



Spawning, larval development and spat settlement in the Venus clam *Gafrarium tumidum* (Roding, 1798) from south-east coast of India

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ABSTRACT

Induced spawning, larval development and spat settlement of the venus clam *Gafrarium tumidum* (Roding, 1798) were studied. Brood clams (27.5-32.8 mm dorsoventral measurement / 5.3-15.8 g) collected from the natural bed were conditioned (24.0 -26.0 °C; 33.5-34.5 ppt) and subjected to thermal stimulation for spawning. Fertilised eggs were spherical in shape and measured, on an average 62 µm in diameter. Day one veliger larvae of mean size 91 ± 1.69 µm transformed into umbo stage on day 7 at a size of 149 ± 6.02 µm, pediveliger on day 11 at 180 ± 6.0 µm size developed functional foot which settled on day 13 at a size of 200 ± 6.945 µm. The growth of post-set clams was monitored and described upto 68 days and the growth equation was derived. Few surviving post-set clams transformed into juveniles and radial ribs were observed with naked eye when they were 2.3 mm in 75 days. These clams reached 6.4 mm in 165 days. Metamorphosis and larval growth are compared with that of other bivalve species reported earlier. Further, specific refining of methodology for post-set clam rearing would pave way for their mass seed production for aquaculture purpose.

Keywords: *Gafrarium tumidum*, Larval rearing, Spat settlement, Spawning, Venus clam

Introduction

Spawning and larval development of various commercially important temperate bivalves have been carried out and reported worldwide (Chanley, 1965; Loosanoff and Davis, 1963; Wong *et al.*, 1986). In India, technologies are available for large scale seed production of the oyster *Crassostrea madrasensis* (Nayar *et al.*, 1984) pearl oyster *Pinctada fucata* (Alagarsami *et al.*, 1983) green mussel *Perna viridis* (Sreenivasan, 1988) clams *Meretrix casta* (Sreenivasan and Rao, 1991), *M. meretrix* (Narasimham *et al.*, 1988) and *Anadara granosa* (Muthiah *et al.*, 1992). Venus clam *Gafrarium tumidum* (Roding, 1798) is an important resource on the shores of the Gulf of Mannar and Palk Bay (Hornell, 1922). It is also being exploited for food in many countries bordering the Indian Ocean (Nayar and Rao, 1985). Hsieh *et al.* (1981) in their study, underlined the potential of these clams for possible mariculture in the littoral zones of Penghu, Taiwan. Work on this species in Indian waters is very limited. Jagadis and Rajagopal (2007 a; b) have reported the reproductive biology as well as age and growth of *G. tumidum* from south-east coast of India. Induced spawning and embryonic development upto pre-set stage has been studied (Jagadis, 2005). Induced spawning, larval development and spat settlement of Venus clam under hatchery conditions were studied in the present investigation.

Materials and methods

Conditioning, spawning and embryonic development

G. tumidum of size range 27.5-32.8 mm dorsoventral measurement (DVM) and 5.3-15.8 g weight were collected from mud flats of Chinnapalam, Pamban, south-east coast of India (latitude 8° 35' – 9° 25' N and longitude 78° 08' – 79° 30' E) with a hand shovel during low tide and transported to the shellfish hatchery of the Tuticorin Research Centre of the Central Marine Fisheries Research Institute. The clams were placed in 100 l fibre reinforced plastic (FRP) tank containing filtered seawater with mild aeration in the conditioning room (temperature 24-26 °C; salinity 33.5-34.5 ppt) and fed once with mixed algal cultures predominantly of *Chaetoceros* spp. @15 litres with a concentration of 2 lakh cells ml⁻¹ (Nayar *et al.*, 1984). After conditioning for a period of 10 days, the clams were used for spawning. In the event of not obtaining spawning, they were transferred back to the conditioning room and the experiment was repeated after another 10 days.

Fifteen to twenty numbers of conditioned unsexed clams were used for spawning experiments. They were placed in 30 litre plastic troughs containing filtered seawater and the temperature was slowly raised 4-5 °C above the ambient level by adding hot seawater (34 ppt). After completion of spawning, samples of eggs drawn and were

microscopically examined for percentage of fertilization. Viable fertilized eggs were then gently siphoned and sieved through a sieve of 4 inches diameter, made of 20 mm nylobolt mesh. The sieved fertilized eggs were then given a gentle wash to remove the excess sperms and transferred to 500 l circular FRP tanks containing 250 l filtered seawater (10 μm ; 34 ppt) and the subsequent embryonic and larval developments were monitored from the larvae of this spawning. The tank was covered with black cloth to avoid light and dust. Samples were drawn every half an hour and observed till they transformed into trochophore larvae.

Larval rearing

The day 1 veliger larvae were filtered through a 30 mm nylobolt mesh and transferred to a glass beaker containing 5 litres seawater. The larval count was estimated using a larval counting chamber marked with 100 squares, which can hold 1 ml of larval sample. The number of larvae in 1 ml of sample was then raised to the total volume of the larval sample for arriving at the number of viable larvae produced in that spawning. The estimated larvae were then stocked in 1000 l fibre glass tank filled with 500 l filtered seawater @ 2 larvae ml^{-1} . Larval rearing was done following Nayar *et al.* (1984). The 'D' shaped veliger larvae were fed with pure culture of *Isochrysis galbana* at the rate of 5,000 cells/larvae/day, umbo stage at the rate of 8,000 cells/larvae/day and from late umbo to pediveliger stage at the rate of 10,000 cells/larvae/day and thereafter at the rate of 20,000 cells/larvae/day. Mixed phytoplankton predominantly of *Chaetoceros* spp. were also supplemented as feed @ 5 litres with 1.0 lakh cells/larvae/day after the settlement stage. Water temperature, salinity and pH in the rearing tank were monitored once in two days. Twenty larvae were measured for height (dorsoventrally, DVM) and length (anteroposteriorly, APM) at regular intervals and the mean length/height were considered for studying the growth of the larvae. Photographs were taken using Labomed binocular microscope.

Results

Conditioning and spawning

Clams maintained in the conditioning room reached ripe conditions suitable for spawning within 10 days on an average. Spawning occurred when thermally stimulated and was completed in 1 h and fertilization occurred during this time. Of the five experiments conducted each with 20 unsexed clams between November 2008 and January 2009, induced spawning was successful in three attempts (60%) and the average percentage of fertilization was 96.

Early embryonic development

Spherical eggs measuring 56 to 63 μm (average 62 μm) in diameter, were released immediately after the

males spawned. Spawning was noticed for 45-60 minutes and the water in the spawning container became milky due to the continuous release of the gametes. Fertilization followed immediately and the fertilized eggs settled at the bottom (Fig. 1a). After 20 minutes post fertilization, the first polar body started developing. (Fig. 1b). Thirty minutes after fertilization, cleavage started and the differential macromere and micromere were observed at this stage and later the two cell stage appeared (Fig. 2c) followed by trefoil stage (Fig. 2d). Subsequently, the 8, 16 and 32-celled stages developed followed by the blastula and gastrula stages and became 'morula' within four hours with a typical 'rotary' movement (Fig. 2e). The morula transformed into 'trochophore' within 7 hours moving in the water column by lashing the flagella. (Fig. 2f).

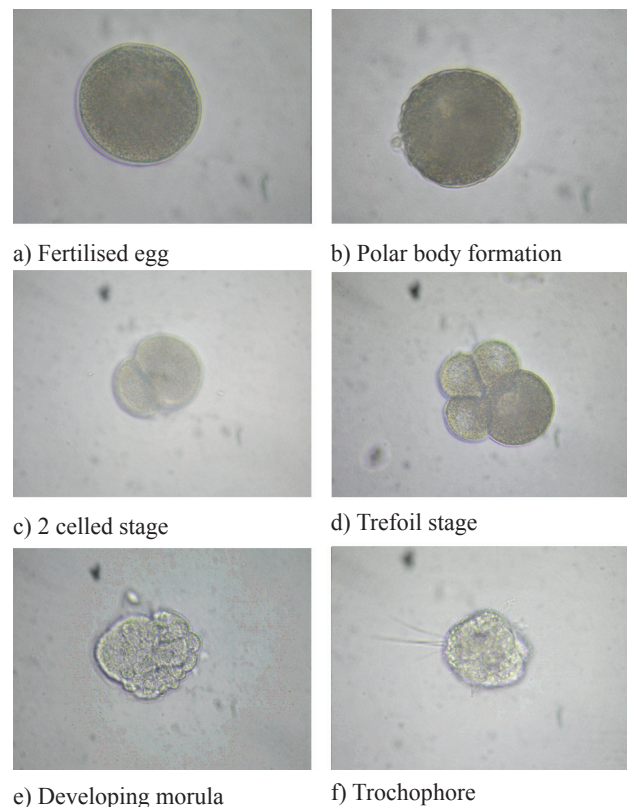


Fig. 1 a-f Embryonic development of Venus clam *Gafrarium tumidum*

Larval development

After 20 h of fertilization, the stocked fertilized eggs got transformed into 'D' shaped veliger (Fig. 2a). The average yield of 'D' larvae was 5.58 lakhs/spawning. The average size of the veliger larvae was 70/91 μm DVM and APM respectively, and had equal shell valves and a well developed ciliated velum with strong musculature. On the fourth day the larvae transformed into 'early umbo' and measured 112/123 μm and the development of umbo was not very prominent. On day 7, they transformed into an

almost ovoid 'umbo' stage and had an average size of 134/149 μm . (Fig. 2 b). On day 9, the larvae reached 'late umbo' and measured 152/172 μm size. On day 11, when the larvae reached 162/180 μm , few of them had developed functional foot and velum was also present in most of the larvae with reduced activity. No eyespot was observed when the larvae were about to develop foot and settle. On day 13, the size increased to 170/200 μm and almost all the larvae had developed functional foot to become 'pediveliger' (Fig. 2c) and descended to bottom confirming set condition (25-28 % of reared larvae). In the present experiment, considerable mortality (above 70%) was observed at this stage. Further rearing of the post-set clam was continued, weekly measurements of the post-set clam was recorded. The set clams reached 244/326; 327/378; 360/438; 425/506; 488/579; 609/694 and 730/838 μm on 20, 27, 34, 41, 48, 58 and 68th days respectively. Till then, the radial ribs on the shell was not observed but the shells had concentric rings on the periphery (Fig. 2d). Gradual mortality occurred and the few surviving post-set clams were collected and reared in a smaller tank without any sandy substratum and they transformed into juveniles. The radial ribs were observed with naked eye when they were 2.3 mm on 75th day. They have grown to a size of 6.4 mm on 165th day (Fig. 2e). Seawater temperature ranged from 28.5 to 32 °C, salinity from 32.5 to 34.0 ppt and pH from 8.0 to 8.5 in the rearing tanks.

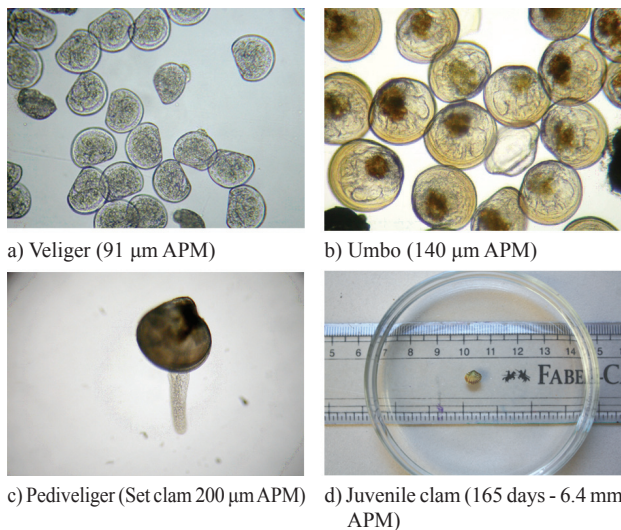


Fig. 2 a-d. Photomicrographs of larval stages of Venus clam *G. tumidum*

The linear relationship between the height (DVM) and length (APM) in *G. tumidum* is described by the equation: $H = 0.863772 \times L + -4.17465$ and the 'r' is 0.9970 (Fig. 3). The growth of the clams was curvilinear and described by the equation: $L = 10.8765 \times D + 74.23875$ and $H = 9.40793 \times D + 59.60576$, where L is the length, H is the height and

D is number of days post spawning. The r^2 values obtained for length and height 0.9958 and 0.994195 respectively, indicated high level of significance (Fig. 4).

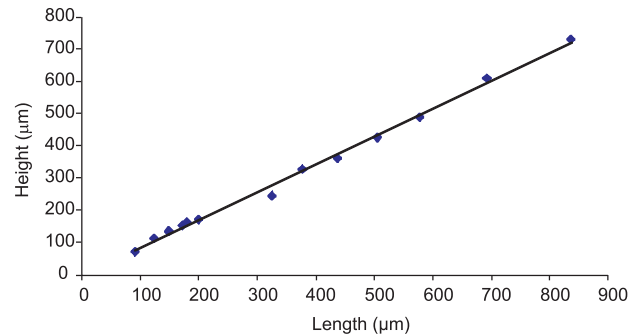


Fig.1. Relationship between length (APM) and height (DVM) in Venus clam *G. tumidum*

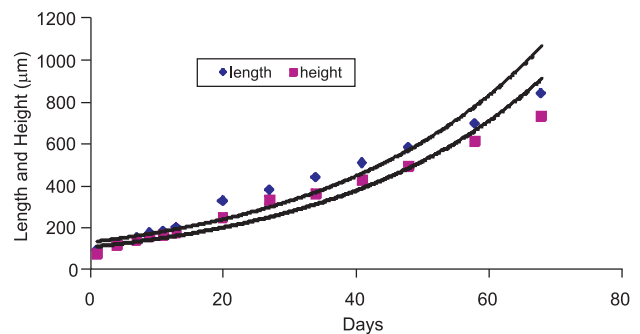


Fig. 2. Growth of Venus clam *G. tumidum*

Discussion

Many bivalve species of aquaculture importance have been studied in detail for their spawning and spat production (Broom, 1985; Narasimham *et al.*, 1988; Sreenivasan and Sathyanarayana Rao, 1991; Muthiah *et al.*, 1992). Thermal stimulation technique is found to be effective to induce spawning in bivalves (Sreenivasan, 1988). Sreenivasan (1988) insists the collection of broodstock from places where they naturally exist. He also suggested that the conditioning of the broodstock should be done at least 5 °C less than the ambient temperature. In the present experiments also, the brood clams were collected from the natural bed and conditioning was done.

The mean size of fertilised eggs of *G. tumidum* measured 62 μm and was more or less similar to that of *A. granosa* (50-60 μm) and much smaller than that of *M. meretrix* (75.9 μm) and *M. casta* (83 μm). The transformation into veliger larvae in the present study took about 19-20 h and the size attained was 91 μm at temperature range of 28.5 to 32.0 °C. Muthiah *et al.* (1992) reported 20-26 h and a size of 80-90 μm at temperatures ranging from 27.0-32.0 °C for *A. granosa*. In the case of

M. casta, it was 14 h with a size of 85 μm and in *M. meretrix*, it was at the 20th hour with a size of 116.4 μm at temperatures 30.5 to 32.5 °C. The size of the veliger larvae of *G. tumidum* was smaller when compared with *M. meretrix*. The time taken for transformation was similar to that of *A. granosa* and *M. meretrix* but longer than *M. casta* though the rearing temperature ranges were more or less similar.

Umbo was not prominent in the present study and is comparable to that of the observation made for great clam larvae (Narasimham *et al.*, 1988). Eyespot was not observed in the larvae of *G. tumidum* as that of *M. meretrix* (Narasimham *et al.*, 1988) prior to setting. Chanley (1965) observed the absence of eyespot in mactrid clam *Rangia cuneata*. Loosanoff *et al.* (1966) also made similar observation in the larvae of dwarf surf clam *Mulinia lateralis*, venerid clam *Pitar morrhuana* and the cockle *Laevicardium mortoni*. In the present study, the functional foot in most of the larvae developed when they reached a size of 200 μm APM (13th day) when compared to 172 μm (6-7days) in great clam.

The number of days required for larval metamorphosis depends on a combination of factors like temperature and food availability. Loosanoff and Davis (1963) observed that the larval metamorphosis in venerid clam, *Mercenaria mercenaria* took place on 16th day at 18 °C and on 7th day at 30 °C after fertilization. In *M. meretrix* and *M. casta*, the duration was short, 7-10 and 8-9 days respectively in temperature ranging from 30.5 to 32.5 °C. In the present study, with more or less similar temperature range (28.5 to 32.0 °C) the larval metamorphosis for *G. tumidum* was observed to be longer (13 days) than that of great clam but shorter than that of *A. granosa* (21-23 and 16-18 days) (Wong *et al.*, 1986; Muthiah *et al.*, 1992).

Development of radial ribs in the shells of *G. tumidum* was not very evident till 68 days in contrast to *A. granosa* in which it appeared on 22nd day (Muthiah *et al.*, 1992). In the present study, the growth of post-set clam upto pre transplant stage to farm (2-2.5 months) was observed to be less (12.3 $\mu\text{m}\cdot\text{day}^{-1}$) than the growth rate (38.6 and 40.7 $\mu\text{m}\cdot\text{day}^{-1}$) reported for *Meretrix meretrix* (Narasimham *et al.*, 1988) and *A. granosa* (Muthiah *et al.*, 1992).

Spawning, larval development and spat settlement is considered basic for spat production on large scale. During the three larval rearing trials, though the percentage of initial settlement ranged from 25 to 28%, only few could survive and transform into juveniles. This warrants further research in refining the methods especially, feeding protocol and disease management for better survival and open possibilities of mass seed production for aquaculture.

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