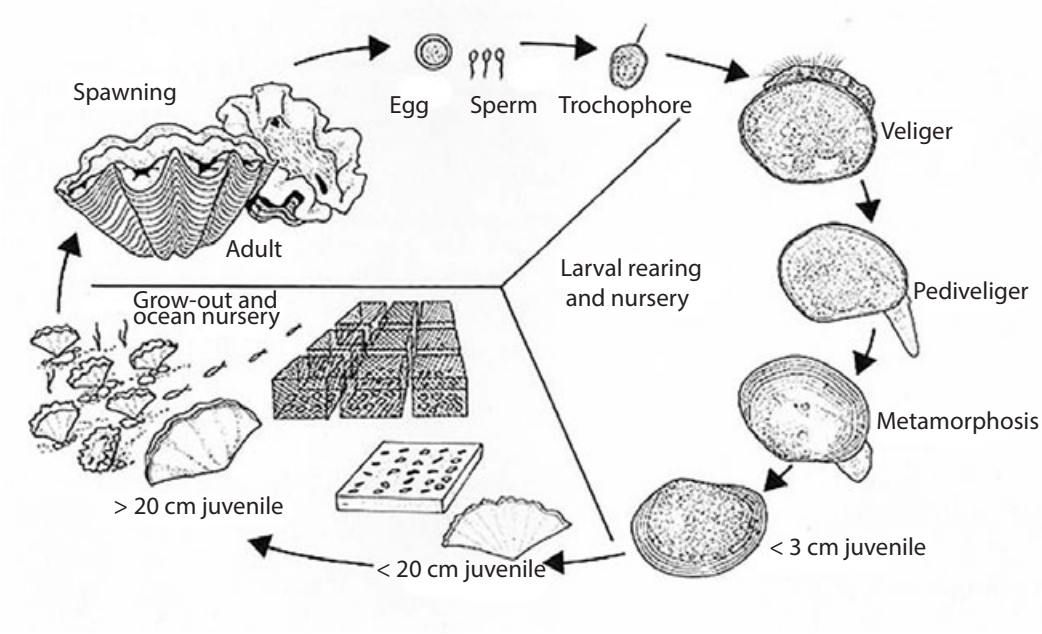

Figure 8. Steps in giant clam farming

9.2 Site selection

In the site selection, biological process factors need to be considered together with legal, social and economic factors. Social factors such as the multiple users of the area, farm security, closeness to communities and reef ownership must be cleared to avoid disputes. The biological indicators are important in assessing the suitability of a site for clam farming. Some positive biological indicator species include, but are not limited to, the presences of wild giant clams, stony coral growth like *Acropora* and *Porites* and sea grass beds. Sites that have the right environmental conditions directly contribute to optimal growth and survival of clams. Some negative biological indicator species include mangrove trees and seedlings; soft corals present in high numbers; high sponge populations and the presence of predator fish such as trigger fish and emperor fish; and some gastropods, especially muricid and pyramidellid snails.

9.3 Shallow depth of water

Ocean nurseries can be located in lower intertidal or shallow sections of inshore reefs with good water flow, and a depth range of 1–5 m is found to be appropriate. These areas can be exposed or covered in the lowest level of a low tide. Shallow water is considerably easier to work with than deeper water. Exposure of cages in low tide lessens the need for cleaning since the direct sun light dries and slows algal growth.

9.4 Clear seawater with right salinity

Giant clams require clear seawater and long periods of exposure to sun light. Light is essential for the zooxanthellae that are living in the mantle of the clams. The zooxanthellae are responsible for providing food for the clams through the process of photosynthesis. Giant clams perform best in oceanic salinity in the range of 32–35 ppt. The giant clams have low tolerance to changes in salinity. Ocean nurseries must be located away from fresh water runoff and river mouths.

9.5 Peaceful seawater with good circulation

Giant clams grow well in areas with quiet seawater and good seawater circulation. When clams are undisturbed, they deliver most of their energy directly towards growth and gonad development. Good water circulation also disturbs temperature stratification and stagnation of the water column and brings fresh water with nutrients for the clams.

Locating the nursery close to rough seawater areas is not a good practice. Strong wave actions can cause widespread damage, cages are destroyed, clamshells are chipped and clams overturned. Areas with very a strong current must be avoided, as clam growth will only lead to thickening of shells.

9.6 Sandy sea grass area

Sandy flats with good water circulation are good areas for ocean nurseries. Certain sea grass beds with firm benthic sand are also good for ocean nurseries. Care must be observed since silt and mud are high in sea grass bed and can limit growth rates.

10. Intertidal ocean nursery

The clam cages in an intertidal culture are exposed to tidal fluctuations. During low tide periods, cages are in shallow water with high temperatures and direct sun light. Cages are normally placed on the seabed or on a low stand where cages are fixed. See Figures 9 and 10.

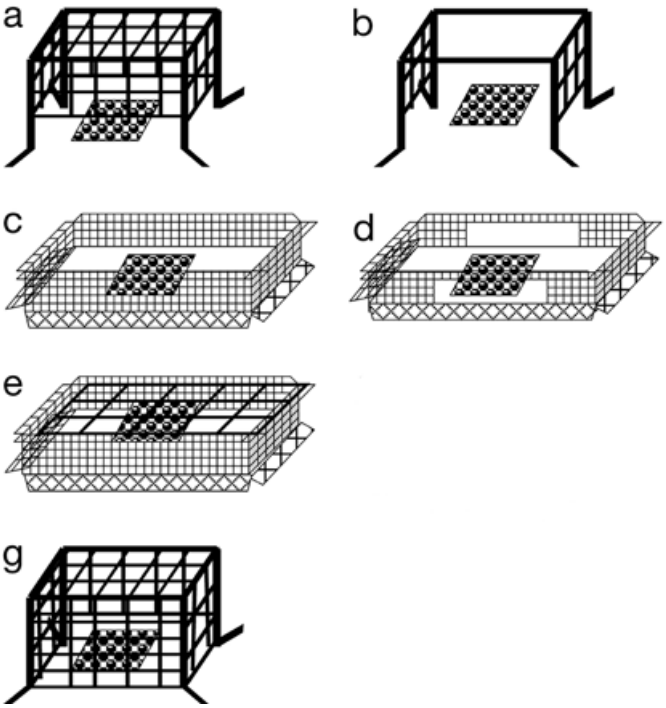

Figure 9. Different types of intertidal culture

Figure 10. Trestle and cages for subtidal culture

11. Subtidal ocean nursery

A protected area with water at a depth of 2–3 m at low tide should be selected for subtidal culture. Giant clams in a subtidal culture are normally cultured in trays and cages.

Similarly, the floating cage culture system provides an alternative culture method to protect clams from benthic predators.

12. Cage culture

A number of cage designs were made and found to be effective for excluding predators in the nurseries. Cages function as protectors of clams from predators such as crabs, snails and crayfish, as well as carnivorous fish. Cages also help to slow down fast currents and thus reduce clam disturbance, which aids good survival and growth.

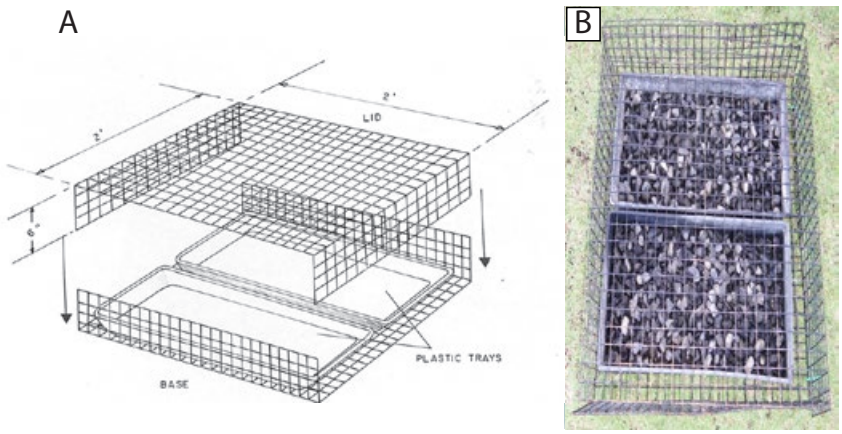

Figure 11. A: Cage with tray. B: Cage with coral/gravel substrate for juvenile settlement

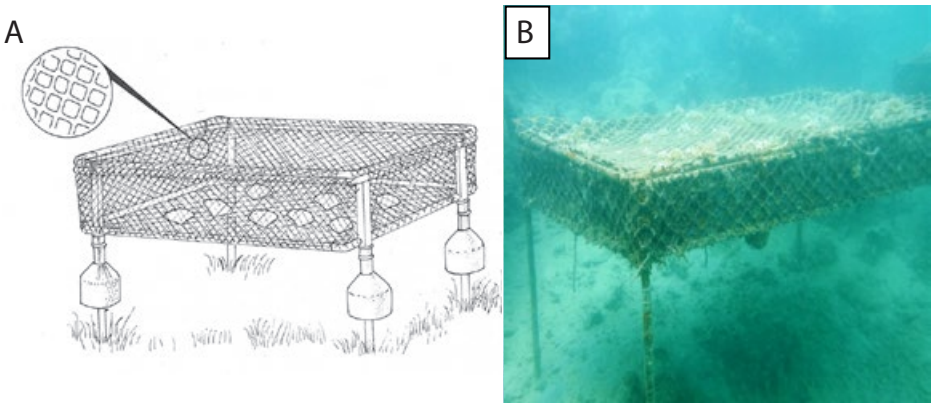

Figure 12. A: Cage culture design. B: Real cage farming in Palau

Figure 13. Broodstock management area

13. Floating ocean nursery

The floating ocean nursery complements the intertidal and subtidal grow-out sites. A floating ocean nursery can be located at the shallow sandy seafloor, sea grass beds or reef beds with a depth of 5–10 m. In the nursery, trays are stocked with juvenile clams and held fixed on the tray carriage, which is suspended from the floating rafter. Depending on the waves and current flow, two anchors are applied as mooring structures for the float. The float should be oriented so that the lead anchor and stern anchor hold it facing the direction of wind, wave and current. Below are different designs of the floating ocean nursery structures.

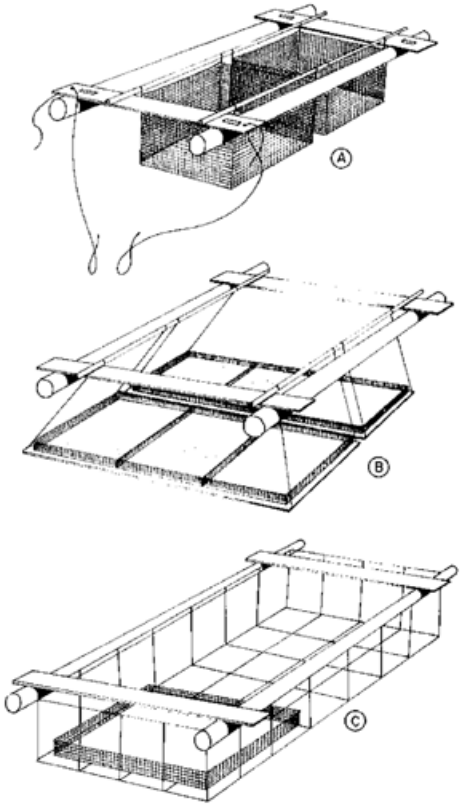

Figure 14. Designs of ocean nursery floating cages

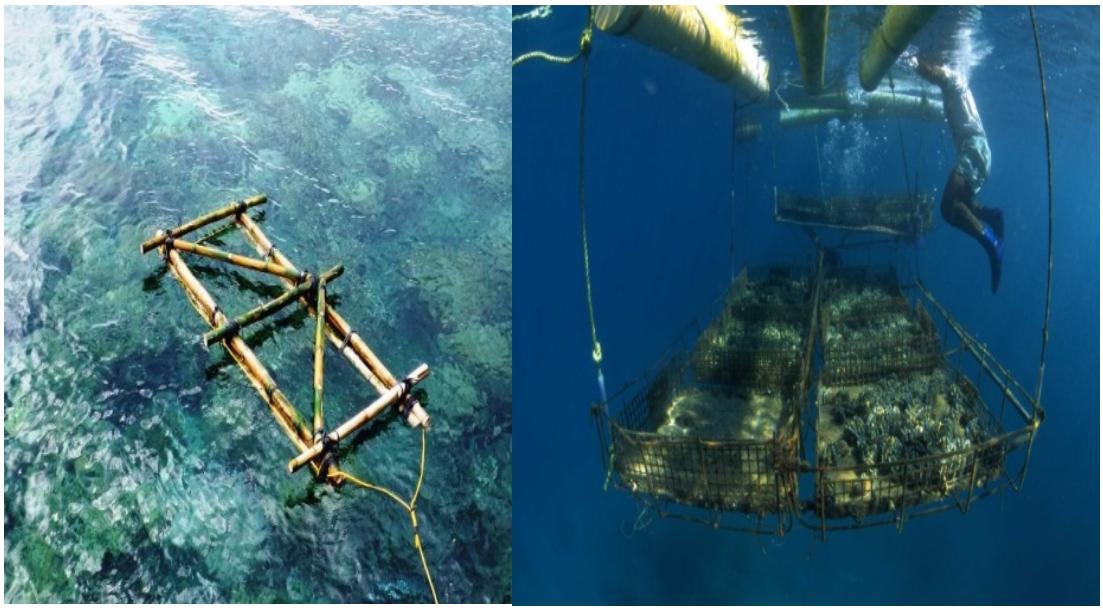

Figure 15. Floating ocean nursery bamboo raft

14. Predator and pest control in the ocean nursery

Predators essentially cause mortality of juvenile clams in farm sites during the ocean nursery phase. The juvenile clams are protected by using strongly built cages from plastic coated wires or galvanised wire. These protection materials can exclude clam-crushing predators including triggerfish (*Balistoides viridescens* and *Pseudobalistes flavimargrinatus*), puffer fish (*Tetradon stellatus*), emperor fish (*Montaxis grandoculus*), wrasses, eagle rays (*Aetobatis narinari*) and crustaceans like crabs and lobsters.

The most serious serial killer of juvenile clams is the *Cymatium muricinum*; it is a gastropod belonging to the family of *Cymatiidae*. *Cymatium muricinum* settles in the culture cages at the larval stage of the clam and grows inside the juvenile clams in the cage, feeding on the clam's tissue. Other known predators include flat worms (*Stylochus* sp.), pyramidellidae (*Turbonilla* sp.)and octopoda (*Octopus* sp.). Boring sponges bore through the shell of giant clams. These sponges are visible on the outer surface of clamshells with holes of orange, yellow or brown colours. Mortalities in the cages are a good indicator of the presence of the predators. Cages should be thoroughly searched or completely harvested to locate the predators.

Table 1. Possible causes of predator related shell damage in juvenile giant clams

Shell damage	Possible cause
Chipping or crushing	Crabs, hermit crabs fish
Drill holes	Muricid snails
Blistering on internal surfaces	Pyramidellids or juvenile ranellids
Layer or thickening along valve	Pyramidellids
Ligament torn and/ or hinge dislocate	Octopus

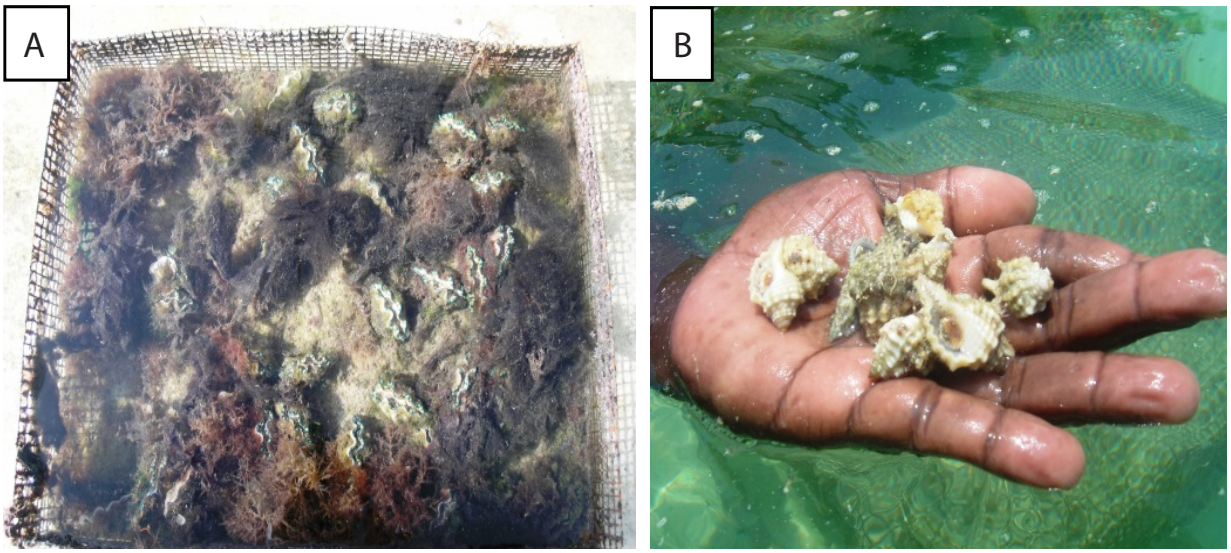

Figure 16. A: Cage with 100% algae fouling. B: *Cymatium* collected from the same cage when cleaned

15. Stocking density

The giant clams with shell lengths ranging from 18–25 mm can be stocked at a density of 1 clam per 2 cm or 500 clams per 1 m^2. When clams grow and cover 75% of the cage surface or more, the cages must be harvested to avoid crowding. They should then be distributed in new cages at a lower density. Stocking density should be reduced as clams grow; clams with a shell length in the range of 30–50 mm should be stocked at 100 juveniles per cage with a substrate size of 0.5 m^2. When clams attain shell length of 70–85 mm, stocking density should be as low as 30 clams in a cage of a substrate size of 0.5 m^2. When the density of clams in a cage is low and clams grow larger, it is time for the clams to be moved to the enclosure. In an enclosure with 9 m^2 of benthic substrate, up to 120 clams should be stocked. Thinning of the clams will be necessary, as they grow larger.

16. Monitoring

Monitoring is a routine part of giant clam husbandry. A well planned monitoring schedule should be made that allows for frequent visits to the farm site. Monitoring activities involves can be seen in "Working procedure 14".

WORKING PROCEDURE 14

- **Clean cages by brushing off algae and other organisms that are growing inside and outside of cages.**
- **Cleave of corals on the cage stand or on the body of the cage.**
- **Remove dead shells.**
- **Using a thin rod of wire, push in between clams to find predators – especially snails.**
- **Thin overcrowding cages and restock in new cages.**
- **Inspect cage for damages, breakages and destroyed anchors for floating nursery.**
- **Immediately inspect farm after storms and strong waves actions.**
- **Take inventory of clam stock.**
- **Measure shell lengths to provide information for work plan, such as time to move clams, next phase of grow-out, when to start preparing to make new cages, etc.**
- **Evaluate possible signs of poaching.**
- **Mass mortality (caused by disease, predators, heavy siltation, etc.).**

17. Record keeping

Keeping an accurate account of all work that is taking place in the farms is crucially important. It is the farmers' responsibility to ensure that all information in relation to farm operation is recorded and kept safe at all times. Records should contain different cohort / batches of clams received from the hatchery, dates, growth and survival, predators, visitation from the fisheries staff members, number of clams sold, number of exporters and a diary of daily activities.

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