Spawning and Early Larval Rearing of Giant Clams (Bivalvia: Tridacnidae)

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Foreword

At the time of writing, a wealth of information is in print on spawning and rearing of giant clams, including two excellent manuals (Heslinga et al., 1990; Braley, 1992a, 1992b). In the years since these manuals were written, clam rearing methods have changed a great deal as have markets for these animals. Many different spawning and larval rearing methods are currently used which blend "appropriate technology" with the unalterable biology of the giant clam. The aim of this manual is not to contradict previous publications but to collate current information and up-to-date farming practices into a series of straightforward, step-by-step accounts of how to spawn and raise giant clams through their early life stages. This manual has an accompanying video also titled "Spawning and Early Larval Rearing of Giant Clams." Additional copies of this manual or copies of the video can be obtained from:

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Introduction

Who is targeted by this manual?

The people targeted by this manual are those with some current interest or involvement in giant clam farming. This publication in conjunction with the references listed should provide enough information for a newcomer to the field of giant clam farming to start raising clams. It is designed to guide an inexperienced culturist through all the steps of clam spawning and larval rearing but at the same time may offer new or alternative information to the experienced farmer.

Note: Highlighted words are described in the glossary in Appendix B.

What are giant clams?

Giant clams are **bivalve molluscs** of the family Tridacnidae with nine living species in only two genera, *Tridacna* and *Hippopus*. They occur in association with coral reefs throughout the tropical Indo-Pacific region. The names and a brief description of the living giant clams is as follows:

a. *Tridacna gigas* is the true giant clam, growing to greater than 1.4 m in shell length. *T. gigas* is easily identified by its size and the triangular projections of the upper margins of the shell. The **mantle** is brown/green with blue or green dots (Figures 1 and 2).

b. *Tridacna derasa* (smooth or southern giant clam) is the second largest species with a shell length of up to 60 cm. The shell is smooth, and the mantle has elongate brown, green and blue patterns (Figure 4).

c. *Tridacna squamosa* (fluted or scaly giant clam) is easily identified by the large fluted scales on the shell. The mantle is generally mottled in blue, brown and green. Sizes reach up to 40 cm (Figure 3).

d. *Tridacna maxima* (rugose or small giant clam) is the most wide-ranging giant clam species, being found from the east coast of Africa to as far east as the Red Sea and eastern Polynesia. It is recognizable by its brightly colored mantle (blue, green and brown) and boring habit (Figure 5).

e. *Tridacna crocea* (crocus or boring giant clam) is similar to *T. maxima* in that it is a boring species and has a brightly colored mantle. This species is generally smaller and more triangularly ovate in shape than *T. maxima* (Figure 6).

f. *Tridacna tevoroa* (deep water devil clam) is a rare species that lives at depths of greater than 20 m in the northern Tonga Islands and eastern Fiji Islands.

g. *Tridacna rosewateri* is a newly described species that is very similar to *T. squamosa* and only occurs on the Saya de Malha Bank in the Indian Ocean.

h. *Hippopus hippopus* (horse's hoof or strawberry giant clam) has a heavy, thick shell composed of triangular **valves** with sharp, jagged teeth. The mantle is a dull yellow-brown and does not extend over the margin of the shell (Figure 7).

i. *Hippopus porcellanus* (China clam) differs from *H. hippopus* by having a lighter, less ribbed shell although the mantle color is similar. The **incurrent siphon** of the China clam is lined with fringing **papillae**. *H. porcellanus* has a very limited range in the region of Indonesia, the Philippines and Palau.

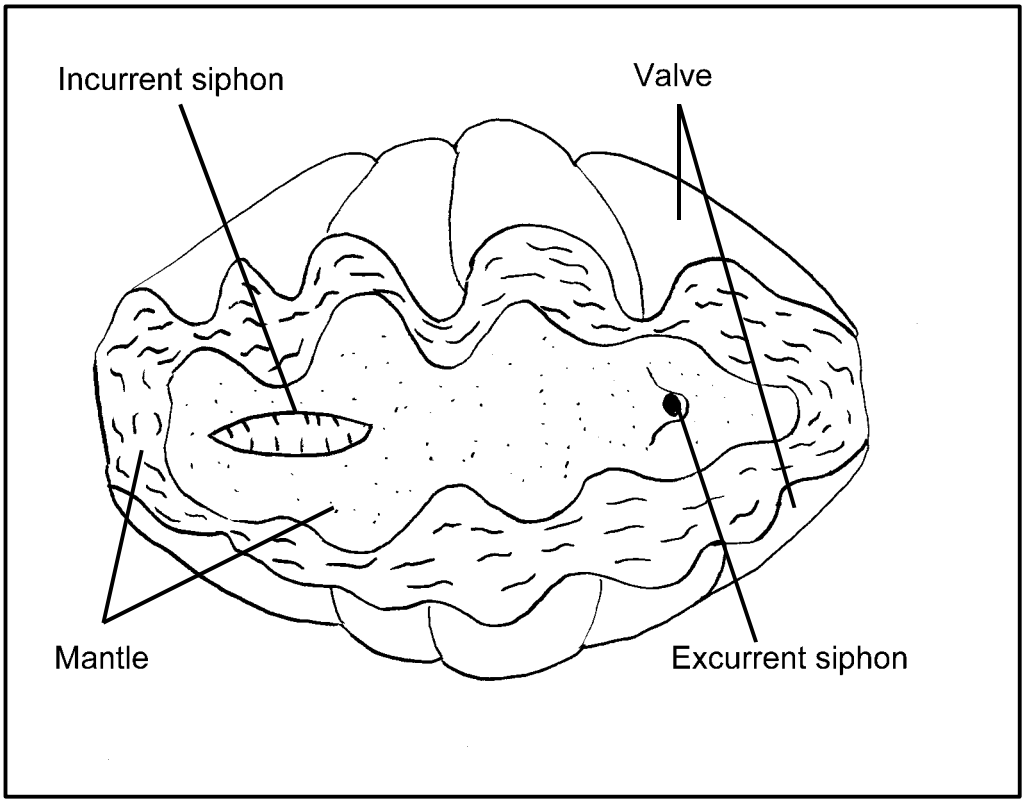


Figure 1. Schematic diagram of a Tridacnid clam showing basic body parts.

Note: Detailed descriptions with illustrations and photographs of giant clam species can be found in Lucas, 1988; Braley, 1992a and Knop, 1996.

Figure 2. Tridacna gigas.

Figure 3. Tridacna squamosa *(photo courtesy of CRRF, Palau).*

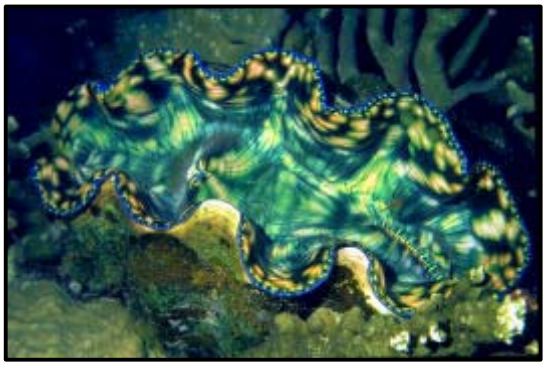


Figure 4. Tridacna derasa *(photo courtesy of CRRF, Palau).*

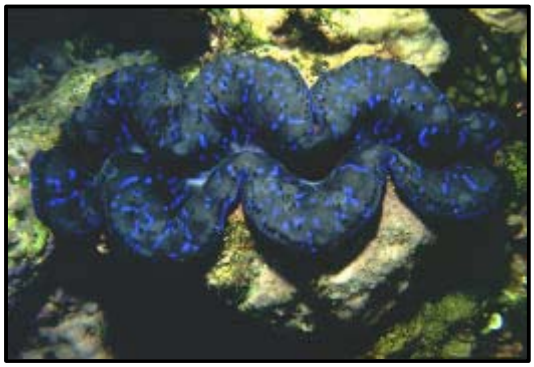


Figure 5. Tridacna maxima *(photo courtesy of CRRF, Palau).*

Figure 6. Tridacna crocea *(photo courtesy of CRRF, Palau.*

Figure 7. Hippopus hippopus *(photo courtesy of CRRF, Palau.*

What are the uses for giant clams?

Giant clams have been traditionally used as a subsistence food source throughout their range. In more recent times, the meat has become a delicacy and is even considered an aphrodisiac in some Asian and Pacific markets. The most recent use for the more brightly colored species is as living decoration in home and public aquariums. The shell also has many uses for both practical and decorative purposes, being used to make dishes, tools, jewelry and ornaments (Heslinga, 1996).

Why raise giant clams?

The initial interest in culturing giant clams stemmed from concerns related to the decline of wild stocks. Increasing coastal populations, pollution and improved harvesting efficiency (power boats and diving gear) are just some of the factors that have contributed to the collapse, and in some cases extinction, of local stocks of giant clams throughout their range. Initial hatcheries were developed with the intent of reseeding depleted reefs and growing clams as a food source to relieve pressure on wild stocks. Giant clams are also grown specifically for sale as aquarium species, the most lucrative market. Today, government and commercial hatcheries exist in most tropical Pacific nations and island groups where giant clams occur (Lucas, 1996).

Biology and Environmental Requirements

Biology

From a biological standpoint, giant clams are well suited for aquaculture. While giant clams filter food through their gills, they differ from most other bivalves in that they derive a substantial portion of their nutrition from a **symbiotic** relationship with millions of **photosynthetic** algae called **zooxanthelle** (*Symbiodinium microadriaticum*) that live in their fleshy, prominent mantle. While zooxanthelle produce mainly complex sugars, they can also produce amino acids and fatty acids, a portion of which are released through the algal cell wall directly into the bloodstream of the clam. The direct benefit of this symbiotic relationship to clam farmers is that giant clams can be grown through their entire life cycle with clean seawater and sunlight as the only sources of input. Another important biological aspect of giant clams that is of value to farmers is their high **fecundity**. Numbers of eggs spawned ranges from millions in small species such as *T. crocea* to hundreds of millions in larger species such as *T. gigas*.

Life-cycle

Giant clams are **protandric hermaphrodites**: they mature first as males in two to three years, then develop gonads with both sperm and egg releasing components. Sperm release precedes egg release during spawning, presumably to prevent self-fertilization. Size and age at maturity varies with species and geographical location.

Early larval development of giant clams is typical of bivalves. Eggs are approximately 100 μm in diameter and hatch into ciliated free-swimming **trochophores** within 12 hours of fertilization (Figure 8). The trochophore develops into a filter feeding, bivalve **veliger** or D-stage larva of 160 μm shell length approximately two days after fertilization (Figure 9). This veliger later develops a foot to become a **pediveliger** (Figure 10) that alternately swims and rests on the substrate, eventually metamorphosing into a 200 μm juvenile clam at day 8-10 post-fertilization. **Metamorphosis** is physically characterized by a sloughing off of the **velar** tissues and **cilia** and marks the beginning of the symbiotic relationship with the zooxanthelle. Growth generally follows a sigmoid curve, starting off slowly then accelerating after approximately 1 year and slowing again as the animals approach maturity.

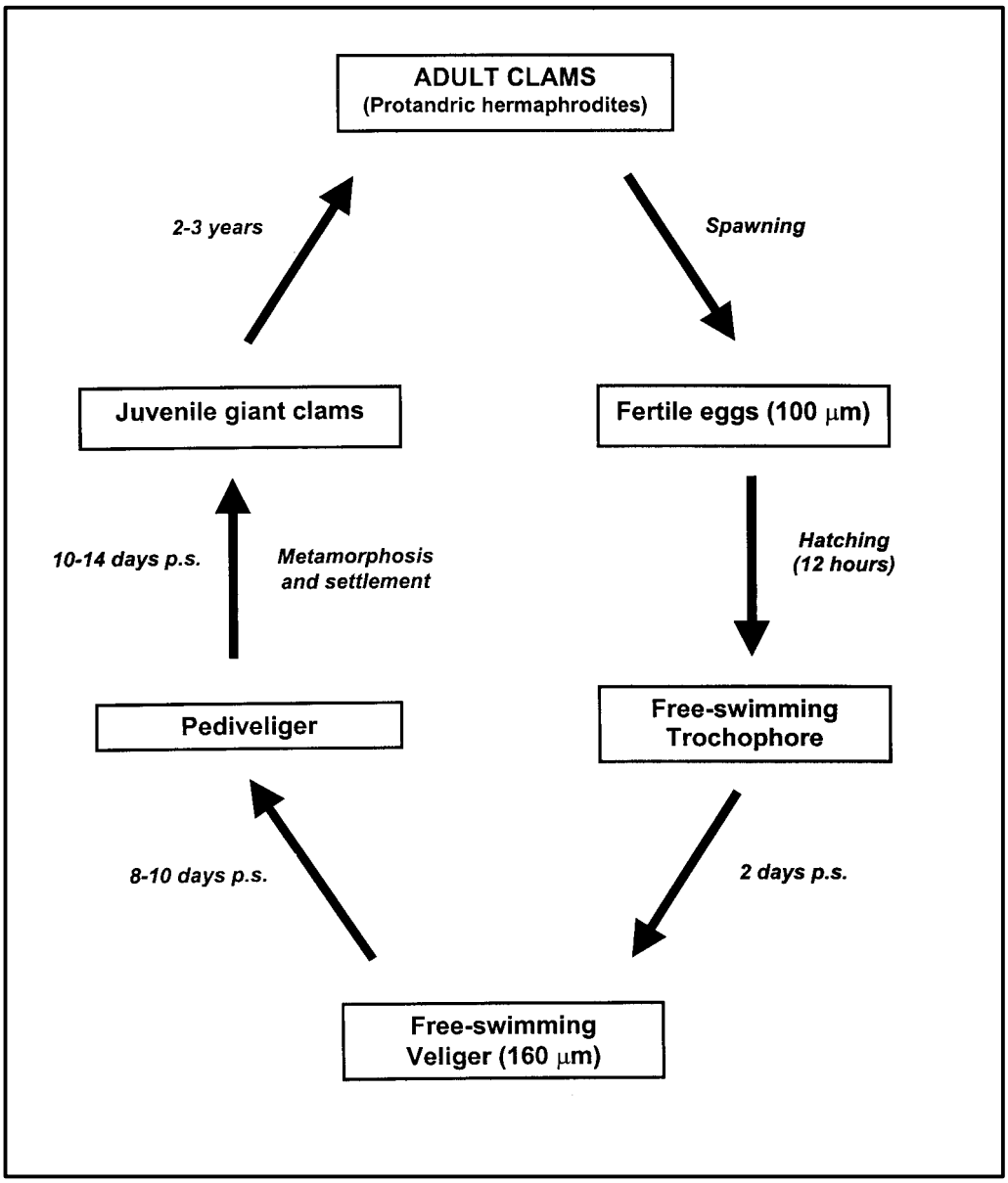


Figure 8. Flow diagram describing the life cycle of Tridacnid clams

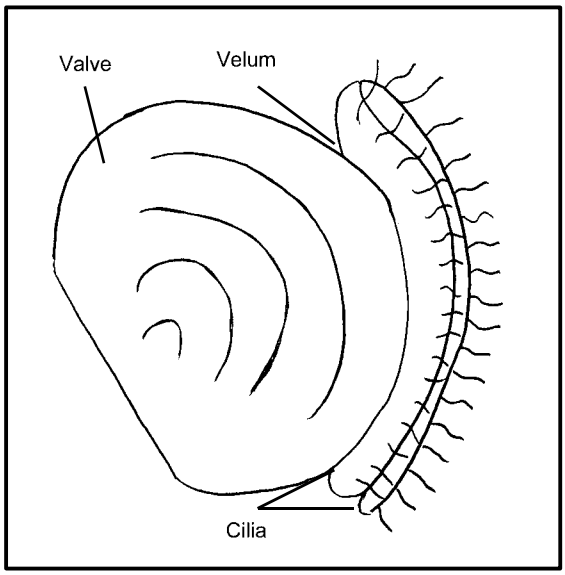
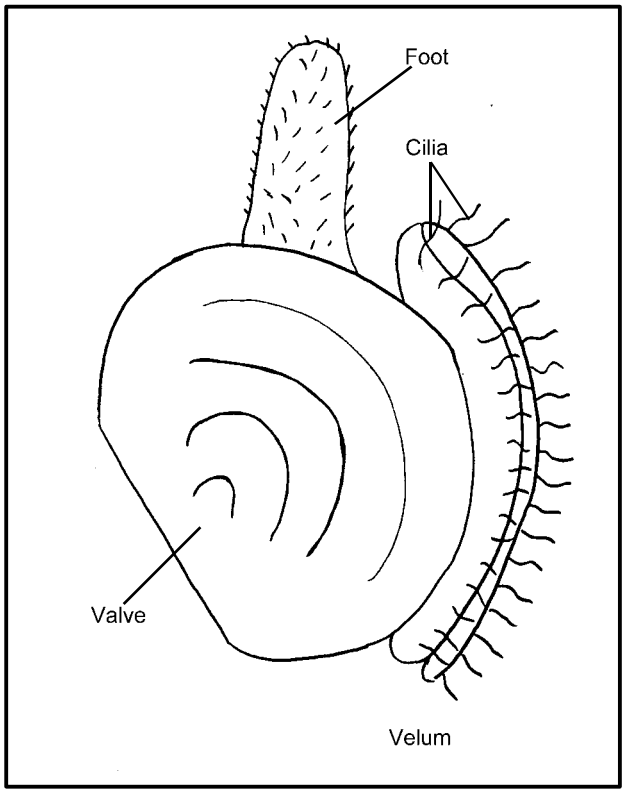


Figure 9. Schematic diagram of a Tridacnid veliger larvae

Figure 10. Schematic diagram of a Tridacnid pediveliger larvae



Environmental requirements

As evidenced by the areas where giant clams naturally occur, they require clear tropical seawater for optimum growth and survival. Water temperature should range from 25-30^{o}C, salinity should range from 32-35 ppt and pH should range from 8.1 to 8.5. Sunlight is important for photosynthesis to occur, and only one species of giant clam, the rare *T. tevoroa*, occurs at depths below 20 m. Giant clams deprived of sunlight quickly die despite the presence of food in the water, indicating the importance of zooxanthelle to clam survival. Mild siltation or turbidity can be tolerated by some giant clam species but clear, tropical, oceanic water is preferable. Giant clam hatcheries should be equipped with a dissolved oxygen meter, a pH meter and a salinity refractometer to monitor these water quality parameters (suppliers 1 and 5 in Appendix A).

Figure 11. The need for clean, pollution free seawater sometimes means drastic measures. This seawater intake at the has been covered with concrete to protect it from the wave surge.

Hatchery Considerations

Site

To spawn giant clams, a suitable site is required for holding broodstock and raising the larvae. While giant clam broodstock can be kept in ocean holding areas, larval rearing takes place almost exclusively in land-based tanks. Criteria to consider for choosing a suitable hatchery site are:

a. access to pollution-free seawater: the hatchery should not be situated close to point sources of pollution such as garbage dumps, sewage out-falls, or dredging sites. Areas with excessive freshwater run-off should also be avoided

b. proximity to a freshwater supply and electrical utilities: while it is convenient to be located in an area with municipal utilities, many giant clam hatcheries operate in remote locations using generators or alternative energy sources such as solar power to supply electricity and rely on rainwater catchment.

c. proximity to main roads and airports: while close proximity to sources of transport are preferred this again is not always possible.

d. land topography: an area should be selected that is close to the seawater source but is high enough not to flood during heavy rains or tropical storms.

e. security: both clams and equipment may go missing if security is not adequate at the hatchery site. Many hatchery sites are fenced or have full-time security measures.

Hatchery hygiene

In all aspects of clam hatchery work, simple hygiene rules need to be followed in order to prevent unnecessary disease and contamination problems. All containers used in the hatchery should be thoroughly cleaned and rinsed prior to use. Non-toxic detergents such as Simple Green® can be used for cleaning but containers must be rinsed thoroughly prior to use. A bleach solution (0.5 mls for every liter of water) can be used for sterilization but containers should again be thoroughly rinsed or preferably dried prior to use. Sun drying of equipment between uses is an excellent method of preventing disease and contamination.

All seawater used in the spawning and larval rearing of giant clams should be filtered to 1 μm.

Hatchery personnel should be careful with use of skin applications. Insect repellents, sunscreens and skin lotions should never be introduced into static cultures such as spawning tanks, collecting containers or larval rearing tanks. It is good practice for hatchery staff to thoroughly wash their arms from the elbow down before undertaking any work with static cultures of eggs or larvae.

Water filtration

The main predators of giant clams are the Pyramedellid and Cymatium snails. These snails enter the tanks as larvae and attack the clams, often by entering the animal through the **byssal opening**. Entry of snail larvae into broodstock tanks can be stopped by filtering the water down to 25 μm. The easiest way to do this is by attaching filter bags directly to the tank inflow pipe (Figure 12). Alternatively, a cartridge or rapid sand filter system can be used. Filter bags, rapid sand filters and cartridge filters can be purchased from vendors 1 and 5 in the supply source (Appendix A).

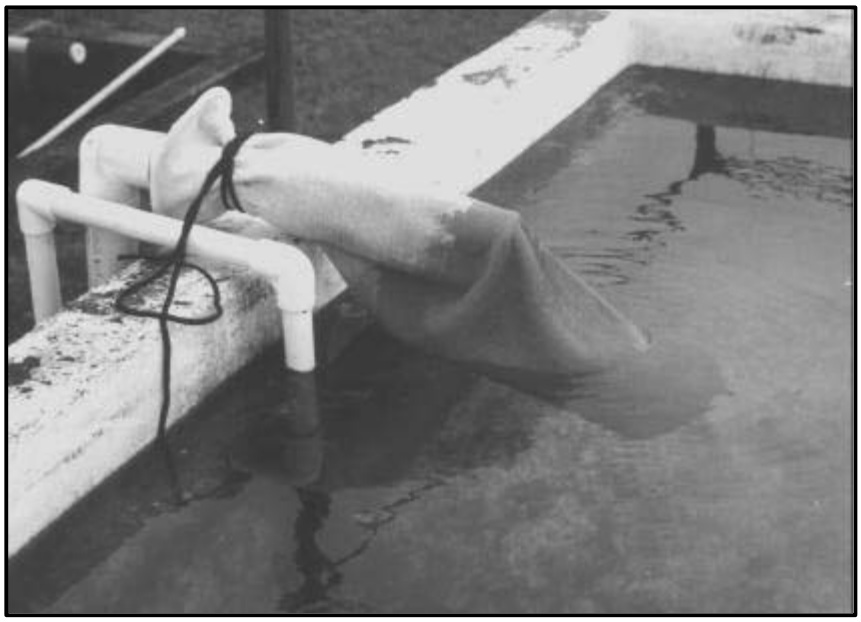


Figure 12. Polyester filter bags such as this one are widely used in giant clam hatcheries to prevent introduction of predatory snails.

Record-keeping and protocol

It is inevitable that technical and disease problems will occur in every hatchery. For this reason it is important that each hatchery develop a set rearing protocol that is strictly adhered to by all technical staff. As advancements are made in rearing methods the hatchery protocol can then be changed accordingly. A daily log of rearing activities with written comments by the technicians is also essential. Many problems with rearing or disease can often be easily traced to one particular activity or event that was recorded in the daily hatchery log.

Broodstock Acquisition and Husbandry

Acquiring broodstock

New broodstock can be collected from the wild or grown from locally reared seed. Alternatively, broodstock can be shipped in from another location. However, this can raise concern over the import of disease organisms with the clams or genetic alteration of local stocks and should be conducted according to local government regulations. Broodstock should be carefully selected for uniformity of shape, appropriate color and general health.

Holding broodstock

Broodstock can be held in an offshore location or in land-based facilities. Offshore locations should be 5-10 m deep in areas of coral growth. Larger animals can be nestled among coral heads while smaller clams should be protected from fish predation by cages or other exclusion methods (Figure 13). The smaller, burrowing clams (*T. maxima* and *T. crocea*) should be allowed to attach to a piece of tile, brick or concrete. Using a reef setting for holding broodstock approximates the conditions they encounter in their natural habitat and optimizes reproductive conditioning. Clams in an offshore holding area should be checked once monthly for predators such as boring algae or snails (see section on Broodstock Diseases, page 20), and cages should be regularly cleaned to remove fouling organisms and the eggs of predatory snails.

Prior to spawning, broodstock are collected from the offshore holding area by divers using SCUBA or snorkeling equipment. A canoe or small motor boat is usually used for collection (Figure 14). Clams are first concentrated in one area then hoisted to the surface using a rope attached to a mesh bag. Once the clams are brought on-shore, their valves are thoroughly scrubbed (Figure 15) to remove algae, snails and other epiphytes. Cleaned clams should be placed in a clean tank or raceway with flowing ambient seawater. Care should be taken not to leave the clams out of the water or expose them to excessive sunlight for a long period (more than a few hours) during the collection process as the stress involved may trigger them to spawn immediately upon re-immersion in water. As a contingency, spawning equipment (see section on Egg and Sperm Collection, page 28) should be on hand in case spawning occurs.

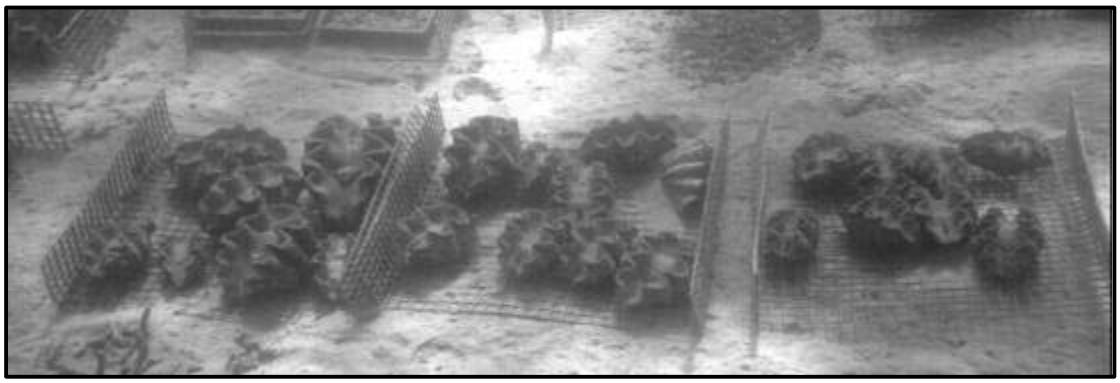


Figure 13. These broodstock T. gigas *are held on an open sand bottom on wire trays placed among coral heads at the National Aquaculture Center of the Federated States of Micronesia in Kosrae.*

Figure 14. Broodstock are retrieved from the offshore holding area using an out-rigger canoe at ICLARM's Coastal Aquaculture Center on Gaudalcanal, Solomon Islands.

Figure 15. Broodstock are thoroughly scrubbed to remove predators and epiphytes before being placed in the spawning tank.

Note: Clams removed from the water should be laid on their side to prevent the mantle sagging into the shell and damaging the viscera.

Land-based holding tanks vary in size and shape. The most popular design is a round, square or rectangular tank constructed of concrete (Figures 16 and 17). However, simple structures of plywood or locally available materials with non-toxic PVC liners are commonly used (Figures 18 and 19).

Figure 16. Rectangular concrete raceways at the Palau Mariculture Demonstration Center in the Republic of Belau.

Figure 17. Round concrete tanks at ICLARM's Coastal Aquaculture Center on Guadalcanal, Solomon Islands.

Figure 18. Simple holding tanks constructed of plywood or wire frame with vinyl pool liners are used at the Palau Mariculture Demonstration Center in the Republic of Belau.

Figure 19. Holding tanks are constructed from coconut logs and vinyl liners at ICLARM's Coastal Aquaculture Center on Guadalcanal, Solomon Islands.

Broodstock husbandry

Husbandry techniques for broodstock giant clams should adhere to the following guidelines to ensure optimal health and conditioning:

a. a minimum of 2-2.5 water exchanges of the tank volume per day to provide essential nutrients and minerals to the clams.

b. filtering the incoming seawater to 25 μm to prevent the larvae of predatory organisms, such as Pyramidellid and Cymatium gastropod snails, and fouling organism from entering the tank.

c. a 20-30 cm clearance from the top of the clam's mantle to the water surface.

d. a minimum 50% light occluding shade cloth over the tank to prevent sun damage.

e. aeration to facilitate water circulation. This prevents low oxygen pockets from forming in the tank and ensures equal nutrient delivery from incoming seawater to all clams.

f. "feeding" of ammonium based nitrogen products. This has been shown to significantly increase clam growth and improve condition in land-based systems (Heslinga et al., 1990; Belda et al., 1993; Fitt et al., 1993; Knop, 1996). A daily addition of 100 μM ammonium nitrate (4 g per 1000 L of tank volume) in the morning is sufficient to raise nitrogen levels for the rest of the day.

g. use of herbivorous animals such as rabbit fish, surgeon fish or *Trochus*, in combination with regular cleaning to prevent excessive algal fouling in the tanks.

Good broodstock husbandry should be a balance between controlling tank fouling and not disturbing the clams too frequently. Tanks should be cleaned when algal fouling is starting to encroach on the clams (approximately every 3-6 months). Fouling is generally dependant on the nutrient level of the water. When fouling becomes heavy, clams should be removed from the tank and the mantles scrubbed thoroughly. They should then be restocked in a clean tank that has been sun-dried prior to use. Care should be taken not to detach the clams from the substrate unless necessary because the energy required to re-grow the **byssus** and reattach to the substrate reduces the energy available for growth and reproductive conditioning.

Note: Primary algal growth can be quite easy to control with grazing organisms. However, secondary algal growth is often characterized by green filamentous algae which can be noxious to grazers and clams alike.

For hatcheries with the goal of mass production, the number of each species that should be maintained varies from producer to producer. Recommendations range from a minimum of 35 to a maximum of 200 clams per species with 25-35% of broodstock being used during each attempted spawning. Smaller species generally require more animals to produce the same number of eggs as larger species because of the direct relationship between clam size and fecundity in giant clams. Broodstock productivity and frequency of spawning can be monitored by individually tagging each clam. Tags can be made of stamped metal or plastic such as Dymo tape (6*) that are attached to the shell with a two part underwater marine epoxy glue such as Petit PolyPoxy ® (7*).

see Appendix A for supply source.

Following spawning, some clams may die, so new broodstock, either wild or hatchery reared, should constantly be added to the existing population. Post-spawning mortality can be reduced by holding clams in on-shore tanks for 1-2 weeks before returning them to the offshore holding area. This allows animals to recover from the stress of spawning and makes them less susceptible to predation.

Broodstock Diseases

Parasitic snails

The biggest disease threat to giant clams kept in land-based tanks or in offshore holding areas is infestation by the parasitic Pyramidellid and Ranellid snails. Problems with specific types of snails tend to be regional, and it is not unusual to find that a troublesome species in one area does not present a problem elsewhere. However, in almost every place where giant clams occur, so do predatory snails of some sort.

Clams should be inspected regularly for signs of snail infestations. Symptoms vary depending on the level and duration of the infestation but include: bleaching or loss of mantle color; an incompletely extended mantle; gaping (the clam valves are too far open, stretching the mantle tissue); and sporadic mortality. If any of these symptoms are observed, the tank or cage should be thoroughly inspected for signs of snails.

Ranellid snails are voracious predators of giant clams and act by injecting a poison which allows the snail to start eating the clam flesh. The best known clam predators in the family Ranellidae are in the genus *Cymatium*. Reaching up to 50 mm in length, these snails occur throughout the tropical Pacific and can enter clams at a size of 1-2 mm. They then proceed to devour the clam until it succumbs and dies. The only method for control of this predator in offshore holding areas is to pick through cages and remove visible snails. Clams can be taken ashore and washed before being returned to a clean cage in the holding area. In some cases, clam cages can be relocated or raised off the ocean floor to prevent adult snails entering the cages. In shore-based holding tanks where filtration has proven ineffective, clams must again be inspected for snails before being thoroughly cleaned and moved to a new tank. If the clams have been held in trays containing gravel, they must be transferred to clean gravel and the old gravel discarded. Maintaining clams on a flat surface such as concrete is

thought by some farmers to provide less habitat for juvenile snails, thereby reducing the rate of predation.

Pyramidellid snails rarely grow over 10 mm and are more of a problem to small or juvenile giant clams. For this reason, they are usually only affect smaller broodstock such as *T. maxima* and *T. crocea*. They feed by using a trunk to suck body fluids from the mantle of the clam. Pyramidellid snail larvae do not spend any time in the plankton but hatch from the egg as juveniles at the site where they were laid. Consequently, an infestation of Pyramidellid snails can proliferate very quickly in land-based tanks. Pyramedellid snails reproduce every 90-120 days. Hatcheries in areas where these snails commonly occur can control infection by breaking the reproductive cycle of the snails. Every three months, the clams should be removed from the tank or offshore holding area and thoroughly cleaned. They should then be returned to a clean offshore cage or land-based tank.

Boring sponges and algae

Certain types of boring sponges and algae can penetrate the valves of giant clams, consequently weakening the shell and increasing the clams susceptibility to other infections. Boring sponges can be recognized by a series of holes approximately 1 mm in diameter that are filled with the yellow, brown or orange tissue of the sponge. Boring algae are hard to recognize externally, but infested animals exhibit a green hue to the inner part of the shell that can be seen under the mantle.

Both boring sponges and algae can be easily controlled by regularly washing the clam shell with an anti-fouling treatment. The most simple form of anti-fouling is freshwater, which quickly bursts the cells of the marine dwelling algae and sponges by osmotic shock. Other treatments include scrubbing the shell with a 1% formalin solution (Braley, 1992b; Knop, 1996) or hydrogen peroxide. It is important not to allow formalin or hydrogen peroxide to enter the clam; exposure to freshwater should also be limited.

Note: Detailed accounts of giant clam diseases and treatments can be found in Braley, 1992b and Knop, 1996.

Induced spawning

Spawning in giant clams has been shown to follow both a **diel** and **lunar** pattern (Heslinga et al., 1990; Braley, 1992a; Matthew Hollis and Cletus Oengpepa, pers. comm.). Spawning activity tends to be highest in the mid to late afternoon and during full and new moon periods. Induction of spawning should be planned around these times to take full advantage of the clam's natural reproductive cycle.

The reproductive condition of giant clams is sometimes monitored by removing a small number of eggs from the gonad. This is called biopsy and involves inserting a hypodermic needle through the mantle into the gonad. Improperly conducted biopsies can easily puncture the kidney or other vital organs causing death. Biopsy is increasingly considered unnecessary, and the less reliable but safer method of observing visceral color is more commonly used. Color of the viscera surrounding the gonad ranges from dull brown to bright orange in color when viewed through the excurrent siphon. Frequently, but not always, a deep orange color indicates a readiness to spawn. Use of visceral color in coordination with diurnal and lunar cycles generally produces reliable spawning results.

Clams that are selected for spawning are placed in a clean spawning tank supplied with flowing seawater. Three methods are recognized for reliably inducing spawning in giant clams: heat stress, gonad extract and serotonin (Heslinga et al., 1990; Braley, 1992a; Gervis et al., 1996; Lucas, 1996). The choice of method depends mainly on the personal preference of the hatchery manager. Some culturists only use serotonin as a last resort, while others use it routinely.

Heat stress

Equipment: Thermometer (1 or 2*)
**see Appendix A for supply source.*

Rapidly changing the internal temperature of giant clams often induces spawning. This can be accomplished by turning off the water flow to the spawning tank, lowering the water level and allowing the sun to heat the water to about 34°C. Alternatively the clams can be removed from the tank and placed on their side in the sun for 1-2 hours (Figure 20). Water flow is then resumed to the

tank or the clams are returned to the spawning tank for a 30-60 minute recovery period. The heating and recovery process can be repeated 3-4 times throughout the day.

Stressing clams in the morning will often produce spawning in the late afternoon. Some hatchery operators prefer a low-level heat stress method, which only changes the water temperature 1-2^{o}C every day over a period of days. Heat stressing can be a time-consuming process but is relatively harmless to the animal and does not require chemical stimulation. However spawning may take hours or even days to induce, and some hatchery managers prefer the more reliable, chemically induced spawning methods. Heat stress is often used in conjunction with other spawning methods to increase the likelihood of spawning.

Figure 20. One method of heat stressing is to leave the clams in the sun for 1-2 hours. Note that the clams are left on their side to prevent the mantle sagging onto the viscera.

Note: Near lethal temperature for giant clams is 35^{o}C, and care must be taken not to exceed this temperature. Signs of excessive heat stress are manifested at a later date by bleaching (expulsion of zooxanthelle) of the mantle and, in some instances, mortality.

Gonad extract

Equipment: kitchen blender (Local purchase)
200 μm nitex screen (1*)
10 ml syringe (2)
**see Appendix A for supply source.*

Giant clams are epidemic spawners and are stimulated to spawn by the presence of giant clam gametes (eggs or sperm) in the water. Hatchery managers can exploit this fact and induce clams to spawn by introducing an extract of ripe clam gonad into the water.

Gonad material is first removed from a sacrificed clam and approximately 20 g is macerated in a blender using filtered seawater (1 μm). This suspension is then poured through a 200 μm sieve to remove large particulates. The water level in the spawning tank is first lowered until it is 2-4 cm above the clam mantles, and the water flow is turned off. The gonad extract is then either added directly to the tank water (Figure 21), or approximately 5 mls is squirted into the incurrent siphon of each clam using a syringe (Figure 22). Spawning generally occurs within 1 hour of the addition of gonad extract.

Remaining gonad or gonad extract can be frozen and used at a later date, thereby preventing the unnecessary sacrificing of broodstock for every spawn. Water containing giant clam gametes can have an effect similar to using gonad extract and can also be frozen for later use. Using gonad extract from one species of giant clam can induce spawning in another species, but results are more variable.

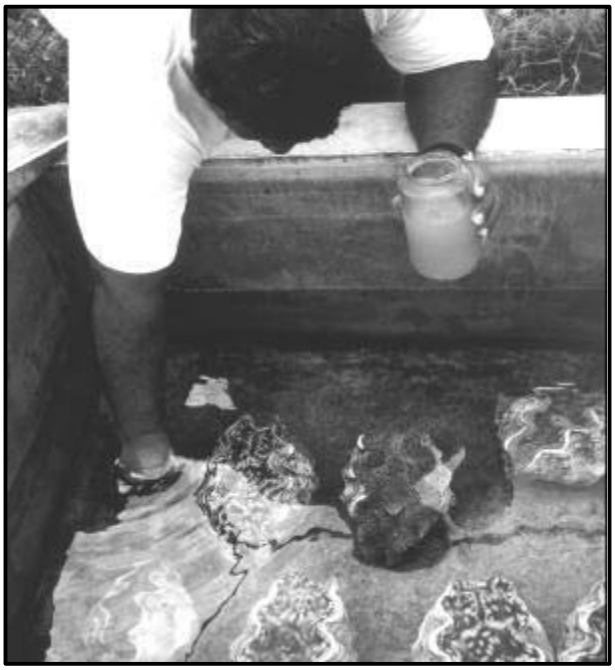


Figure 21. Gonad extract being poured into a tank of broodstock T. derasa*.*

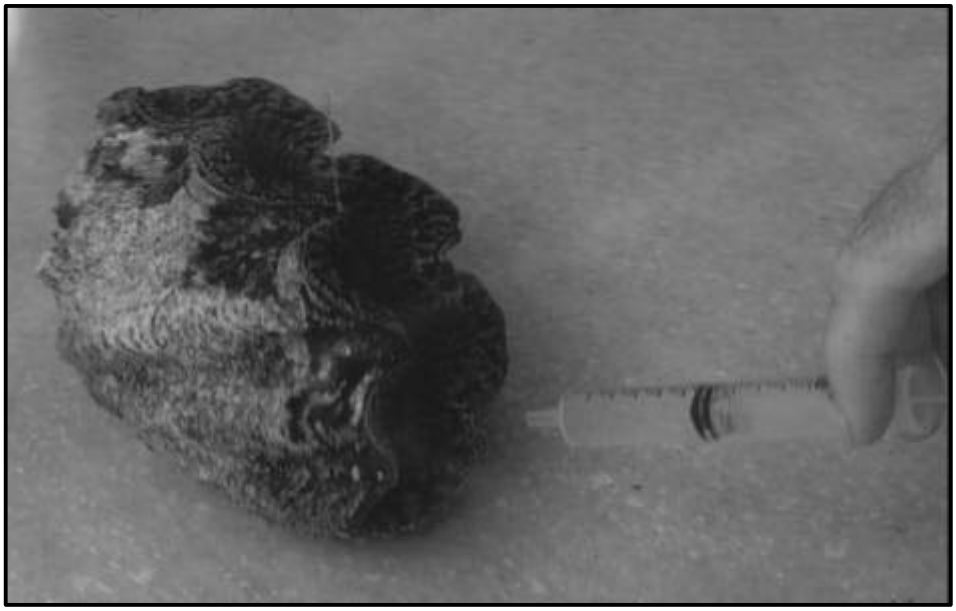


Figure 22. Gonad extract being injected into the incurrent siphon of a broodstock T. maxima*.*

Serotonin

Equipment: Serotonin (3*)
Plastic syringe (2)
150 μm hypodermic needles (2)
Electronic balance (1, 2 or 5)
Refrigerator (local vendor)
Isopropyl alcohol (local vendor)

see Appendix A for supply source.

Serotonin (5-hydroxytryptamine creatinine sulphate complex) is a neurotransmitter that will produce spawning in any giant clam that has ripe or developing gonad material (Braley, 1985). Because the eggs may be released at sub-optimal stages, egg quality and larval viability may be poor if the gonad is not in spawning condition. Gonad condition is governed by the time since the last spawn and diel and lunar phases.

A 20 μM concentration (15.7 mg in 20 ml of 1 μm filtered seawater) of serotonin is injected directly into the gonad of the clam using a 150 μm bore hypodermic needle attached to a plastic syringe. The amount of serotonin injected depends on the size of the animal. Generally 2 mls is used for larger clams (*Hippopus spp.*, *T. gigas*, *T. derasa* and *T. squamosa*) and 1 ml for smaller clams (*T. maxima* and *T. crocea*). The syringe and needle should be flushed with isopropyl alcohol between injection of each clam (Figure 23). Serotonin injected into a ripe animal will induce sperm release usually within 5-10 minutes.

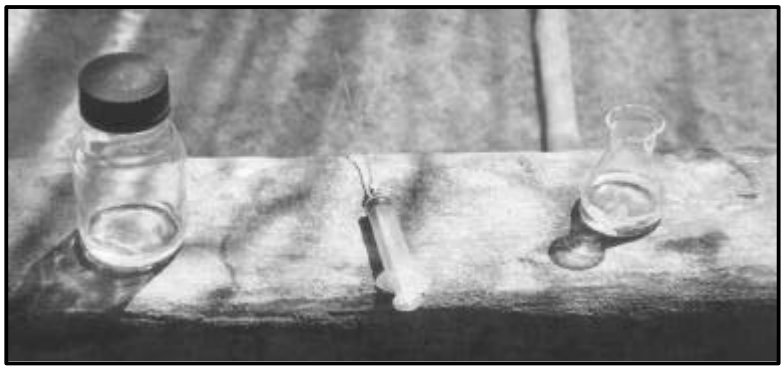


Figure 23. A long hypodermic needle and syringe (center), with isopropyl alcohol (left) and serotonin solution (right) are needed for injecting larger giant clams such as T. gigas *and* T. derasa *.*

Injection of serotonin can cause broodstock mortality, as an inexperienced technician can easily puncture the heart or viscera of a giant clam while administering the drug. In *Hippopus spp.*, the gonad can be clearly seen through the exhalent siphon, making injection relatively easy. In *Tridacna spp*., the serotonin can be administered in one of two ways. Using the first method, the needle is inserted through the mantle below the excurrent siphon in order to avoid the heart. Once the needle has passed through the mantle, the next point of resistance will be the gonad. The needle should be inserted 1-2 mm into the gonad tissue prior to injection.

The second method can be applied only to species with a large byssal opening such as *T. maxima*, *T. crocea* and *T. squamosa*. Using this method, the needle is inserted into the gonad through the muscle covering the byssal opening (Figure 24).

Figure 24. Broodstock T. maxima *being injected with serotonin through the byssal opening. Note the large byssal opening of the clam in the foreground.*

Note: Serotonin in the powdered form should be kept refrigerated or frozen. Excess serotonin solution can be stored in a refrigerator for up to one month or frozen for longer periods. Serotonin solution is clear and should be discarded after it turns yellow or brown.

Gamete Release and Collection

Egg and sperm collection

Equipment: 20 L buckets or larger tubs (local vendor or 1*)
Labeling tape and markers (local vendor or 2)
**see Appendix A for supply source.*

Prior to spawning, approximately 10 clean collecting containers should be placed adjacent to the spawning tank for egg and sperm collection. The size of the container should be proportional to the size of the clam. For example, 20-L buckets are usually adequate for smaller clams such as *T. maxima* or *T. crocea*, whereas larger tubs may be needed for the bigger animals such as *T. gigas*, *T. derasa* or *H. hippopus* (Figure 25).

Figure 25. A typical spawning tank scene at ICLARM's Coastal Aquaculture Center on Guadalcanal, Solomon Islands. Note the individual collection containers and ready supply of filtered seawater.

After spawning induction, by whatever means chosen, clams are placed in a spawning tank and closely observed for signs of gamete release. Initial signs of spawning will be gaping and contraction closely followed by sperm release. Egg release is always preceded by sperm release in giant clams. Both egg and sperm release are often characterized by a series of contractions, with ever increasing numbers of gametes being released during each contraction. This is followed by reduced gamete release with each contraction, as the animal becomes spent.

Once an individual clam is seen releasing large quantities of sperm, it is removed from the spawning tank and placed in a collection container partially filled with 1-μm filtered seawater. To ensure adequate fertilization rates and genetic variation, sperm should be collected from at least three clams, each placed in a separate container. Large quantities of sperm are not required for artificial fertilization in giant clams. Each clam should be allowed only one or two strong, sperm releasing contractions in the collection container before being returned to the spawning tank.

Note: Collection containers should be filled with water just prior to the addition of clams. This reduces the risk of temperature shock to the clam and gametes. Clams in the spawning containers should also be left in a shaded area to reduce the risk of water heating and temperature shock.

As the rate of sperm release starts to diminish, clams should be closely monitored for the presence of eggs in the milt. This will be manifested by a granular appearance to the sperm. Over progressive contractions, sperm release will diminish and eventually cease, while egg release will gradually increase. When only eggs are being spawned, the clam should again be transferred from the spawning tank into a clean collecting container partially filled with 1 μm filtered seawater. Each egg-releasing clam should be placed in a separate collecting container, and egg release should be allowed to continue until the animal is spent. Some species of giant clam, such as *T. gigas* and larger specimens of *T. derasa*, are too heavy to be moved to a collection container. In this situation, eggs and sperm are collected by holding the opening of a polyethylene bag, such as a clean garbage bag, over the excurrent siphon of the clam.

Note: When spawning large numbers of clams, organization is essential. Labeling of spawning containers is highly recommended so that gametes from

individual clams can be quickly identified. Labeling tape and indelible markers can be purchased locally or through Carolina Biological Supply (Appendix A).

Fertilization

Equipment: 100 ml graduated cylinder (1, 2 or 5*)
**see Appendix A for supply source.*

At this point in the proceedings the culturist should have sperm and eggs held in separate containers. Fertilization is the process of adding sperm to the eggs. Giant clam sperm can remain active for up to one hour from release but eggs are viable for a shorter period. Therefore, sperm should be added to the eggs within 15 minutes of egg release. One of the greatest dangers involved in fertilizing giant clam eggs is the addition of too much sperm. This results in a condition known as polyspermy, where the egg surface becomes choked with sperm, resulting in death. Recommendations for the quantity of sperm to use range from adding 0.1 to 1% sperm water, by volume, to the container of eggs (Heslinga et al., 1990; Braley, 1992a). This equates to adding 1-10 mls of sperm water per liter of eggs. If there is any doubt about sperm concentration, it is better to add a lower volume of sperm water to the eggs. Experienced culturists try to determine the sperm concentration by the color of the water and add sperm water to the eggs accordingly.

If sperm was collected from more than one clam, then eggs should be fertilized with sperm from all the clams unless the eggs and sperm came from the same clam. Self-fertilization, the mixing of sperm and eggs produced by the same animal, generally produces very poor fertilization and survival rates in giant clams. Labeling the collection containers can help ensure that self-fertilization does not occur. After fertilization, eggs are left in a shaded area for at least one hour before being counted.

Note: When using sperm from more than one clam, the sperm water added from each clam must be reduced proportionally to account for the number of sperm donors. For example, if a total of 100 ml of sperm water is needed for fertilization and there are three sperm donors, then 33 ml (100/3) of sperm water should be added from each sperm donor.

Counting

Equipment: Stereo dissecting microscope (1 or 5*)
12- or 24- well tissue culture plate or counting chamber (1 or 5)
1 ml plastic pipette (1 or 5)
pipette pump (1 or 5)
hand held counter (1 or 5)

*see Appendix A for supply source.

It is important to know how many fertilized eggs are available for stocking. To do this, the volume of the collecting containers must be known. This is easily accomplished by filling each container with a known volume of water and marking the container at the water level. For instance, if 20- L buckets are being used for collection, each bucket should be filled with 20 L of water and marked on the outside of the vessel at the water level. Containers of eggs can then be "topped off" with filtered seawater to the known volume mark prior to counting.

The contents of each container must be thoroughly mixed prior to taking samples for counting. Mixing can be done with a beaker, stirrer or even a clean arm (Figure 26). It is important not to stir in a circular motion which will concentrate the eggs in the center of the container. A 1 ml pipette is used to take at least five 0.5 ml samples from each container, and each sample is placed in one well of a tissue culture plate or counting chamber.

Figure 26. A "plunger" style egg mixer ensures an unbiased sample is taken by avoiding a circular stirring motion.

The numbe e culture tray or counting chamber is then counted using a stereo dissecting microscope.

Fertile eggs that are developing normally will have cleaved into 2 and 4 cells within 2 hours of sperm addition and are most easily distinguished from infertile eggs at this time. Unfertilized eggs will not be cleaved and may appear irregular in shape or have clear areas in the yolk. The percentage of fertile eggs is often used as an indicator of egg quality and expected larval survival. Fertility of high quality eggs should be greater than 80%.

Note: Counting the eggs can be made easier by using a scalpel or razor knife to scratch regularly spaced lines, approximately 1 mm apart on the underside of the tissue culture tray or counting chamber.

How to determine the number of eggs in a container

a) Average number of fertile eggs = (total fertile eggs counted in all 0.5 ml samples) / (total number of samples). Minimum of five samples.
b) Average number of infertile eggs = (total infertile eggs counted in all 0.5 ml samples) / (total number of samples). Minimum of 5 samples.
c) Multiply the number of liters in the collecting container by 2000.
d) Total fertile eggs per container = a x c
e) Total infertile eggs per container = b x c
f) Total eggs in the container = d + e.
g) Average number of infertile and fertile eggs = a + b.
h) Percent fertile eggs = a / g x 100.
i) Percent infertile eggs = 100 - h.

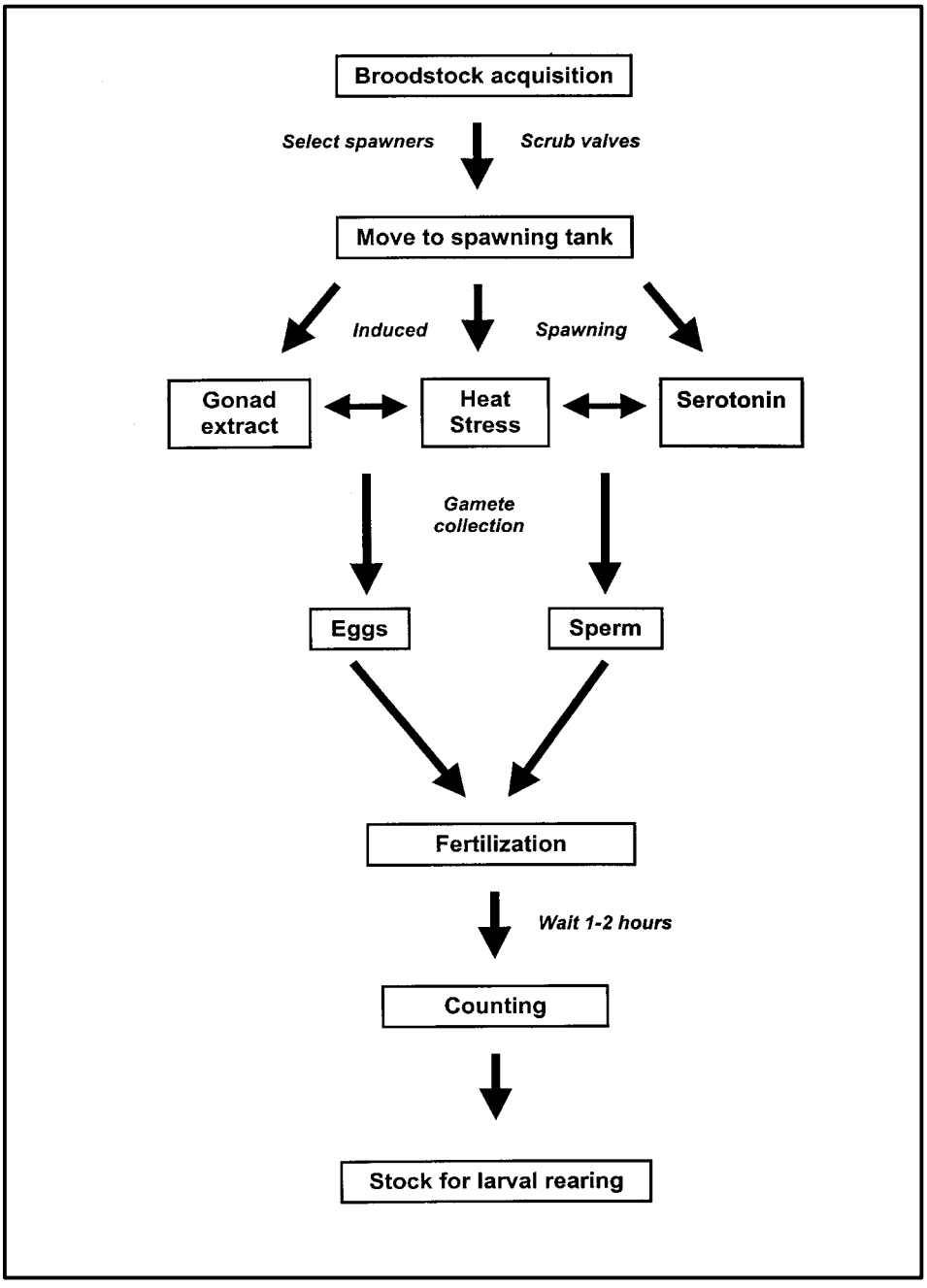


Figure 27. Flow diagram describing sequence and events of induced spawning in giant clams.

Larval Rearing through Metamorphosis

There are many ways to raise giant clam larvae through metamorphosis. These range from complicated or “intensive” rearing methods through simple or “extensive” rearing protocols. In general, intensive rearing methods produce higher survival but are more labor intensive and costly. Some culturists feel that giant clam fecundity is so high that survival is not an important issue and prefer the less costly, extensive rearing methods. Many clam hatcheries also employ a more intermediate or “semi-intensive” protocol. The level of intensity of larval rearing will be determined by many factors such as facility size, production goals, technical skill available, labor costs and desired capital investment. For example, a facility that has few broodstock but must produce a set quota of seed clams every month would most likely opt for a more intensive larval rearing regime.

Intensive larval rearing

While this is the most costly method of rearing giant clam larvae through metamorphosis, intensive larval rearing tends to produce higher and more consistent larval production.

Equipment: larval-rearing tank (see section on Rearing tanks, page 35)
2.5 cm diameter siphon tubing (1 or 5*)
plastic harvest tub approx. 50 x 70 x 30 cm (l x w x d) with a 3 cm drain hole approx. 15-20 cm up the side wall (local purchase)
80 μm sieve constructed of 36 cm PVC pipe
small aquarium air pump (1 or 5)
3 mm air tubing (1 or 5)
small airstones (1 or 5)
Neomycin or Streptomycin antibiotic (3)
electronic balance (1, 2 or 5)
disposable transfer pipettes (1 or 5)
counting equipment (see section on Counting, page 31)
20-L buckets (local purchase or 1)
binocular compound microscope (optional; 1, 2 or 5)

see Appendix A for supply source.

Rearing tanks

Rearing tanks for intensive culture vary greatly in shape (square, rectangular or round) and size (500 to 2000 L in volume), a factor which is usually dependant on locally available materials and operating budget. The most important factor in construction of larval rearing tanks is that they are lined with a material that is smooth, inert and non-toxic to the clam larvae. For this reason, fiberglass resin is widely used to construct or coat the walls of larval rearing tanks. Epoxy paints or vinyl liners offer a non-toxic alternative to fiberglass coatings.

A typical larval rearing tank would be round with a slightly cone-shaped bottom, approximately 750-1000 L in volume and constructed of fiberglass with a smooth epoxy gel coat (Figure 28). Because giant clam larvae are not sight feeders, tank color is not important, although dark tanks may enable hatchery staff to better monitor tank conditions i.e. larval motility, bacterial blooms and detrital build-up. Tanks should be placed on a stand or be equipped with a harvest sump to facilitate easy draining and should be placed under a covered structure, out of direct sunlight or rain, and with enough ventilation to prevent excessive heating during the day.

Figure 28. A locally made fiberglass larval rearing tank at ICLARM's Coastal Aquaculture Center on Guadacanal in the Solomon Islands.

Rearing Protocol

***Day 0 (stocking*)**

Larval rearing tanks should be thoroughly cleaned, sterilized and left to dry prior to use. Only seawater filtered to 1 μm should be used during all phases of larval rearing and all tanks should be filled with filtered seawater immediately prior to stocking the eggs. Temperature of the incoming seawater should be compared to that of the container of eggs. If temperatures vary by more than 1°C between the larval rearing tank and the container of eggs, stocking should be delayed until temperatures have equilibrated. This lowers the possibility of temperature shock to the eggs.

Eggs are stocked by pouring the water from the collection container gently into the larval rearing tank, through a fine mesh aquarium net or a 200 μm sieve to remove mucous and other unwanted debris. Eggs should be stocked at a density of no more than 20-30 eggs/ml (30 million per 1000 L of tank volume). Each tank should be equipped with one or two airstones to provide light to moderate aeration. Aeration at this point is mainly intended to circulate the water in the tank rather that to impart oxygen to the water. Heavy aeration may cause air-bubbles to become trapped in the valve of the developing larvae, resulting in mortality. Larval rearing tanks should be covered at all times to prevent introduction of insects, animal waste and other debris.

The use of antibiotics in improving larval survival has been documented by Fitt et al., 1992. Antibiotics should be used for at least the first two days of larval rearing. It is recommended that a mixture of 10 mg/l (10 g per 1000 L of tank volume) each of streptomycin and neomycin should be added to the larval rearing tank at stocking as this has produced higher survival of clam larvae than other antibiotics applied singly or in combination. Other antibiotics that have been used successfully are penicillin and rifampin (Fitt et al., 1992).

Note: excessive and improper use of antibiotics has been shown to lead to resistant strains of bacteria in hatcheries worldwide, often making problems of bacterial infection worse. For this reason, antibiotic use should be minimized where ever possible and experiments done in each hatchery setting to determine the optimum use of these drugs.

Stocking example:

To stock 20 eggs/ml into a 750-L larval rearing tank from a 20-L collecting container with 50 million eggs, you must determine:

a) eggs required to stock a 750-L tank at 20 eggs/ml = 750 x 1000 x 20 = 15 million eggs
b) volume of the egg container required to stock each tank = (15 million / 50 million) x 20 L = 6 L

Day 1 post-spawn (p.s.)

On day 1 p.s. the aeration level should be checked and adjusted if necessary. Aeration should be light to moderate. The water surface of the tank should be cleaned using two strips of polystyrene, which are brought together across the tank surface, trapping surface debris between them. A sample of larvae should be removed from the water column and examined using a compound microscope for rate of hatch, vigor and deformity. Activity of larvae in the water column can also be viewed by shining a flashlight into the tank.

Day 2 p.s.

By day 2 p.s., larvae will be in the veliger stage and can be harvested from the tank, counted and restocked into a clean larval rearing tank. This should take place no earlier than 40 hours post-fertilization. Larvae are collected on an 80 μm sieve, which is made by stretching and gluing a piece of 80 μm, nylon screen across the aperture of a piece of PVC pipe 36 cm in diameter and 30 cm long. The sieve is placed inside the harvest tank, which is a plastic tub approximately 50 x 70 x 30 cm (l x w x d) with a 3 cm drain hole placed 15-20 cm up the side wall. The 80 μm sieve is supported approximately 5 cm above the tank bottom (Figure 29) . As the rearing tank water is drained through the sieve and exits the harvest tank through the hole in the side the veligers are retained on the 80 μm screen. The water level in the harvest tank should always be higher than the screen level so that harvest water will not flow directly onto the screen. This will prevent larvae being mashed against the screen. Tanks can be drained using

either 2.5 cm diameter siphon hoses or a central drain attached to a harvesting drainpipe made of 2.5 cm PVC (Figure 29).

The harvest is divided into a “top” and “bottom” portion. Water is drained from the larval rearing tank through the 80 μm sieve at a rate of approximately 20 L/min until approximately 40 L remain in the larval rearing tank. This is the “top” portion of the harvest which tends to have the majority of healthy veligers. The sieve should be emptied twice during harvest of the “top” portion and the retained larvae should be rinsed through a 300 μm sieve (to remove debris) into a clean 20-L bucket containing filtered seawater. The bucket is filled to the 20-L mark and the larvae are counted in the same manner as on day 0.

The remaining 40 L of water in the larval rearing tank is then drained into the sieve. The tank is then rinsed with seawater, and this water is also collected in the 80 μm sieve. This constitutes the “bottom” portion of the harvest, which tends to have a higher amount of dead and deformed larvae as well as high levels of debris. Contents of the 80 μm sieve are then rinsed through a 300 μm sieve into a separate 20-L bucket. Larvae in the “bottom” portion are counted and also evaluated for survival, health, level of deformity and quantity of debris in the water. If larvae look healthy and debris levels are low, they are restocked with the “top” portion of the harvest into a clean larval rearing tank. If the bottom portion of the harvest has many dead or deformed larvae or excessive debris, it is discarded. Aeration levels and other tank conditions are maintained in the same manner as on day 0. The use of antibiotics after day 2 p.s. is optional.

It is important to evaluate larval health and survival through the veliger stage. Larval survival should be at least 50% on day 2 p.s., and in a healthy batch of eggs upward of 80%. A particular tank of larvae that is surviving poorly may not be worth saving and should be discarded at this point to save time and labor. The decision to keep or discard a batch of eggs will depend on many issues such as size of the spawn (number of eggs), importance of the eggs to hatchery production and the reproductive status of remaining broodstock.

Figure 29. Draining a tank of giant clam larvae during an intensive rearing run at ICLARM's Coastal Aquaculture Center on Guadalcanal, Solomon Islands. Note the central PVC drainpipe and the positioning of the harvest tub and 80 μm sieve.

A note on feeding giant clam larvae

Giant clam larvae can be raised to metamorphosis without any external source of food. However, feeding giant clam larvae starting on day 3 p.s. has been shown to greatly improve survival. If live algae is available, a combination such as 10,000 cells/ml each of Tahitian *Isochrysis galbana* and *Chaetoserus muelleriI* should be fed. Dried foods such as 0.8 g each of Frippak Booster (4[*]) and dried *Tetraselmis* (5[*]) per 5 million larvae can also be used (Cletus Oengpepa, personal communication). Feeding should take place once every two days until zooxanthelle populations are well established in the gut of the larvae (see section on Zooxanthelle extraction, page 40). Despite evidence showing improved survival of larvae that are given food, many culturists still choose not to feed larvae, citing that the extremely high fecundity of giant clams reduces the importance of improved larval survival. [*]*see Appendix A for supply source.*

Day 3 p.s.

Unless larvae are being fed, the only activities on day 3 p.s. should be to check the level of aeration and to observe the larvae using a compound microscope to ensure continued good health and development.

Day 4 p.s.

As mentioned previously, giant clams derive a substantial portion of their nutritional requirements from symbiotic zooxanthelle that live in their mantle. Giant clam larvae do not inherit zooxanthelle from the parent, so extracted zooxanthelle are seeded into the larvae on the afternoon of day 4 p.s.

The larval rearing tank is first harvested using the same methods as those employed on day 2 p.s. Then zooxanthelle are added to the larvae held in a 20-L bucket after harvest. The larvae should be left for 2 hours in the bucket with the zooxanthelle under mild aeration before being restocked into a clean larval-rearing tank.

Zooxanthelle extraction

Zooxanthelle are extracted from the mantle of a living donor clam. In most instances the clam is killed, but in some areas where giant clams are rare, small pieces of mantle may be cut from a living clam (Braley, 1992a). The factors that determine giant clam mantle color and the relationship between donor mantle color and larval mantle color are poorly understood at this time. Mantle color is of paramount importance in species being raised for ornamental purposes. Although hatchery technicians routinely extract zooxanthelle from one or more donor clams that have a highly desirable color or patterning in the hope that this will produce clams with a similar color, there is little evidence that this works.

The mantle is first cut from the clam and any excess tissue or mucus removed (Figure 30). Zooxanthelle are then extracted from the mantle by macerating the tissue in a kitchen blender. The blender is partially filled with filtered seawater and the tissue is macerated in short bursts until the mantle appears white in color (a sign that the zooxanthelle have been extracted). At this point the water in the blender will be a dark brown color. Excessive clam tissue in the larval culture can decay and cause bacterial infection. To avoid this the

zooxanthelle/seawater mix is strained through a 25 μm sieve into the bucket of clam larvae.

Figure 30. Mantle tissue being removed from a brightly colored T. maxima *for seeding zooxanthelle into larval clams.*

Day 5 p.s.

On day 5 p.s., the larvae are checked using a compound microscope for the presence of zooxanthelle in the gut. Zooxanthelle will appear as golden-brown spheres in the gut or developing mantle (Braley, 1992a). Aeration levels in the tank should be checked and adjusted, if necessary, and the larvae should be fed if feeding is being practiced.

Day 6 p.s.

By day 6 p.s., larvae should be developing into the pediveliger stage, and starting to settle to the bottom of the tank. A final harvest should be done following methods described for day 2 p.s. Zooxanthelle are added to the larval culture again using the same methods described for day 4 p.s. in order to ensure that all larvae have taken up a resident population of the algae. Larvae are not restocked into a larval rearing tank but are now moved outside into a settling tank.

Settling and metamorphosis

Between days 6 and 14, giant clam larvae will settle to the bottom of the tank and metamorphose into juveniles. Stocking density is very important at this stage as the clams are now dependent on ambient sunlight and available nutrients in the water for the majority of their growth. Over-stocking the tank can lead to stunting, reduced growth rates and less saleable animals. Under-stocking the tank leads to lower productivity per tank and increased production costs. Recommended stocking rates into settling tanks are between 5 and 10 larvae/cm^2 of bottom surface area (50,000 – 100,000 per m^2).

Like larval rearing tanks, settling tanks must be made of a non-toxic material. The most common and cost-effective design is a shallow round, rectangular or square concrete tank coated with non-toxic epoxy paint (1 or 5*) or a PVC liner (1 or 5*). Tanks should be cleaned and sun-dried prior to use and filled to a depth of at least 50 cm with 1 μm filtered seawater immediately prior to stocking. A 50% light occluding shade cloth should be placed over the tank to help reduce algal growth.

Days 7 to 14 p.s.

A water sample should be checked daily for the presence of larvae in the water column. When larvae can no longer be found swimming, a water flow of 1 μm filtered seawater can be started. The exchange rate should be 2.5 times the tank volume per day. Aeration can be increased to a more vigorous level after settlement has occurred.

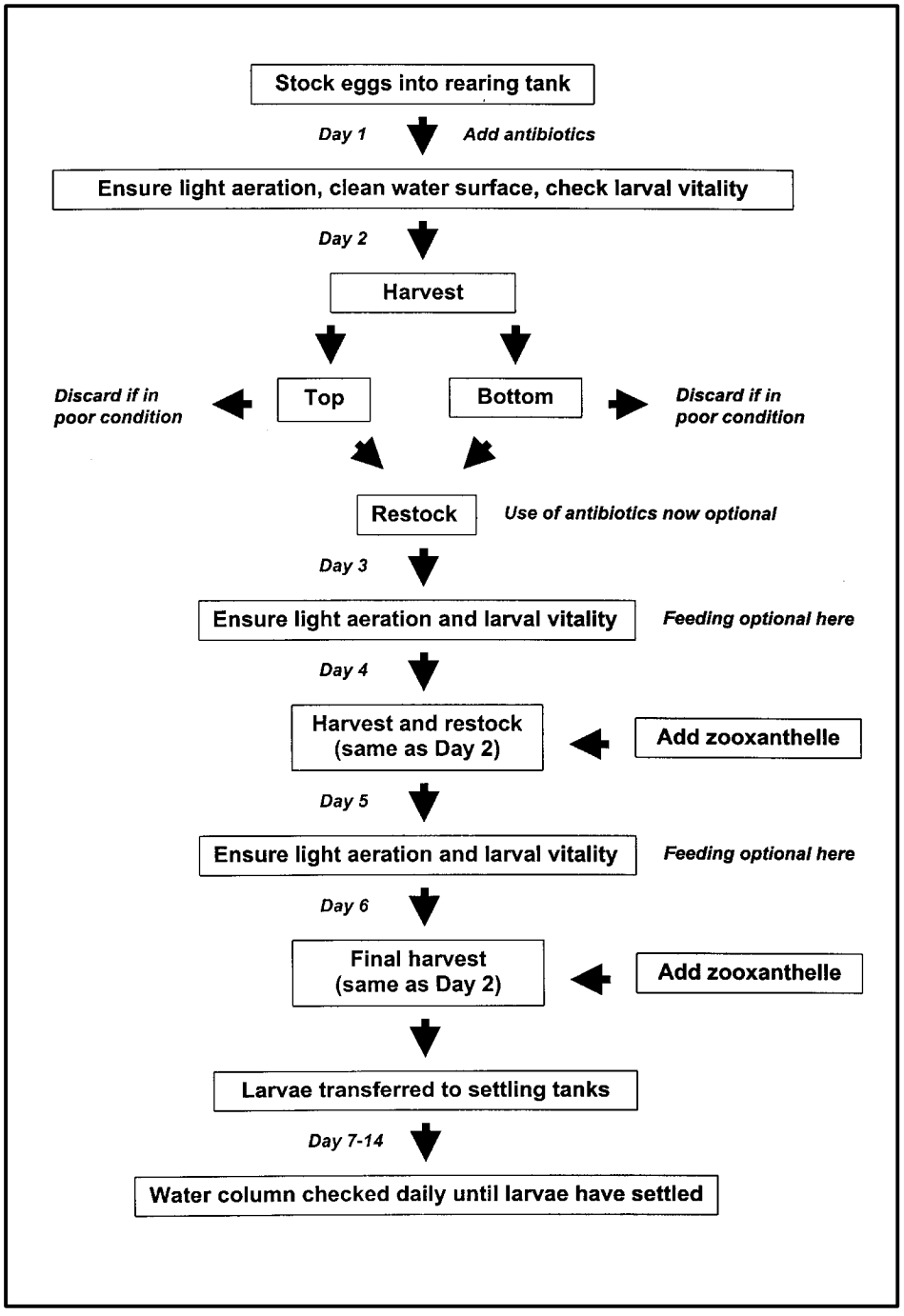


Figure 31. Flow diagram describing sequences and events of intensive larval rearing of giant clams

Semi-intensive larval rearing

Semi-intensive larval rearing has the advantage of rearing the larvae through the critical first two days post-spawn using antibiotics but then utilizing low cost, extensive rearing methods by transferring the larvae directly to the settling tanks. Larval health and survival can also be assessed before stocking into the settling tanks to determine if the quality of the larvae is sufficient to continue grow-out. This can save time and labor if the larvae are in poor condition.

Semi-intensive culture follows the rearing protocol for intensive larval rearing for days 0 – 2 p.s. (see section on Intensive Larval Rearing, pages 34-43). After harvest on day 2 p.s., the larvae are transferred directly to a settling tank that has no aeration or water flow until the larvae have settled (see page 42). Zooxanthelle are isolated using methods described on pages 40, and are added directly to the settling tank on days 4 and 6 p.s. Feeding is generally not practiced and antibiotics are not used after day 2 p.s.

Extensive larval rearing

Extensive larval rearing is the simplest, cheapest way to raise giant clams. It is possible for a large hatchery to operate successfully using extensive rearing practices. Because it saves labor and equipment, it should be seriously considered as an alternative to the more intensive methods of rearing. However, extensive methods tend to be less reliable than intensive rearing, which can be a deterrent to hatcheries that have rigorous production targets.

Extensive larval rearing uses only settling tanks for the entire larval cycle. The settling tank is filled with 1 μm filtered seawater immediately prior to eggs being stocked at a density of 1 egg/ml. The tank is left static with no aeration or water flow until the larvae have settled. Zooxanthelle are isolated using methods described on page 40 and added directly to the settling tank on days 4 and 6 p.s. Feeding is not practiced and antibiotics are not used.

Note: Static outdoor tanks can be prone to rapid changes in salinity during heavy rains. To prevent this, a portion of the tank (up to 50%) can be covered with a waterproof, non-toxic material such as polyethylene to prevent excessive dilution (Figure 32). This material should be removed when the larvae have metamorphosed and water flow is initiated to the tank.

Figure 32. To protect outdoor, static cultures from extreme salinity changes during heavy rainfall a waterproof material, like this shade tarp at the RRE Wau Mariculture facility in Majuro, RMI, is sometimes used to cover all or part of the tank until water flow is started.

Nursery and grow-out

Once metamorphosis is complete the juvenile clams must now be grown to a size suitable for market or stock enhancement. Nursery and grow-out methods are detailed in the CTSA publication titled “Nursery and Grow-out Techniques for Giant Clams.” However, the following brief description is intended to give the reader a general idea of the involvement and duration of the next phase of giant clam culture.

Juvenile clams remain in the settling tanks for up to 5 months before their first harvest. Precautions are taken during this time to prevent excessive algal build-up in the tanks. After the first harvest the clams are redistributed at lower densities for further grow-out in land-based nursery tanks. The land-based nursery phase may last as little as 12 months or until the clams are large enough to go to the open-water nursery (2-3 cm). Some facilities choose to grow their animals entirely in land-based systems. Clams that are grown as seed-stock for satellite farms or for stock enhancement efforts will leave the facility after the nursery phase. Animals grown for the aquarium trade or for food will require a further 1-2 years of grow-out before harvest and sale.

Whether clams are grown in an open-water or land-based setting, constant cleaning and monitoring is required to keep the clams healthy and growing adequately. Cleaning and checking for parasites usually takes place on a 3 month schedule for both types of system. However, this may vary depending on the level of algal fouling and disease incidence.

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Appendix A. Suppliers of Equipment Listed in this Manual.

1. Aquatic Eco-systems Inc.
   1767 Benbow Court
   Apopka, FL 32703-7730, USA
   Tel. 407-886-3939 or 1-800-422-3939
   Fax. 407-886-6787
   e-mail: aes@aquatic-eco.com
   web site: http://www.aquatic-eco.com
   Comments: Suppliers of a large range of aquaculture and laboratory products.

2. Carolina Biological Supply Company
   2700 York Road
   Burlington, NC 27215, USA
   Tel. 1-800-334-5551
   Fax. 1-800-222-7112
   Comments: Suppliers of basic laboratory equipment and animal specimens.

3. Sigma Chemical Company
   P. O. Box 14508,
   St. Louis, MO 63178, USA
   Tel. 1-800-325-3010 or 314-771-5750 (call collect outside USA)
   Fax. 1-800-325-5052 or 314-771-5757
   Comments: Suppliers of chemicals for laboratory and technical use.

4. INVE Aquaculture
   598 W. Clarke St., P. O. Box 1306
   Grantsville, UTAH 84029, USA
   Tel. 801-884-3406
   Fax. 801-884-6492
   Comments: Suppliers of dietary products for the larval aquaculture industry.

5. Aquaculture Supply

33418 Old Saint Joe Road
Dade City, FL 33525, USA
Tel. 352-567-8540
Fax. 352-567-3742
e-mail: ASUSA@Aquaculture-Supply.com
Web site: http://www. Aquaculture-Supply.com
Comments: Suppliers of a large range of aquaculture and laboratory products.

6. Quill Corporation
P. O. Box 94081
Palatine, IL 60094-4081
Tel. 1-800-789-1331
Fax. 1-800-789-8955
web site: http://www.quillcorp.com
Comments: Suppliers of office equipment.

7. E and B Discount Marine
201- Meadow Road
Edison, NJ 08818, USA
Tel. 1-800-BOATING
Fax. 1-408-761-4421
web site: http://www.westmarine.com
Comments: Suppliers of a full line of fiberglass and marine products.

8. Performance Diver
One Performance Way
Chapel Hill, NC 27514
Tel. 1-800-933-2299
e-mail: service@performancediver.com
web site: www.performancediver.com
Comments: Suppliers of a full range of diving equipment.

Appendix B. Glossary

Bivalve mollusc: animal of the mollusc family with two shells that are hinged dorsally.

Byssal opening: area underneath the clam where it puts out threads that attach it to the substrate. This is area where predatory snails can enter the clam. The byssal opening is particularly large in *T. maxima, T. crocea* and *T. squamosa.*

Byssus: a tuft of long, tough filaments that certain bivalves use to attach themselves to the substrate.

Cilia: small hair that can be moved in conjunction other cilia to allow the larva to swim.

Diel: relating to a 24 hour period that usually covers a day and the adjoining night.

Dinoflagellate: group of unicellular algae having two whip like antennae used in some case for mobility.

Excurrent siphon: opening where water flows out of the clam after passing over the gills.

Fecundity: pertaining to the reproductive capability or number of eggs an animal can produce.

Incurrent siphon: opening where water is drawn into the clam.

Lunar: of or relating to the moon.

Mantle: portion of the animal responsible for secreting the shell; in the case of Tridacnids it is also the colored fleshy tissue that houses the zooxanthelle.

Metamorphosis: the physical transition of the clam from a larva to a juvenile.

Papilla: a small projecting part of the body or skin of a plant or animal.

Pediveliger: development stage in some marine invertebrates where a velum is still present but the foot has started to form.

Photosynthetic: pertaining to the ability to convert sunlight into energy, as in the case of plants.

Protandric hermaphrodite: an animal that matures first as a male then later develops a gonad with both sperm and egg releasing components.

Symbiotic: pertaining to the intimate living together of two different species, which is generally mutually beneficial to both parties.

Trochophore: a free-swimming ciliated larval stage typical of many marine invertebrates.

Valve: one side of the shell of a bivalve

Velar: pertaining to the fleshy, veil like tissue that houses the cilia in the veliger larval stage.

Veliger: larval stage of some marine invertebrates when a velum is present.

Zooxanthelle: group of symbiotic dinoflagellate algae that live inside the cells of other animals especially reef building corals, soft corals and giant clams.