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Giant Clam Aquaculture: a Review on Induced Spawning and Larval Rearing

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Abstract Giant clams are the largest extant bivalves and widespread inhabitants of the Indo-Pacific coral reefs. These organisms are particularly sought after by the food industry because of their large mantle and adductor muscle, while also are relevant to the aquarium trade market. The aquaculture of giant clams is dependent of a land-based hatchery divided in several tanks with different purposes for larval rearing. Prior to reproductive events, broodstock are conditioned and gametogensis is stimulated by frequent food additions, increased photoperiod and intense physical-chemical stability at optimum conditions. Several different methods can be employed to induce spawning, most notoriously the intragonadal injection of serotonin, thermal stress and addition of macerated gonadal tissue of a conspecific. The success of the method is based on spawning occurrence, time for response and events of broodstock mortality. After spawning, gametes are fertilized and a succession of planktonic larval stages is cultured in hatching tanks and raceways. Embryonic development lasts for approximately 12 hours until the hatch of free-swimming and non-feeding trochophore larvae. After 24 hours post-fertilization larvae morph into veliger stage, made evident by the presence of calcium carbonate shells and velum. Veliger larva are fed with live or preserved phytoplankton and must also acquire symbiotic zooxanthellae. The last stage is the pediveliger stage at approximately one week post-fertilization, when settlement takes place and metamorphosis is soon attained.

Keywords Giant clam; Tridacna; Aquaculture; Spawning; Larval development

1 Background

1.1 Giant clams

Organisms belonging to the family Tridacnidae are known as giant clams and represent the largest living bivalves. The tridacnid clams are easily distinguishable among bivalves by having a hypertrophied siphonal mantle, which is also colourful given the fact that it houses symbiotic dinoflagellates known as zooxanthellae. Giant clams are distributed across the tropical coral reefs of the Indo-Pacific, where the water column is characterized by physical-chemical stability. Nutrient concentrations are low, salinity circles 34 ppt and temperature averagely ranges from 24℃ to 30℃. Specimens can be found in intertidal zones as well as in deeper offshore reefs at depths below 30 m, often attached to consolidated substrates or associated with hermatypic coral colonies.

The family Tridacnidae is comprised of ten species placed in two genera, *Tridacna* and *Hippopus*. The smallest and slowest growing species is *T. crocea* (Hart et al., 1998), while the largest and fastest growing is The True Giant Clam (Beckvar, 1981), *T. gigas*, that can reach over 130 cm in shell length (Ruscoe, 1962). While most species are physically similar, their identification can be easily made based on shell morphology and mantle characteristics (Braley, 1992; Calumpong, 1992).

1.2 Importance

There is a worldwide interest in giant clams, both as a food source and also for ornamental purposes. The mantle and adductor muscle are sold in many Asian restaurants while live organisms are traded for displaying in home and public aquaria. Larger species such as *Tridacna gigas* and *T. derasa* are especially sought after for their higher meat content while *T. maxima* and *T. crocea*, which present the highest

variation in mantle pattern and colouration, are the most popular choices for decorating aquariums. The marine ornamental trade has experienced a significant growth in the past decades and giant clams are among the best-selling invertebrates (Olivotto et al., 2011). So much that approximately 200,000 live giant clams were exported from the Pacific in the year of 2007 (Friedman and Teitelbaum, 2008).

Along with the increase in exports comes an increasing pressure on the natural stocks. As a result of overexploitation, giant clams are locally extinct in several Asian and Micronesian countries (Heslinga et al., 1984; Gomez et al., 1994). Considering that larger specimens are especially targeted and that the recruitment rate in tridacnids is slow (Braley, 1988), the recovery of populations has been hampered. Aquaculture and restocking programs were established in Micronesia and seeds were transplanted into the adjacent reefs (Heslinga et al., 1988), but not enough to restore the previous natural conditions. As a consequence, the major constraint in successfully aquaculturing giant clams has been the acquisition of large, healthy and numerous broodstock.

1.3 Ecology

Protandrous simultaneous hermaphrodites (Nash et al., 1988), giant clams at first can function solely as a male and eventually mature the female gonads, only then functioning simultaneously both as a male and a female. As broadcast spawners, the gametes are released in the water column and externally fertilized. In a spawning event, an individual releases sperm and after around 15 to 30 minutes undergoes gamete reversion, when eggs are first released.

The fecundity among tridacnids is high, with a single individual of *Tridacna gigas* reported to spawn 500 million eggs in a single event (Crawford et al., 1986). Mean egg size for giant clams is 100 μm (Lucas, 1988) and once fertilization occurs, an embryonic development of approximately ten hours (Fitt and Trench, 1981) begins to take place. At 12 hours post-fertilization (pf), the first of three larval stages, the trochophore larva, is hatched (Lucas, 1988; Mies et al., 2012). The trochophore larva is planktonic and free-swimming, but with incomplete digestive tract and therefore non-feeding. At 24 hours pf the veliger stage is attained (Fitt et al., 1984; Mies et al., 2012), made evident by the presence of calcareous shells and a more complex locomotion apparatus composed by velum and cilia. Newly formed veliger larvae use both endogenous and exogenous food sources, especially by filter-feeding on live phytoplankton (Heslinga et al., 1984; Crawford et al., 1986). During veliger stage, symbiotic zooxanthellae are also acquired through filter-feeding (Fitt and Trench, 1981). The last larval stage is the pediveliger stage, usually reached at one week pf (Fitt et al., 1984). Pediveliger larvae are marked by the presence of a foot and can crawl more frequently than swim, nearing settlement. Metamorphosis is completed ca. 15 days pf (Fitt et al., 1984) and is evidenced by the upward positioning of the shell, a consequence of a regulation of balance by the statocyst. At metamorphosis, an individual averages 200 μm in shell length (Heslinga et al., 1984). Natural larval mortality is high among giant clams, generally with less than 1% survival from egg to juvenile (Beckvar, 1981; Mies et al., 2012).

Giant clam larvae of larger species such as *Tridacna gigas* and *T. derasa* have been successfully reared without any external food additions (Heslinga et al., 1990). However, for most of the cases the addition of phytoplankton has proved essential for larvae to survive metamorphosis (Fitt et al., 1984; Fitt, 1993). While there have been no quantifications, it has also been determined that symbiotic zooxanthellae also contribute to the nutrition of giant clam larvae with translocated metabolites (Mies et al., unpublished data). Adult clams are mixotrophic filter-feeders and organic carbon is acquired by feeding on phytoplankton and dissolved organic matter (Klumpp et al., 1992; Klumpp and Griffiths, 1994). Additional sources of food are found in photosynthates translocated by symbiotic zooxanthellae, such as glucose and glycerol (Muscatine, 1967; Ishikura et al., 1999). It has been shown that such endosymbiotic association can supply over 50% the amount of carbon required by an adult giant clam (Klumpp and Lucas, 1994).

The symbiotic relationship between zooxanthellae and juvenile or adult giant clams is obligatory for the host.

The loss of zooxanthellae in the mantle tissue is a phenomenon called “bleaching”, in which the mantle colouration turns white and the host perishes as a consequence of massive tissue fragmentation and necrotic events (Sudek et al., 2012**)**. However, the nature of such relationship is slightly different when the host is still in its larval development. The gametes released by an adult giant clam are aposymbiotic, with total absence of zooxanthellae cells (Fitt and Trench, 1981; Fitt et al., 1986; Mies et al., 2012). Zooxanthellae are first acquired during veliger stage by filter-feeding in the water column. The symbionts are resistant to digestion (Fitt and Trench, 1981; Fitt et al., 1986) and eventually establish symbiosis prior to metamorphosis (Mies et al., unpublished data). Both larvae and adult individuals are able to establish symbiosis with different strains and isolates of zooxanthellae, originally extracted from corals, anemones and especially other giant clams (Fitt et al., 1986).

2 Hatchery System

2.1 Scale

Many different aquaculture systems have been described for the production of giant clam seeds. Large-scale systems (Heslinga et al., 1990) tend to be outdoors and connected to the ocean with no artificial lighting or manipulating of water physical-chemical parameters. Rearing protocols can be either of intensive or extensive character, with both yielding proper results (Heslinga et al., 1990). Small-scale systems (Mies et al., 2012) are generally located indoors and completely recirculated without any water exchange. Artificial lighting is provided and water parameters are thoroughly regulated through the use of protein skimmers and water heaters/chillers. Rearing techniques are of extremely intensive character. Independently of the scale, a few prophylactic filtering practices are commonly employed in all production systems, such as the installation of ultraviolet sterilizers and mesh filters. Both artificial and natural seawater have been used in the culture tanks.

2.2 Components

The most popular and successful systems used in giant clam aquaculture were comprised of five different types of tanks (Figure 1), each one for a particular purpose (Heslinga et al., 1990; Braley, 1992; Mies et al., 2012).

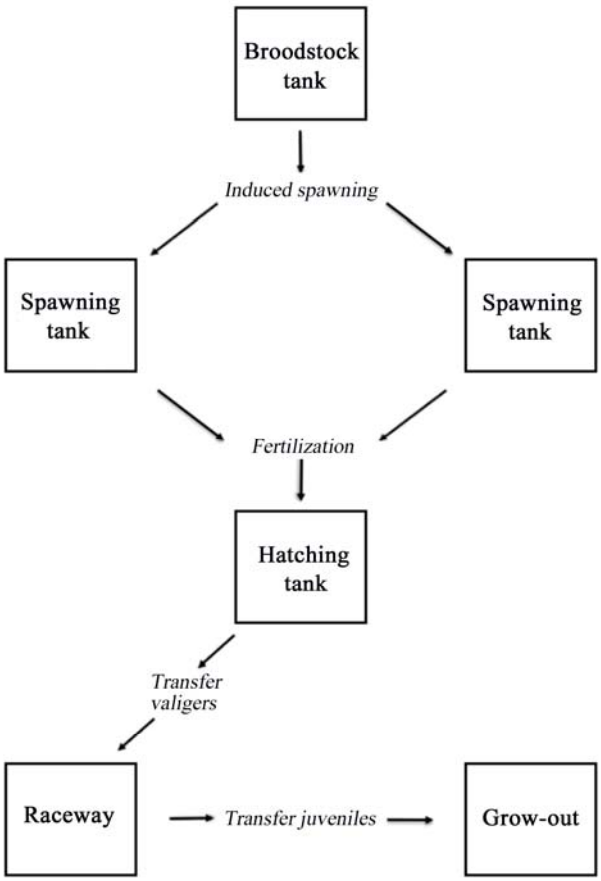

Figure 1 Diagram of the five sets of tanks generally used in giant clam aquaculture and the main tasks and processes involving each one of them.

The first is a broodstock tank (1), in which adult and mature clams are kept. The broodstock tank is generally shallow to facilitate light penetration and made of rigid material, *e.g.* concrete, in order to hold large and heavy specimens. Spawning broodstock are transferred to spawning tanks (2), which are small volume vessels of easy handling used for gamete collection and transportation. Gametes are fertilized in the hatching tanks (3), where eggs and trochophore larvae are stocked. Hatching tanks are generally round and made of plastic or fiberglass. When the majority of the larvae have reached the veliger stage they are transferred to shallow and flow-through raceways (4) also made of rigid material to withstand large water volumes. Raceways are provided with

appropriate irregular and rugose substrata to facilitate larval settlement and metamorphosis. The substrate provided is often cut in smaller pieces for removal and viewing under stereomicroscope. The last tank suits the purpose of grow-out (5) and is shallow and well lit to stimulate zooxanthellae growth.

3 Induced Spawning

3.1 Broodstock

Individuals chosen for broodstock are generally very large in order to spawn a good amount of quality and ripe eggs. Broodstock must also be very healthy and allocating energy for reproduction and not for healing. With the purpose of evaluating the quality and ripeness of the eggs stored in the gonads of an adult individual, a gonadal biopsy is usually performed (Braley, 1984). A thin hypodermic needle is inserted through the excurrent siphon aperture and a small tissue fragment is removed. Mature and ripe eggs are spherical and developing eggs are irregular (Crawford et al., 1986).

Bivalve broodstock are generally submitted to a conditioning process before induced spawning (Utting and Millican, 1997; Sühnel et al., 2012) with the principle of optimizing egg production. In the case of giant clams, suggested techniques include the regular addition of cultured microalgae, increase in photoperiod and keeping physical-chemical fluctuations at a minimum (Mies et al, 2011). While there are no reports on the frequency of spawn of giant clams *in situ*, several studies recommend that an individual is given at least six months to recover from one induced spawning event to another and successfully undergo gametogenesis (Gwyther and Munro, 1981; Heslinga et al., 1990). A rotation of broodstock may be necessary in order to keep production continuous.

3.2 Evaluation of the method

Several techniques may be employed to induce spawning in tridacnid clams and mixed results are obtained. The technique may have a chemical, biological or physical character. The parameters that should be considered for the evaluation of the success of a method should contemplate whether spawning took place, the spawning response time and the occurrence of broodstock mortality events (Table 1). Independently of the method, the individuals are rinsed clean of epibionts before induced spawning and placing in the spawning tank to avoid pollution of the aquaculture system.

3.3 Chemical methods

The most successful method of induced spawning giant clams is of chemical character, the intragonadal injection of 1 mL of a 1.0 g L^{-1} solution of serotonin (5-hydroxytryptamine), a neurotransmitter naturally found in mollusks (Welsh and Moorhead, 1960; Braley, 1992). There are two different techniques for injecting a clam with serotonin, depending on the syringe positioning and place of insertion. The needle can be inserted through the mantle cavity (Braley, 1985) or through the byssal orifice (Mies et al., 2011). The mantle cavity injection is based on the insertion of the needle through the excurrent siphon, in a downward angle, until the gonadal tissue is reached. At this point the needle is allowed to pierce a few millimeters into the gonadal layer and the syringe contents are ejected. The second technique is based on the injection through the byssal orifice, an aperture found underneath the shells from which the byssal filaments and foot are exposed. The clam is held and the needle is inserted through the byssal orifice, piercing a muscle layer and then reaching the gonads. The main difference between both techniques is the fact that the mantle cavity injection sometimes reports broodstock mortality (Ellis, 1998). That is a consequence of misplaced injections that inadvertently pierced vital organs, while there are no major organs in between the byssal orifice and the gonads, with broodstock mortality yet unreported in the byssal orifice method. Both serotonin techniques yield high response rate and quick spawning, with the byssal orifice technique often inducing the release of gametes in less than a minute (Mies et al., 2011).

Hydrogen peroxide has also been reported as an agent of induced spawning in tridacnid clams. By means of a syringe, 10~20 mL of a 3% solution is introduced to the incurrent siphon (Beckvar, 1981). Spawning does occasionally take place, but the majority of the individuals does not respond and still show many signs of stress (Fitt and Trench, 1981; Gwyther and Munro, 1981).

Table 1 Parameters considered for the evaluation of the success of an induced spawning method performed in tridacnid clams.

Method	Spawning occurrence	Average time for spawn	Broodstock mortality events	Character	Reference
Serotonin injection (mantle cavity)	high	minutes	yes	chemical	Braley (1985), Ellis (1998), Mies et al. (2011)
Serotonin injection (byssal orifice)	high	seconds/minutes	no	chemical	Mies et al. (2011)
Hydrogen peroxide	low to medium	minutes/hours	no	chemical	Beckvar (1981), Fitt and Trench (1981), Gwyther and Munro (1981)
Macerated gonads	medium to high	minutes/hours	no*	biological	Fitt and Trench (1981), Gwyther and Munro (1981), Heslinga et al. (1990)
Thermal stress	low to medium	hours	yes	physical	Fitt and Trench (1981), Heslinga et al. (1990), Ellis (1998)

Note: *while no induced clams perish due to the employment of this method, it does require the sacrifice of adult broodstock.

3.4 Biological methods

When an adult individual begins spawning activity, non-described pheromones are also released along with the gametes. These substances trigger spawning activity in other nearby individuals, resulting in an epidemic spawning event. The introduction of macerated gonads of a conspecific is a technique based on the simulation of an epidemic spawning event. This technique requires the sacrifice and removal of ripe gonads of conspecifics. The gonadal tissue is then macerated and suspended in a solution of ca. 50 g of gonadal tissue L^{-1} (Heslinga et al., 1990) and finally approximately 10 mL are introduced to the incurrent siphon of a broodstock clam. This procedure is relatively successful (Fitt and Trench, 1981; Gwyther & Munro, 1981), but spawning may take place hours after stimuli and it requires the sacrifice of broodstock.

There are alternative biological methods employed in the induced spawning of other bivalve species, such as the simulation of a phytoplankton bloom (Velasco et al., 2007), but most of them remain untested in tridacnid clams.

3.5 Physical methods

Stressful conditions often cause giant clams to spawn, as it is made evident with traveling and transported broodstock. Most physical techniques are based on the generation of a stressful event. The thermal stress, or heat shock, is widely used in the oyster and scallop industries and it is the sole physical technique successfully used for induced spawning in giant clams. This can be accomplished in two different manners, either in raising the water temperature or exposing the individuals to sunlight (Heslinga et al., 1990). After a few hours under heat stress, the individuals are returned to the original tank and natural conditions, where spawning activity is expected to commence. While having the advantages of being costless and producing low mortality, this procedure is frequently unsuccessful (Fitt and Trench, 1981) and it generally takes several hours for individuals to respond and start spawning (Ellis, 1998).

4 Larval Rearing

4.1 Early stages

Induced broodstock are placed in the spawning tanks once stimuli have been made. Spawning activity is marked by the release of gametes through the excurrent siphon (Figure 2). Individuals spawning sperm are kept in separate tanks from those spawning eggs. Sperm is the first gamete released and is active only for approximately one hour (Ellis, 1998). After sperm release is ceased, egg release commences and can last several hours. Egg cells, however, are viable for a shorter period, around 30 minutes (Ellis, 1998)

Figure 2 Sperm release by broodstock of *Tridacna crocea* 40 seconds after induced spawning by means of an intragonadal injection of serotonin through the byssal orifice.

and therefore must be quickly fertilized by fresh sperm. The fertilization procedure is performed in the hatching tanks. The recommended protocol is to store eggs at a density of 25 mL^{-1} and add sperm considering a volumetric ratio of 1:200 for egg:sperm (Heslinga et al., 1990), in order to avoid polyspermic events. At two hours post-fertilization (pf) the fertilized eggs have all sank to the bottom (Mies et al., 2012). The top layer of the water column of the hatching tanks can be siphoned to remove floating debris. Aeration spots can be turned on to keep the oxygen levels high. With a homogenous water column, eggs should be sampled and a minimum 80% should be fertilized to yield a commercially viable batch (Ellis, 1998).

After fertilization, a daily addition of 5 ppm of broad-spectrum antibiotics has greatly improved survival (Fitt et al., 1992). Cephalosporin and streptomycin are suitable choices (Fitt et al., 1992; Mies et al., 2012). Antibiotics can be used on a daily basis during the first week and gradually removed the following days. Tanks should be kept undisturbed during the embryonic development and the trochophore stage. A simple sampling can be performed to evaluate hatch rate and trochophore activity.

4.2 Veliger stage

After all larvae have reached the veliger stage, approximately 30 h pf at 27℃ (Mies et al., 2012), the hatching tanks are drained and larvae are transferred to the raceways. This procedure is performed slowly, by submerging a 70-μm mesh cloth in a bucket containing filtered seawater and siphoning with a hose. The veligers are then placed in the raceways and stocked at a density of 10 larvae mL^{-1} (Fitt et al., 1984). Slightly stronger aeration can be turned on.

Veliger larvae have fully formed digestive tract and feed on available phytoplankton. To increase survival rates, green algae, haptophytes and diatoms may be added to the larval tank at a final concentration of approximately 5×10^4 cells mL^{-1} (Fitt et al., 1984; Murakoshi, 1986). The haptophyte species *Isochrysis galbana* has proven the most suitable and with an adequate fatty acid profile (Fitt et al., 1984; Fitt et al., 1986). Added phytoplankton can be either live and cultured or dead and preserved as found in concentrated products. Giant clam larvae fed every two days until metamorphosis have yielded the fastest growth rates (Mies et al., 2012).

Giant clam larvae are aposymbiotic and during veliger stage first acquire symbionts (Fitt and Trench, 1981; Heslinga et al., 1984; Mies et al., 2012). The symbiotic relationship is established within 24 hours after zooxanthellae acquisition, translocating photosynthetically fixed carbon in the form of glycerol (Mies et al., unpublished data). The importance of the early seeding of zooxanthellae is highlighted by Mies et al (2012), where is shown that veliger larvae containing symbionts in the digestive tract grow 25% faster than aposymbiotic veliger larvae.

Zooxanthellae from different taxa have been successfully seeded into giant clam larvae (Fitt et al., 1986; Mies et al., 2012). Freshly isolated and highly motile cells from a conspecific have produced the higher acquisition and survival of veliger larvae (Fitt and Trench, 1981; Fitt et al., 1986). Zooxanthellae can be retrieved from the tissue of other organisms by blending and filtering in a 40-μm mesh. This symbiotic relationship has proven so fundamental that no juvenile tridacnid clam has ever been found without zooxanthellae in its mantle tissue. Zooxanthellae additions are recommended on days four and six pf (Mies et al., 2012) and also occasionally after larvae have metamorphosed

(Crawford et al., 1986). After zooxanthellae offering, artificial lighting must be turned on in indoor systems. Photoperiod should simulate tropical reef conditions and light distribution must be homogenous across the raceways. Considering veliger larvae are positively phototactic, patched lighting may result in aggregation and overcrowding in some areas. Daily sampling for the determination of zooxanthellae acquisition by veliger larvae is recommended (Figure 3).

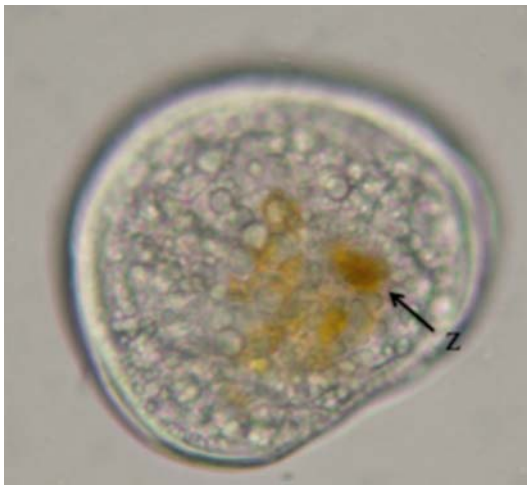

Figure 3 Veliger larva of *Tridacna crocea* with defined calcareous shells at ca. 100 h post-fertilization. Digestive tract is partially full containing several zooxanthellae cells (Z).

4.3 Pediveliger, metamorphosis and grow-out

Pediveliger larvae require as much attention as that provided for the veliger stage. Feeding and zooxanthellae additions once every two days greatly improve survival (Crawford et al., 1986; Mies et al., 2012). At approximately 14 days pf the metamorphosis is complete and a thin mantle will be extended beyond the shells and visible. At this point the substrate can be removed and transferred to the grow-out tank for seed production. Strong lighting and intense water movement aid in the growth of juvenile clams, as well as slightly elevated nutrient concentrations (Fitt et al., 1993). It has been shown that a concentration of approximately of 4.0×10^{-4} g L^{-1} of NH_4NO_3 stimulates zooxanthellae growth and significantly increases shell length (Heslinga et al., 1990; Grice and Bell, 1999).

In aquaculture establishments near the shore, juveniles at 2 cm of shell length can be transferred to open ocean grow-out cages (Calumpong, 1992). It has been determined that giant clams tend to grow much faster in natural conditions (Munro et al., 1993). Large cages made with PVC or plastic can be placed in clear, shallow and quiet tropical areas and stock individuals at 1,000 individuals m^{-2} (Heslinga et al., 1984). Covering the cages with 25-mm mesh is recommended to avoid predation by fish and benthic invertebrates (Calumpong, 1992). The cages are no longer necessary once clams reach 20 cm in shell length considering that the risk of predation is significantly reduced at this point.

Authors' Contributions

M Mies and PYG Sumida both wrote and reviewed the presented article.

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