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Comparative Study of Available Spawning Methods of the Giant Clam *Tridacna squamosa* [Bivalvia: Tridacnidae] in Makogai, Fiji

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Abstract: In this present study, the intra-gonadal injection of serotonin, heat stress and macerated gonads methods were compared to determine the best method of induced spawning in terms of lowest larval mortality rate in *Tridacna squamosa*. Broodstock were induced to spawn using the three methods. The gametes were collected, fertilized and stocked (10 larvae/ml) into larval rearing tanks to monitor percentage mortality of the different treatments. Larvae were fed twice with the zooxanthellae during the veliger stage. The addition of zooxanthellae was decreased significantly ($P \leq 0.05$) the mortality in *Tridacna squamosa*. Initial mortality of the clams was significantly higher than before the first and second additions of zooxanthellae; however, there was no significant difference ($P \geq 0.05$) between the first and second additions of zooxanthellaes. The macerated gonads, heat stress and serotonin methods of induced spawning differed significantly ($P \leq 0.05$) with mean value of (6657.56±2766.527%), (7355.56±3045.012%) and (8979.56±3641.121%) respectively. High mortality in the veliger stage (48 hrs post fertilization) may have been caused by bacterial infection. Addition of zooxanthellae was seen as an important procedure especially when feeding was not done in the hatchery phase of giant clam larvae production. Thus, the best method of spawning in terms of lowest mortality rate was macerated gonads followed by heat stress and serotonin respectively.

Key words: Tridacna squamosa · Serotonin · Heat Stress · Macerated Gonad

INTRODUCTION

Giant clams have a long traditional and cultural history and it is classified as an important resource throughout the tropical Indo-Pacific. Their meat has been traditionally used as a subsistence food source. According to Ellis [1] the shell is used to make dishes, tools, jewellery and ornaments and 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 of giant clam is as a living decoration in home and public aquariums. Global demand for the aquarium trade was 200 000 pieces in the year 2007 and 69 000 pieces of the demand was exported from Pacific region [2].

Giant clams and belong to the family Tridacnidae and there are nine species occurring in two genera, *Tridacna* and *Hippopus* which are well distributed in the Indo-pacific region and the Red sea [1]. The genus *hippopus* has two species; Hippopus hippopus and Hippopus porcellanus. According to Adams [3], the genus *Tridacna* has seven species; *Tridacna crocea, Tridacna derasa, Tridacna gigas, Tridacna maxima, Tridacna squamosa, Tridacna tevoroa and Tridacna costata*. Out of the two genuses both are presently found in Fiji which includes *Tridacna derasa, Tridacna squamosa* and *Tridacna gigas*. However, *Tridacna tevoroa* and *Hippopus hippopus* are also present in Fiji, but not yet documented.

Initially interest developed in researchers to culture giant clams by concerns such as depletion of wild stocks by eminent fishing pressure such as the use of SCUBA gear. Giant clams have been reproduced in land-based facilities by natural and artificial methods of spawning.

Tridacna squamosa, also known as the "*fluted or scaly clam"*, is distinguished by its large and well-spaced scutes (scaly projections on the shells), maximum shell length of about 40cm and has mantle which tends to be

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mottled in various mixes of green, blue, brown, orange and yellow [4]. It originated from Indo-Pacific region [5] and the distribution extends from eastern Polynesia to the Red Sea and to East African shores [6].

T. squamosa prefers fairly sheltered lagoon environments and usually associated with coral reefs [4]. Giant clams are 'protandric hermaphrodites' that is they mature as males in first 2-3 years and later also develop gonads [1].

In the central tropics there is no evidence of any seasonality in reproduction [7, 8] and there is very little information on the size at maturity of giant clams [9].

There are 6 reported methods of induced spawning in giant clams. Wada [10], Jameson [11] and LaBarbera [12] have successfully induced spawning in tridacnids using fresh and macerated gonads as the stimulus for spawning. Braley [13] induced spawning in giant clams by intra gonadal injection using 20 μM. Exposure to a rapid temperature change also induces spawning [1]. Mechanical irritation of the posterior abductor muscle [7] and administering a mild electric shock [12] also induces spawning.

MATERIALS AND METHODS

The study site Makogai Mariculture Station (MMS) was located on Makogai Island; east coast of Viti Levu on the Lomaiviti archipelago with coordinates 17.26° south and 178.58° east. Broodstock was collected from the reefs around Makogai Island, transported to the hatchery in tubs containing sea water and conditioned for 2 days. Sea water was filtered to 1 μm to eliminate possible sources of bio fouling agents and predators such as pyramedellid and cymatium snails [1]. Water parameters (Temperature, pH and Salinity) were recorded [1, 5, 14].

Broodstock were given four treatments to induce spawning which included injecting the mature gonads with Serotonin, placing the clams in the sun and stressing them for 1-2 hours, introduction of matured macerated gonads and introduction of hydrogen peroxide. After giving the four treatments to the brood stock, observations were made to see signs of spawning. Initial signs of spawning was an observation of gaping and contraction closely followed by sperm release [1].

For all treatments eggs and sperm were collected in separate containers and fertilized in a 1:200 sperm: oocyte ratio by volume [1]. Fertile eggs were homogenized and sub samples were taken to count the number of fertile eggs in the fertilization containers 2 hours post fertilization and stocked into Larval Rearing Tanks (LRT) with a stocking density of 10 larvae/ml where each treatment had 3 replicates.

Daily activities during larval rearing included 50% water exchange every 12 hours, monitoring of water parameters and counting the number of larvae every 24 hours. Zooxanthellae extract was added at 96 and 144 hours post fertilization [15]. After 5 hours of zooxanthellae addition at 96 and 144 hours, the larvae was examined under the microscope to check for the presence of zooxanthellae in their guts. No feeding was done apart from the two zooxanthellae additions during the culture period.

Water Parameters (Temperature, pH and salinity recorded [1, 5, 14] SPSS 18 was used to compare the means of survivability for the different treatments to show if the difference between the treatments were significant. The Tukey's post hoc test was then run to compare which means differ amongst each other.

RESULTS AND DISCUSSION

Water Parameters recorded were observed to be in accordance to the finding of [5], [1] and [14] as shown in Table 1.

Raw mortality was converted to percentage and was plotted against time as summarized in Figure 1. This illustrated that before adding zooxanthellae, at 24 hours of stocking the larvae into the larval rearing tanks, the mortality for Treatment 1 (Serotonin), Treatment 2 (heat stress) and Treatment 3 (macerated gonad) were 69.20%, 73.40% and 79.30% respectively. In the next 24 hours the mortality decreased to 53.50%, 51.59% and 53.60% for treatments 1, 2 and 3 respectively.

At 72 hours the percentage mortality for all treatments were approximately the same as at 48 hours with values of 51.50%, 52.395 and 53.60% for treatments 1, 2 and 3 respectively.

Table 1: Summary of water parameters followed during broodstock acclimation, spawning and larvae culture of *T. squamosa*.

Parameter	Temperature	pH	Salinity	Dissolved Oxygen
Value	26.5 – 27.5 °C	8.3 - 8.4	33.8 ppt	4 ppm
Reference	[5]	[1]	[14]	-

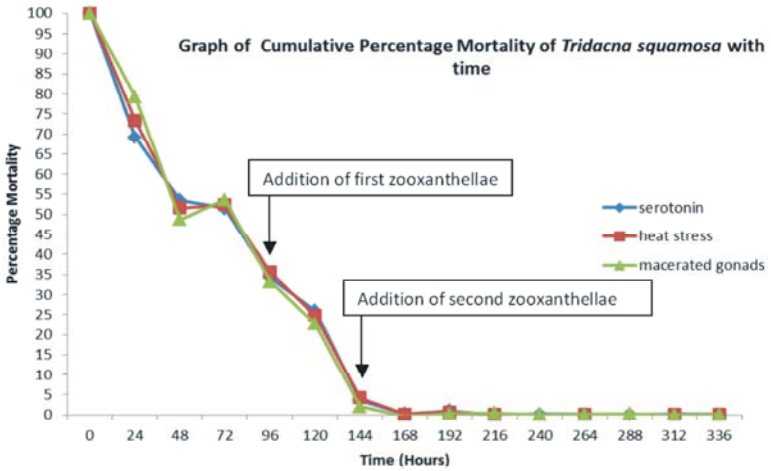

Fig. 1: Cumulative percentage mortality of serotinin, heat stress and macerated gonad induced spawning methods in *Tridacna squamosa* with time.

Zooxanthellae extract was added at 96 hours and the mortality further reduced to 34.30%, 35.59% and 33.21% for treatments 1, 2 and 3 respectively. Percentage mortality for treatments 1, 2 and 3 reduced to 25.90%, 24.80% and 22.70 respectively. A second dose of zooxanthellae was given at 144 hours and it further reduced the percentage mortality to 3.60%, 4.29% and 2.10% for treatments 1, 2 and 3 respectively. After the addition of second zooxanthellae, that is from time 168 hours to 336 hours the percentage mortality for all treatments were generally less than 1%.

The addition of zooxanthellaes significantly decreased the mortality in *Tridacna squamosa*. Initial mortality of the clams was significantly higher than before the first and second additions ($P \leq 0.05$) of zooxanthellae however there was no significant difference ($P \geq 0.05$) between the first and second additions of zooxanthellaes.

The three methods of induced spawning differed significantly ($P \leq 0.05$). Macerated gonads (6657.56±2766.527%) had significantly lower mortality than heat stress (7355.56 ± 3045.012%) and serotonin (8979.56±3641.121%).

The results of this study showed that successful spawning occurred in serotonin and macerated gonad methods and gametes were obtained within 2 minutes after giving treatments to the brood stock. In this study the time taken to induce spawning for heat stress method was consistent with that of Ellis [1], that is spawning occurred approximately 3 hours after the broodstock was stressed and put back in water (26.5°C).

The larval mortality could be high in the early stages because of bacterial infection. [16], showed that a range of bacteria, mainly from the genus *Vibrionaceae* from the larval culture environment, initiate disease and cause mortality in healthy larvae in the veliger stage (48 hour post fertilization).

In this study, larvae were not fed with microalgae before and after addition of zooxanthellae since there was no provision for on-site microalgae production. Thus this could have contributed to very high mortality rate (0-72 hours) until the acquisition of zooxanthellae at 96 hours (veliger stage). Mies [17] observed high mortality rate despite the larvae being fed with microalgae before addition of zooxanthellae and mortality decreased when zooxanthellae was added at 96 hours. This is also supported in the findings of Ellis, Knop and Fatherree [1, 5, 14] who state that zooxanthellae must be seeded into the clams as early as day four, otherwise mortality can be considerably higher. Fitt *et al*. [18] initially stated that larval growth is enhanced after live zooxanthellae are ingested by the larvae and hypothesized that photosynthetically fixed carbon might be translocated from zooxanthellae to the veligers.

Zooxanthellae translocate products of photosynthetic activities to host and are the major source of nutrition in tridacnids [19]. Addition of zooxanthellae was seen as an important procedure especially when feeding was not done in the hatchery phase of giant clam larvae production. No difference was observed after 2nd addition of zooxanthellae since it had no impact on mortality. This observation can be attributed to, zooxanthellae not being detected well during observation after the 1st addition.

It was expected that all treatments would have similar percentage mortality but in the experiment it was proven otherwise. Serotonin method dominated the lowest percentage mortality when compared with macerated

gonad and heat stress. It is assumed from the observation that serotonin that was injected into the gonads might have some sort of effect on the gametes and later affect the larvae. However, further research should be done to prove this.

Better results (lower mortality rates) would have been achieved if the larvae were fed with micro-algae before zooxanthellae extract was added. A re-circulating system would also have aided in achieving a more stable culture environment in terms of water parameters for larval rearing. Additionally, high mortality in the early stages could have been decreased by giving a 4 ppm dose of antibiotic cephalosporin every 24 hours as suggested by Fitt *et al*. [18].

CONCLUSION

The main objective of this investigation was to compare the three methods to determine the best method of induced spawning of *T squamosa* in terms of lowest larval mortality rate and this was achieved. The experiment showed that macerated gonad method dominates survival rates (lowest percentage mortality) over the heat stress and serotonin methods. Other researchers can now benefit from this research and can develop further research as suggested in the discussion on induced giant clam reproduction. Hatchery operators can adapt to macerated gonad method that showed highest survival rates and that may reduce hatchery costs and maximize production.

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