Standard Operating Procedures

for the spawning of giant clams, *Tridacna maxima* and *Tridacna derasa* at the Aitutaki Marine Research Center

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1.0 Purpose

The purpose of this document is to outline the process and requirements for the spawning of giant clams at the Aitutaki Marine Research Center (AMRC). The Standard Operating Procedures (SOP) will provide the necessary steps and procedures for all individuals involved in the spawning process and will ensure that monitoring and management processes are in place to promote successful spawning practices.

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2.0 Procedures

2.1 Broodstock collection and preparation

- Clams collected for breeding should be healthy and at least 25cm in length of mantle.
- Clams may be transported in or out of water dependent on length of transportation period. If transportation period is greater than a couple hours, transport in water and/or in shade to reduce heat stress.
- Clams transported out of water should be placed on their side to prevent the mantle from collapsing inwards and causing damage to the internal organs of the clam.
- Care should be taken to avoid excessive stress on the clam from direct sunlight, and excessive bouncing during transport.

Figure 1: Harvesting of *T.derasa* for spawning. Photo by ©MMR
**Note: Clams lying on its side to prevent the mantle from collapsing*

2.2 Broodstock preparation for spawning

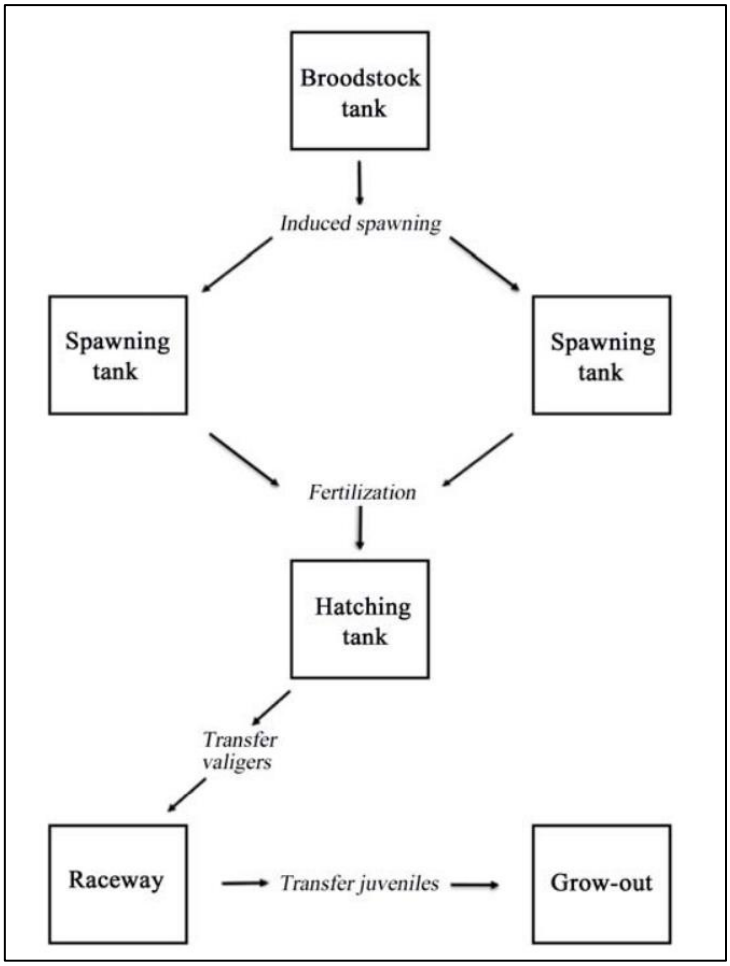

Figure 2: Diagram showing tanks used for spawning process. Reprinted from Mies and Sumida, 2012

- Broodstock, spawning and hatching tanks should be cleaned with chlorine several hours prior to spawning and then filled with 1µm filtered seawater.
- All broodstock clams should be scrubbed clean and any foreign marine organisms chipped off (i.e. tube worms, snails, crabs hiding on the mantle, etc.).
- Chlorine should be applied onto the outer valve of each shell by using a small paint brush. Care should be taken to avoid getting chlorine inside the mantle.
- Let it stand for 10 minutes.
- Rinse off chlorine with 1µm filtered seawater before they are placed in the broodstock spawning containers.
- Spawning tanks should be numbered to keep track of clams (Fig. 4).
- Collection cups for sperm should be ready and labelled corresponding to numbers on container for identifying clams.
- Black containers (as shown in Fig. 5) are preferred for spawning as it is easier to see eggs and sperm (which are white).
- All spawning and larval rearing equipment should be prepared and cleaned with chlorine prior to spawning.
- All larval rearing tanks should be filled with 1µm filtered seawater.

Figure 3: Cleaning of *T. maxima* prior to spawning. Photo by ©MMR

3.0 Spawning (release of both male and female gametes)

There are a number of artificial methods that have been developed and have been successful in liberating gametes from giant clams and other tropical bivalves. These methods include heat stress, serotonin injection, and the use of gonad extract (Ellis, 1997). Serotonin injection is the preferred method by the AMRC to induce spawning.

3.1 Serotonin injection method

Serotonin is injected into the gonad of mature clams either from the top (T. derasa, T. maxima) or from the bottom (T. maxima).

- Serotonin dosage varies between species, size, and health of clams.
- 0.5ml-1ml for T. maxima and 3ml-5ml for T.derasa clams.
- Needles also vary between species (small skinny needles for T. maxima and long needles for T. derasa).
- Needle should be cleaned with fresh water and then sterilised using alcohol after each use.

Figure 4: *T. maxima* clams ready for spawning in labelled container with fresh 1µm seawater. Photo by ©MMR

**Note: Circled section shows the number corresponding with clam for identification.*

Figure 5: *T. derasa* clams ready for spawning in labelled container with fresh 1 µm seawater. Photo by ©MMR

Figure 6: Spawning method using serotonin injection into *T. maxima* to induce the release of male and then female gametes. Photo by ©MMR

Figure 7: Collection of sperm from *T. derasa* using a syringe. Photo by ©MMR

4.0 Fertilisation

All giant clam species are functional hermaphrodites (possess both viable male and female gametes). Therefore, care must be undertaken to prevent self-fertilisation during spawning.

4.1 Collection and fertilisation of eggs

Each clam should be given an identification number to prevent confusion of individual broodstock. The numbering of each clam is very important to prevent self-fertilisation when gametes are released.

- Sperm is generally released before eggs from an individual clam, but this is not always the case. Sperm should be collected from each male and stored out of the sun in a labelled container marked with the same number as the clam.
- Sperm can remain viable in these containers for 30 minutes and should be replaced periodically to maintain fresh sperm for as long as the individual is producing sperm continuously.
- Sperm quality varies between individuals and within an individual during one spawning event.
- If sperm concentration in spawning tank becomes too dense, the water should be exchanged using 1µm filtered sea water. This will greatly reduce sperm concentrations in the spawning tank or containers reducing the possibility of polyspermy and provide a better environment (oxygen, temperature, etc.) for the release of eggs.
- When clams begin to release eggs, the individual clams (all species) should be removed from the broodstock spawning tank, rinsed with 1µm filtered seawater and place in a cleaned chlorinated container that has 1µm filtered seawater with a known volume.
- The clam must be fully submerged in this container.
- Eggs will be released into the water and sperm (from a separate individual) should be added directly to the container once the clam has finished releasing its eggs.
- The actual amount of sperm added to the container depends on the density of egg and sperm and requires visual inspection during spawning with the aid of a waterproof torch.
- Sperm should be added from at least two different individuals for each individual releasing eggs (it should be ascertained that the sperm is not from the clam releasing eggs).

Figure 8: Two *T. maxima* clams releasing eggs in 1µm filtered seawater. Photo by ©MMR

4.2 Estimating the number of eggs

Once the sperm has been added to the eggs, a small hand-held plunger is used to attain an even distribution of eggs and sperm for even fertilization. Six samples are then taken and used to obtain an estimate of the number of fertilised eggs collected. The rest of the eggs in the spawning tank are then carefully transferred to the hatching tank.

- Take 6 x 1ml pipette samples from the spawning tank after fertilization and place them directly into a counting chamber.
- Place the counting chamber under a microscope and, using a hand counter, count the number of eggs visible in each of the six samples.
- Calculate the average number of eggs and then multiply by the total volume of the spawning tank (e.g. if the tank holds 40L of filtered seawater, multiply average by 40,000 ml).

Figure 9: Counting of fertilised eggs under microscope. Photo by ©MMR

5.0 Larval rearing

Fertilised eggs are stocked into larval rearing tanks (20-50 eggs per ml) and cultured from fertilised embryo, trochophore to D-stage veliger. Once the D-stage veliger (48 hours) larval is attained, healthy swimming veligers are selected from the larval tanks and transferred, at much lower stocking density rate (1-2 per ml), to outdoor flow-through larval rearing tanks where they are cultured through to settlement and juveniles.

- An additional 5-8 days are required to attain the pediveliger stage and another week (10 days plus) before metamorphosis is completed into a juvenile.

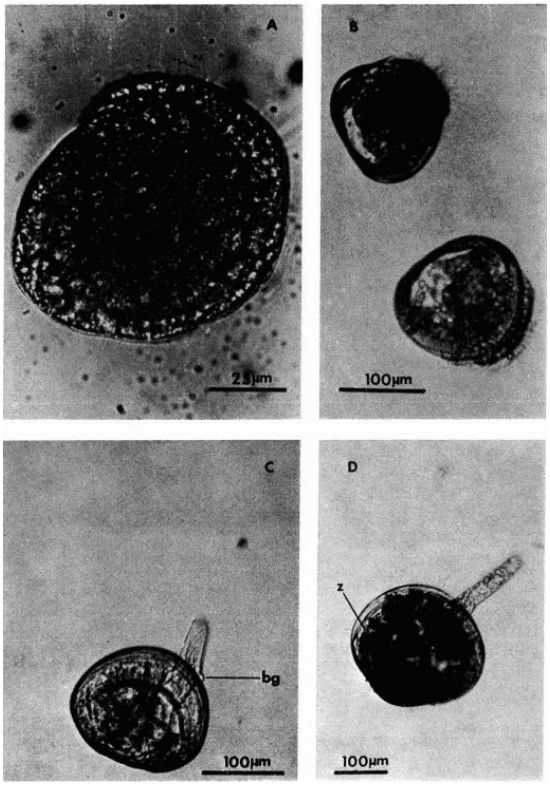

Figure 10: Early life stages of *Tridacna crocea*. A) trochophore; B) veliger; C) pediveliger; D) juvenile. In these photos, bg = byssus gland and z = zooxanthellae. Photo reprinted from Jameson, 1976.

Timing of each larval stage is dependent on water temperature, species and local environment conditions.

Figure 11: Transfer of fertilized eggs from spawning tank (blue) to hatching tank (grey) with aerator. Photo by ©MMR

<u>Life stages of all giant clam species:</u>

- Egg + Sperm = Fertilized eggs
- Trochophore – swimming (~20-24 hours)
- D-stage Veliger – swimming (~48 hours)
- Pediveliger – (~5-8 days)

- Juvenile (10 days plus)
- Adult (5-15 years mature)

5.1 Stocking rates

Stocking densities of fertilized eggs into a larval rearing tank should be between 10-30 eggs per ml. Stocking rates should not exceed 50 eggs per ml.

- All larval rearing tanks should be moderately aerated for the first 12 hours of egg development and be reduced throughout their entire larval cycle.
- Aeration prevents eggs accumulation on the bottom of the tank and suffocation.
- Plunging of larval rearing tanks every 2-3 hours is a good practise to assist the egg development process until trochophore larvae hatch. Care should be taken when plunging (slow and steady while not going all the way to the bottom) to prevent damaging the larvae.
- Aeration should be reduced (but still on) once the trochophore larvae stage is reached (20-24 hours). Aeration remains reduced through to the D-Veliger larval stage (48 hours.).
- Once trochophore larvae have hatched, the bottom of each larval tank should be visibly inspected for the accumulation of undeveloped eggs (appears as a pink spot on the bottom of the tank). If present, the tank bottom should be siphoned where the spot is observed to reduce water contamination.
- Trochophore and veliger larvae can be viewed in the larval tank with the use of a torch.

5.2 Transfer of veliger larvae

Veliger larvae hatch within 36-48 hours after fertilisation. Once this stage is reached, the larvae are ready to be transferred to a runway tank. It is advised to take a small sample of larvae from the tank to view under the microscope and determine if the larvae have reached the veliger stage.

When transferring the veliger larvae to a runway tank:

- Place a 37 micron sieve into an overflow container of 1 micron filtered seawater. This is to ensure that larvae are kept in water.
- If there is a leak in the pipes while draining, an additional container should be placed under the leak to catch any larvae that may spill through and be poured onto the larvae collecting screen.
- When number of larvae becomes too concentrated on the screen, the drainage tap is turned off and the screen is removed, and larvae are rinsed off into a container of 1 micron filtered seawater.
- Counts should be taken on these larvae and distributed into the raceway tank
    - Using procedure mentioned below (5.2.1).
- Care must be taken to prevent larval mortalities caused by excess force
    - I.e. slow and steady, and do not press plunger to bottom.
- The bottom 1-2cm of water in the spawning tank should not be collected to avoid contaminating the raceway tank with dead larvae.

5.2.1 Collecting and counting of veligers

- Take 6 x 1ml samples of the larvae holding container (while simultaneously plunging the water to ensure even distribution of eggs) using a clean volumetric pipettor into 6 counting well.

- Take the average of the 6 samples and multiply by the volume of the container the samples were taken from.
- This procedure can be repeated until all larvae are collected. If process is repeated, add final calculations from each replicate together to determine total count of veligers.

5.3 Stocking of veliger larvae into nursery tanks/raceways

It is important that all equipment and tanks utilised for the larval rearing are chlorine cleaned prior to the transfer of larvae.

- Raceway tank should be filled with 1 micron filtered seawater and moderately aerated throughout.
- Water should be filtered again at outflow going into the raceway tank using a 1-micron filter bag inside of a 10-micron bag. This will prevent debris inside the pipes from flowing into the tanks.
- Flow rate through filter bags should be **approximately 2 litres per minute**.
- Filter bags should be changed and cleaned daily (more frequently if there is a lot of debris inside the pipes).
- The filter bags should be turned inside out and back flushed with fresh water to remove all material caught in the bags, placed into a chlorine bath, rinsed again and sun-dried before reusing them.
- Filter bags should be inspected for holes and excessive wear before reuse.
- A 50-micron filter flow-through screen should be used to prevent larvae from being washed out of the tank and airline pipes or circular air stone should be located at the bottom of the screen to provide a mechanism of sweeping larvae off the screen.
- Flow-through screen should be carefully wiped clean by gently brushing by hand or a soft brush daily.
    - To replace a flow-through screen, it is important to turn off the water and wait until the water stops flowing to prevent loss of larvae.
- Always clean and rinse with 1 micron water anything that is going to enter the raceway tanks to prevent contamination.
- Shade cloth of 5 percent should be placed above raceway tank to prevent debris from falling into the tank and contaminating the water during the larval cycle and reduce sunlight and heat.
- During heavy rain, aerator system must be turned off to prevent mixing of freshwater with seawater and pump turned on to help drain freshwater out (freshwater is less dense and will sit on surface).

Figure 12: Mounting a 10 micron and 1 micron filter bag onto water inlet PVC taps. Photo by ©MMR

5.4 Zooxanthellae Feeding

Giant clams, like many other tropical reef invertebrates, possess a unique symbiotic relationship with a unicellular alga. Both algae and clam benefit from this relationship which persists for the entire life of a clam. The algae are not passed from adult to offspring and therefore these algae must be administered.

Zooxanthellae are found in all tropical coral reefs and therefore the water delivery system will supply these algae to the clams. However, additional zooxanthellae need to be supplied to the tanks in a hatchery to provide adequate numbers of algae to all clams.

- Select a clam (can be same or different species) and retrieve the zooxanthellae from the mantle tissue.
- Mantle tissue is removed, and then zooxanthellae are scraped off using a knife (or mantle can be finely blended).
- Aerator should be turned off for an hour before zooxanthellae is added for feeding to prevent zooxanthellae from flowing out of the tank.
- Liquid is decanted off from the chopping board using a squirt bottle filled with filtered 1 micron of seawater into a container.
- When pouring zooxanthellae into the raceway tank, a 37-micron sieve is used to remove all large tissue debris.
- Zooxanthellae should be added to the larval tanks on the **1st day** in pediveliger stage and again for **3 more times every second day** (for a total of 4 feedings).
- 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 developing mantle.

6.0 Larval settlement

Veliger larvae will develop into pediveliger larvae and will stop swimming and settle to the bottom of the tank.

- Once settlement has occurred (5-8 days, depending on water temperature and species), the 10- and 1-micron filter bags can be replaced with a single 25-micron filter bag and flow-through screen should be removed.

7.0 Juvenile nursery culture

Giant clams become visible to the naked eye at around 2-3 months of age and mortalities can still be high. The biggest problem is fouling on the bottom of the tanks and the consequent effects on the clams.

- Incoming seawater should be filtered to 25 microns using filter bags for the first 2 months in nursery tanks.
- Nursery tanks must be aerated and have at least one water exchange per 24 hours.
- Remove any predator snails if observed in the tank.
- Aeration system to be increased to improve water circulation.

7.1 Estimating clam numbers in the nursery tanks

- Should be undertaken every 2-3 months or when juveniles are removed from the nursery tanks to provide information on clam density, count, health, and growth.
- To obtain an estimate, a template (circular or square of 10 x 10cm) of a known surface area should be placed randomly within the tank.
- Template should be non-toxic to the clams.
- Area within the template should be siphoned into a small 100-micron sieve screen.
- Juveniles should then be washed into a petri dish and viewed under the microscope.
- Four samples should be taken for each tank and the average number of clams should be multiplied to account for the entire surface area of the tank.

7.2 Cleaning protocols for algal fouling

The growth of algae, especially filamentous species, needs to be limited to maintain high survival and growth of clams in the nursery tanks. The assemblages of fouling algae differ between tanks and with time. The use of shade cloth over the tanks reduces rapid growth of these algae, however light is required for clam growth and therefore it is a balance that must be maintained. Manual cleaning and/or polyculture with herbivores are the main methods utilised.

7.2.1 Manual cleaning (Siphon)

It is recommended **NOT** to attempt to manually cleaning the bottom of the nursery tanks until juvenile clams are at least 2 months old. Cleaning the tanks before this stage is detrimental to the clams. Juvenile clams therefore should be left undisturbed during the first 2 months. Breaking and/or removing clam byssal threads will result in growth checks, reduced growth rates and higher mortality levels.

- After 2 months, tank bottom should be cleaned regularly to remove excess algae and faecal material.

- Algae can be removed and/or reduced by wafting the algae gently with a hand just above the bottom of the tank to loosen the algae.
- The tank/raceway is then stirred with a paddle or arm to suspend the algae.
- Three quarters of the tank should be drained to remove the suspended algal material.
- Suspended material is collected using a 250-300 microns mesh sieve to remove any clams that may be attached to the algae.
- Any material that was not removed by this process can be siphoned from the tank.
- Tank/raceway should be refilled as quickly as possible to prevent excess stress on the clams.

7.2.2 Herbivore polyculture

To aid in the cleaning process, the use of herbivorous animals has been successful for all species of giant clams. When choosing a particular algal grazer the method of feeding in relation to the size of the clam is very important.

- Large Trochus are very efficient grazers but are not practical to use with small clams as they will crush and kill small clams.
- Careful monitoring and management of algal grazer populations in each juvenile clam tank is required to maintain populations that are effectively removing the algae.
- Too few grazers will not control the algae whilst too many will cause death of the grazers (limited food) and will be detrimental to the water quality of the tank.
- For small juvenile clams (<5mm) Amphipods are recommended and should be added to all tanks after 2 months.
- Once clams have reached at least 5mm small gastropod grazets (e.g *Trochus niloticus*), sea hares (*Alypis sp*.) and small herbivorous surgeon fish (e.g *Acanthurus triostegus* – convict tang) should be used.

Figure 13: Herbivore polyculture help the removal of algae growing in giant clam tanks. Photo by ©MMR

7.3 Nutrient additions

Nutrient levels in tanks that have high densities of giant clams and benthic algae are limited and can directly affect clam growth. However, trials undertaken over the past decade have concluded that the addition of nutrients substantially improve clam growth, health and survival.

- Nutrient additions should be added at least twice weekly to each nursery tank at a concentration of 20g of ammonium sulfate (or ammonium nitrate) for each 5,000L of water.
    - These additions should be administered in the morning.
    - Nutrient additions should not be administered to clams which are less than 2 months old.

NOTE: The addition of nutrients will stimulate algal growth and hence fouling from benthic algae will increase. It is therefore mandatory to have the correct algal cleaning regime in place to control algal growth in the tanks.

8.0 Harvesting juvenile clams

Clam densities must be monitored. If densities of clams are high then thinning must occur or growth and survival rates will be affected. Clams are gregarious and will clump. These clumps should be thinned periodically and clams redistributed within the tank. Care must be observed when removing juvenile clams from the tank bottom. All juvenile clams of all species attach themselves to the substrate when they are young and therefore a flexible, sharp knife (e.g. sharpened butter knife) must be used to remove the clams. Clams should **NOT** be pulled from the substrate.

8.1 Volumetric and weight count estimates of juvenile clams

Once juvenile clams have been harvested and cleaned, they should be counted (measurement can also be taken if required) before they are placed back into an additional tank.

- Remove clams from raceway tank and place in a beaker with seawater to measure volume.
    - Note: Only the volume of the clams is measured. The seawater is just to keep the clams from being left in open air. They should also **NOT** be left in this container for longer than necessary to get the measurement. They should then be moved to a larger container to spread out so they are not stacked on top of each other.
- Randomly select a subsample of a known volume of clams and count the number of individuals.
- Multiply by the count by the total volume to get an estimate of the total number of juvenile clams.

8.2 Tray or concrete slab culture of juveniles

All species of giant clam attach via byssal threads to the substrate during the early stages of their life. The smaller species, *T. maxima* and *T. squamosa* attach to the substrate for their entire life. These species therefore should be provided with a substrate that allows permanent attachment without affecting the cleaning and maintenance procedures.

There have been several methods developed to allow these species of clams to attach to a permanent substrate. The various methods have both positive and negative aspects. The most widely used substrate in the past has been coral rubble and/or basalt gravel (placed in plastic trays). This substrate has had reasonable success. However the surface area of the substrate is large and provides a huge

surface area for benthic algae to colonise. The result is increased cleaning effort to combat the increased algae growth. Furthermore, this substrate provides a habitat for predator gastropod snails and flat worms to hide, increasing mortality rates. This substrate worked well for the larger species of clams when larger than 15 cm (e.g. *T. gigas*, *T. derasa*, *H. hippopus*) because they do not strongly attach to the substrate so they can be removed with little effort and damage to the clam. The smaller clams, however, require much more time and care to be removed and mortality occurs. Therefore, it is **NOT** advised to use this substrate for *T. maxima* and *T. squamosa*.

Concrete slabs with or without ridges placed in the top surface are the preferred substrate for these clams. Although they are more expensive, they have a reduced surface area for algal fouling, do not provide a habitat for predators to hide and therefore reduce cleaning effort. These are therefore recommended for *T. maxima* and *T. squamosa*.

In addition, it is recommended that all species cultured at the AMRC should be settled onto the concrete slabs when they are harvested for the first time. The settlement onto these slabs will make the management and maintenance of the juvenile nursery tanks considerably easier for the hatchery staff as well as simplify the process of moving the clams, when older, to the nursery grow-out ocean sites and/or farms.

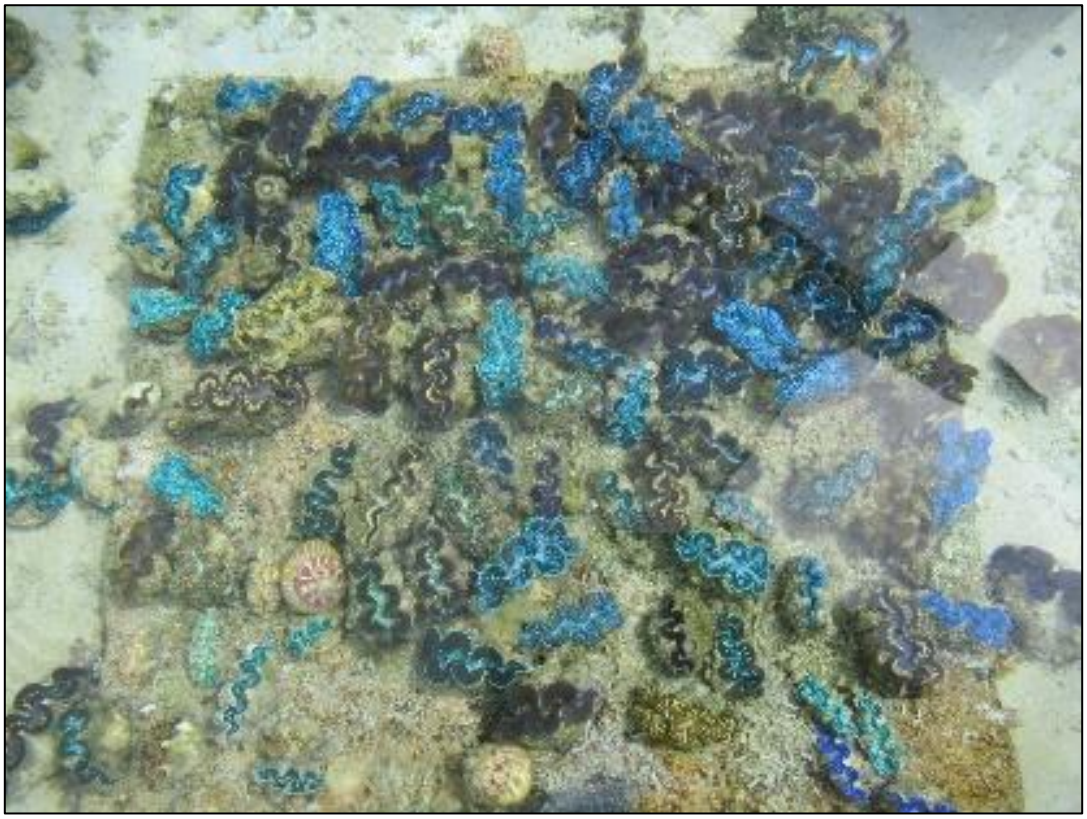

Figure 14: Giant clams on concrete slabs in AMRC raceway with other herbivores. Photo by ©MMR

8.3 Juvenile field culture and recommendations

The age at which clams should be removed from the land-based nursery and distributed to the AMRC ocean nursery field site or to the private farms is directly related to the clam species and size.

T. gigas, *T. derasa* and *H. hippopus* clams between the ages of 6-8 months are of suitable size to be distributed to the field sites while *T. squamosa* and *T. maxima* juveniles should be distributed between

the ages of 8-10 months assuming they have been settled and are attached onto the concrete slabs (as described above).

It is recommended that AMRC keep a small number of clams from each batch cultured for each species in the land-based nursery tanks until such time that the clams have attained a size that will greatly reduce their susceptibility to predation from the marine gastropod snails (e.g *Cymatium sp.*) in the ocean. This will ensure the genetic integrity of each batch of clams cultivated at the AMRC. It is especially important that the first several batches of *T. gigas*, *H. hippopus*, and *T. squamosa* be managed under this protocol to ensure the cultured juveniles will survive and have a greatly increased chance of reaching maturity. These batches of clams should also be used as future broodstock for the AMRC.

All juvenile clams attached to the concrete slabs should be positioned directly into the ocean nursey cages currently deployed by the AMRC at the protected area within the Aitutaki lagoon. The current design of the wire cages and its supporting steel frame is suitable for the culture of juvenile giant clams. The purpose of the wire cages is to provide protection from predators while providing environmental conditions that maximise growth and survival of the clams.

The location and position of the steel frames and tables within the shallow water reef areas located within this area should be constantly evaluated to provide the best possible growth rate for the animals, maintain the lowest mortality rates and minimal maintenance work for the staff. It is therefore recommended that all clams cultured at the AMRC field site be located on steel frames (rebar) situated in water between 2-4 meters in depth. The steel frames should be at a minimum located 1 meter off the substrate and be at least 1 metre below the water surface during low spring tides.

The use of steel frames positioned off the bottom will greatly reduce sedimentation deposit and decreases the habitat for the gastropod snail (e.g. *Cymatium sp.*) to reside. The steel frame must be built to withstand considerable water movement during inclement weather conditions (e.g. cyclones).

T. maxima will need to remain attached to the concrete slabs for the duration of the ocean nursery phase (up to 5 years) at which time the animal can be carefully removed from the slab and relocated onto a suitable reef for reattachment.

T. squamosa, *T. gigas*, *T. derasa* and *H. hippopus* juveniles should be transferred from the hatchery attached to concrete slabs and be cultured for an additional 6-8 months (i.e. 12-14 months old) before they can be removed and placed directly into the clam cages without the slabs. Once they reach a shell length of approximately 15-20 cm, they can be moved out of the clam cages and placed directly on the reefs. This is the approximate size when they lose their ability to attach.

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Appendix 1: Recommended sequence in inducing giant clam to spawn

1. Combination of temperature shock and serotonin injection (based on Steve Lindsay "*Giant Clam Production in Aitutaki - Hatchery and Grow-out Operations Manual*)

It is recommended that all breeders should be placed in the sun for 30 minutes to 1 hour before serotonin is used. In addition, one serotonin injection per clam is required and if no eggs are released, the breeders should be returned to either the nursery or tanks.

Appendix 2: Example of calculation to determine the stock density of eggs or larvae (modified after Braley, 1992)

Example I

Assume there are 30 litres of fertilized eggs in seawater collected from a spawning. The eggs are moderately dense. The water is stirred evenly with a clean glass rod as six 1ml pipette samples are taken. The average counts of eggs are 62 per ml.

62 eggs per ml x 30L x 1000ml/L = 2,170,000 eggs

where 62 eggs per ml is the average obtained from the egg counts

30L is the volume of the container holding the seawater and eggs

1000 ml/L is the conversion from millilitres to litres

Example II: Dilution factor

Assume there are 30 litres of eggs collected from a spawning. The density of the eggs is very high. Therefore, it is necessary to dilute the samples before counting the eggs. The 1 ml pipetted samples are taken as mentioned above. However, these samples are placed in a 100ml graduated cylinder. A 10:1 dilution factor is used by adding an additional 90ml to the 10ml sample.

This 100ml sample is stirred and one 1ml pipette sample is taken. Say, the average (man) counts of eggs from the diluted samples are 30. Therefore, by multiplying the average by the dilution factor, the number of eggs per ml can be acquired. 30 eggs per ml (average count) x 6 dilution factor = 300 eggs per ml

300 eggs per ml x 30L x 1000ml/L = 9,000,000 eggs

where 300 eggs per ml is the average obtained from the egg counts

30L is the volume of the container holding the seawater and eggs

1000ml/L is the conversion from millilitres to litres

Appendix 3: Example of calculation to determine the stocking density for fertilised eggs and/or larvae (modified after Braley, 1992)

Assuming there is an estimate of 1.25 x 10^8 fertilised eggs in a 100L volume. The aim is to stock eggs at 35 per ml in 500L rectangle larval tanks.

Example I: To calculate how much concentrated egg volume is needed per hatching tank

Use the following formula:

$D_1V_1 = D_2V_2$, where;

$V_1 = D_2V_2 / D_1$

V_1 = unknown volume of concentrated egg water needed per larval tank

D_1= mean estimate of fertilized eggs per ml = 1.25 x 10^8 eggs/(100L x 1000ml/L)

= 1250 eggs per ml

V_2= volume of each larval rearing tank, 500L x 1000 ml

= 5000,000 ml

D_2= the desired stocking rate of fertilized eggs per ml

= 35 eggs per ml

V_1= 35 x 500000/1250 eggs ml

= 14,000 ml

= 14.0L egg water per 500L hatching tank

Example II: To calculate how many larval tanks are required for the above example

A = B/C where

A = number of tanks required

B = volume of egg water 100L

C = volume of egg water required for 35 eggs per ml

A = 100/14

A = 7.1

Therefore, 7.1 500L larval tanks are required.

Appendix 4: Example of calculation to determine water flow of tanks

To calculate the flow rate of a tank:

Example I: What flow rate is required to achieve 1 ½ exchanges per 24 hours for the following tank?

Tank dimensions: Rectangle tank

9m x 2.5m x .7m = 15,750 litre

Flow rate required 1.5 exchanges per day

15,750 x 1.5 = 23,625 litre required per 24 hours

Flow rate per minute = 23,625 divided by 24 (time)

= 984 litres per hour / 60 minutes

= 16.4 litres per minute

Therefore, 16.4 litres per minute is required.

Example II: To measure the flow rate of a tank

Place a large (20L) bucket under the delivery system of a tank and measure how many litres are discharged in 1 minute. Multiply this number by 60 and an hour/litre rate can be found. By adjusting the ball valve on the tank intake, flow rates can be altered. By dividing the tank volume by this number, the time it takes for a complete water exchange can be found.

Flow rate 12 litres a minute, in a tank of 15,000 litres, how long will it take before one complete water exchange occurs?

Multiply 12 x 60 = 720 litres per hour

15,000/720 = 20.8 hours for a complete water exchange.