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Spawning, larval development and spat settlement of the clam, *Meretrix casta* (Chemnitz) in the laboratory

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ABSTRACT

The clam, *Meretrix casta* (Chemnitz) was induced to spawn in the laboratory on 2 occasions by increasing water temperature by 3°–7°C from ambient temperature. Spawning was intense on both the occasions. The fertilized ovum measured 0.085 mm. After passing through early stages of development, the trochophore stage was attained by 6 hr after spawning. D-shaped free-swimming veliger larva was formed by 14 hr. The larva started feeding on the microalga, *Isochrysis galbana*, from the second day after spawning. The larvae settled as spat on eighth or ninth day measuring 0.216 mm × 0.201 mm (length × height). It attained a size of 2.5 mm × 2.35 mm by 50th day. Pigmentation of the periostracum varied leading to many morphs, of which 5 major types could be recognized. Spat numbering about 50000 were produced in each experiment.

The clam, *Meretrix casta* (Chemnitz) of Family Veneridae, is one of the commercially important bivalves in India (Alagarswami and Meiyappan 1989). It is widely distributed along the east and southwest coasts of India and forms sustenance fishery in the maritime states of Karnataka, Kerala, Tamil Nadu and Andhra Pradesh. Besides fishing of live clams, extensive quarrying of sub-fossil deposits of this species is carried out along the coasts.

There have not been any attempts on *M. casta* for developing hatchery techniques. Experiments were conducted on *M. casta* and the results are presented in this paper.

MATERIALS AND METHODS

Fully matured 87 specimens of *M. casta* were collected from the area of confluence of Buckingham Canal with sea near Kalpakkam on 6 April 1989. Since sex could not be determined externally, random samples of 20 clams measuring 32.0–37.0 mm in size were kept separately in a 50-litre FRP tank filled with filtered sea water. They were fed with mixed phytoplankton culture of *Chaetoceros* sp. and *Skeletonema* sp. at 10 litres/day. The water was changed daily at 10 00 hr and algal culture was supplied for feeding soon after.

After conditioning the clams for 4 days, induced spawning was attempted on 10 April 1989, by increasing the ambient water temperature from 28.1°C to 35.0°C, by adding warm seawater slowly. Profuse spawning took place after 30 min. Salinity of the seawater was 34.39‰.

In the second experiment, 20 specimens of *M. casta* measuring 32.0–35.0 mm were brought to the laboratory from the Muttukadu Fish Farm of CMFRI on 29 April 1989. They were cleaned and transferred to a 50-litre FRP tank having filtered seawater. A rise in water temperature from 26.0° to 29.0°C induced the

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clams to spawn intensively. Salinity of the water was 35.80‰.

Soon after spawning, the clams were removed and eggs in suspension were kept undisturbed for 1 hr for completing fertilization. The eggs were then transferred to clean tanks of 50-litre capacity containing filtered seawater at the rate of 15 eggs/ml.

Water was changed on every alternate day around 10 00 hr which helped in controlling ciliates. The larvae were filtered carefully through fine-meshed bolting silk filters of appropriate size, after carefully determining the size of the larvae and spat. Aeration was provided after the complete settlement of larvae. The tanks were always kept covered except during water change.

The phytoflagellate *Isochrysis galbana* was fed from second day of spawning at 3 000 cells/larva/day. Concentration of the microalga was determined with haemocytometer. Concentration was gradually raised to 6 000 cells/spat/day by 20th day. After 25th day, spat were fed with mixed phytoplankton culture of *Chaetoceros* sp. and *Skeletonema* sp. at 30 000 cells/spat/day gradually raised to 45000 cells/spat/day by 50th day.

Sampling of the early development was done at every 30 min, but after the formation of D-shaped larvae, sample was collected once in a day. Samples were preserved in 2% seawater formalin.

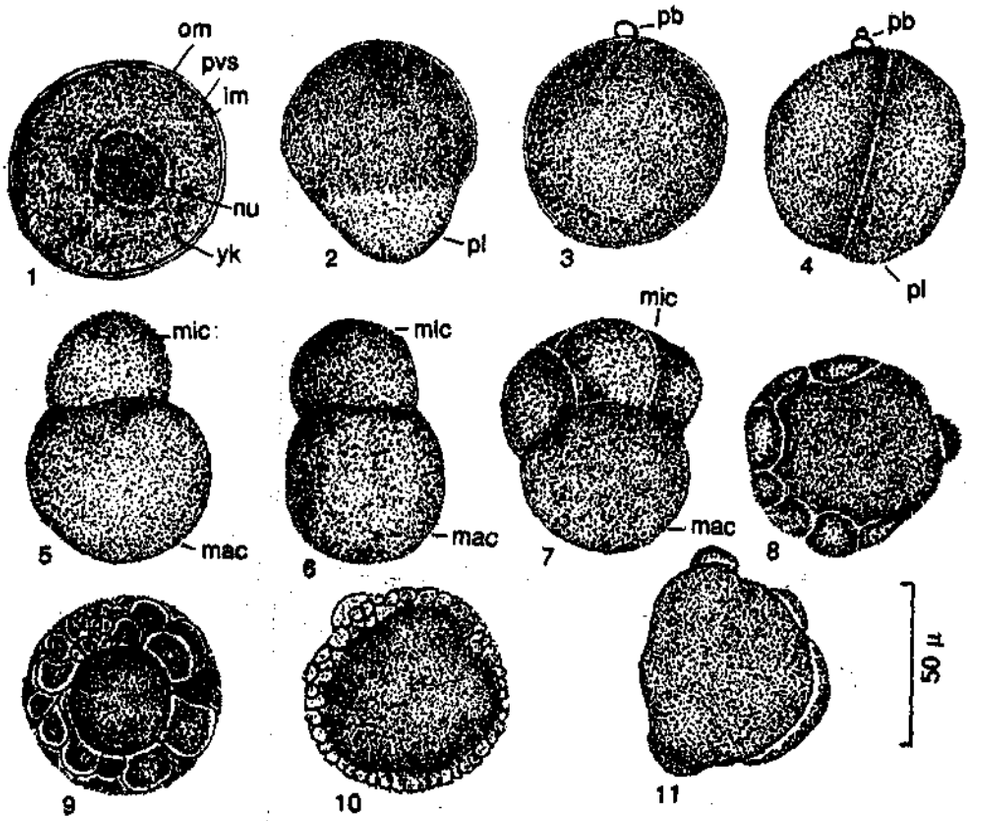
RESULTS

Spawning

Induced spawning of the clam, *M. casta* was achieved on 2 occasions. Raising the ambient water temperature by 3° – 7°C appeared to be effective in inducing spawning in *M. casta*. On both the occasions females released eggs first and males released sperms subsequently. Release of gametes by one clam triggered spawning of other clams. Clams spawning on the first and second occasion were 5 and 3 respectively. Spawning continued for more than 20 min turning the whole water pale cream. By this time, the egg concentration in suspension reached 200 – 250 eggs/ml. To avoid further concentration, the parent clams were removed from the tank.

Early development

The fertilized egg measured 0.08 – 0.095 mm, with a mean value of 0.083mm (only mean values are given in further descriptions). The eggs were spherical and telolecithal. The yolk was pale yellow, granular and reticulate in appearance (Fig. 1). Soon after spawning, the eggs tended to sink to the bottom. Fertilization took place throughout the water column. Development commenced after 15 min, with the formation of the polar lobe at the vegetal pole (Fig. 2) followed by the formation of polar body at the animal pole (Fig. 3). Cleavage commenced in about 30 min after fertilization, resulting in 2-celled stage (Figs 4, 5, 6). Further cleavages led to 4-celled stage and subsequently to 8-celled stage (Fig. 7). At this stage differentiated macromeres and micromeres were evident. The ciliated blastula was formed in 60 min (Figs 8, 9) which rotated on its axis. Gastrulation began after 90 min and was completed by 120 min (Figs 10 – 13). The archenteron was evident in this stage. Further development led to the formation of trochophore larva in 5 – 6 hr (Fig. 14) which measured 0.085 mm. The larva was oval and slightly produced in anteroposterior direction. It was ciliated throughout. The apical tuft of cilia and metatrochal band of cilia were distinct. During late trochophore stage, the shell gland began to secrete the shell (Fig. 15). Further development led to complete enveloping of the soft parts of the larva by the shell. The larva attained early straight-hinge stage in about 10 hr (Figs 16, 17) and D-shaped stage by 14 hr (Fig. 18).

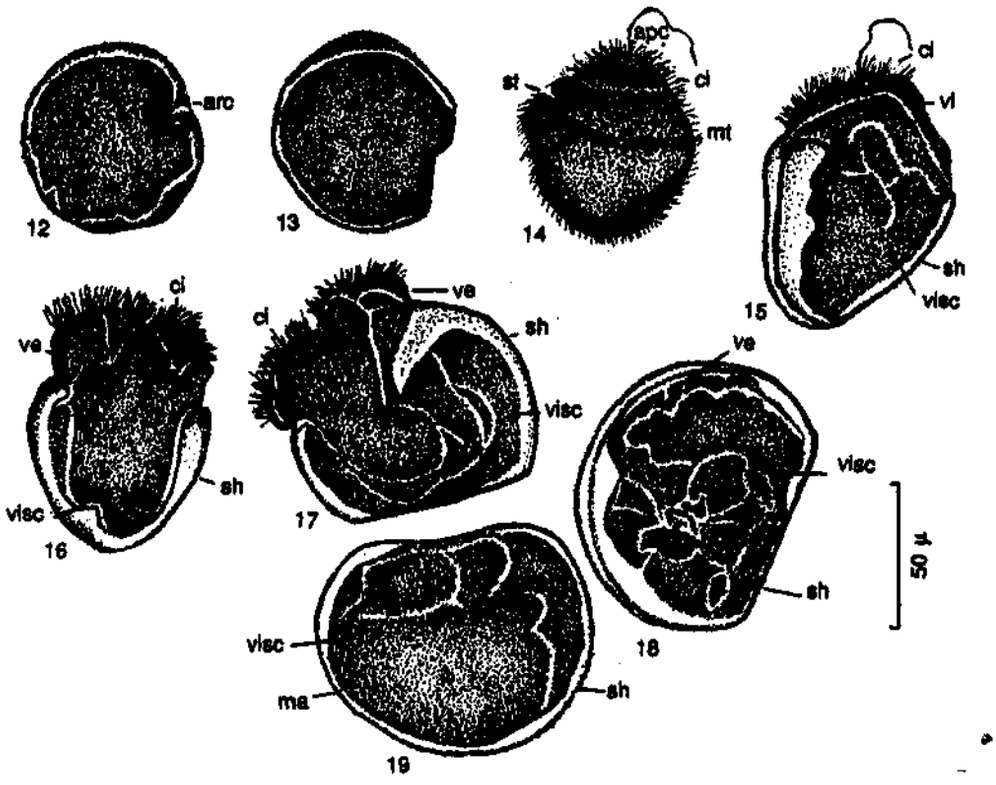


Figs 1–11. 1, Fertilized ovum of *M. casta*. 2, First polar lobe formation. 3, Polar body formation. 4, 5, 6, Formation of 2-celled stage. 7, 8-celled stage. 8, 9, Blastula formation. 10, 11, Gastrulation. aam, anterior adductor muscle; apc, apical cillia; arc, archenteron; asp, anterior siphon; ci, cilia; f, foot; g, gut; grl, growth lines; ht, hinge teeth; in, inner membrane; ma, mantle; mac, macromeres; mic, micromeres; mk, markings on the shell; mt, metatroch; nu, nucleus; om, outer membrane; pam, posterior adductor muscle; pb, polar body; pl, polar lobe; psp, posterior siphon; pvs, perivitelline space; sh, shell; st, stomodaeum; um, umbonal end; ve, velum; visc, visceral organs; vl, velar lobe; yk, yolk.

Free-swimming veliger

The D-shaped larva measured 0.085 mm× 0.076 mm (length × height). The hinge region was slightly narrower than the length. D-shaped larva was pale yellow due to yolk material visible through the soft transparent embryonic shell. There was a well-developed bi-lobed velum with strong musculature and powerful cilia, with the help of which the larva swam in the water column.

The larval size increased to 0.121 mm × 0.1 mm after 2 days of spawning (Figs 19, 20). It started feeding on the microalgal diet (*Isochrysis* sp.) supplied, and became more ovoid by third and fourth days (Fig. 21). By fifth day, the larva acquired almost spherical shape because of the growth of the shell in the anteroposterior axis; the size of hinge got reduced (Fig. 22). The larva measured 0.165 mm × 0.154 mm at this stage and the anterior and posterior adductor muscles were clearly visible. The gut contents could be distinguished from the food-laden stomach and intestine. There was reduction in the size of the velum.



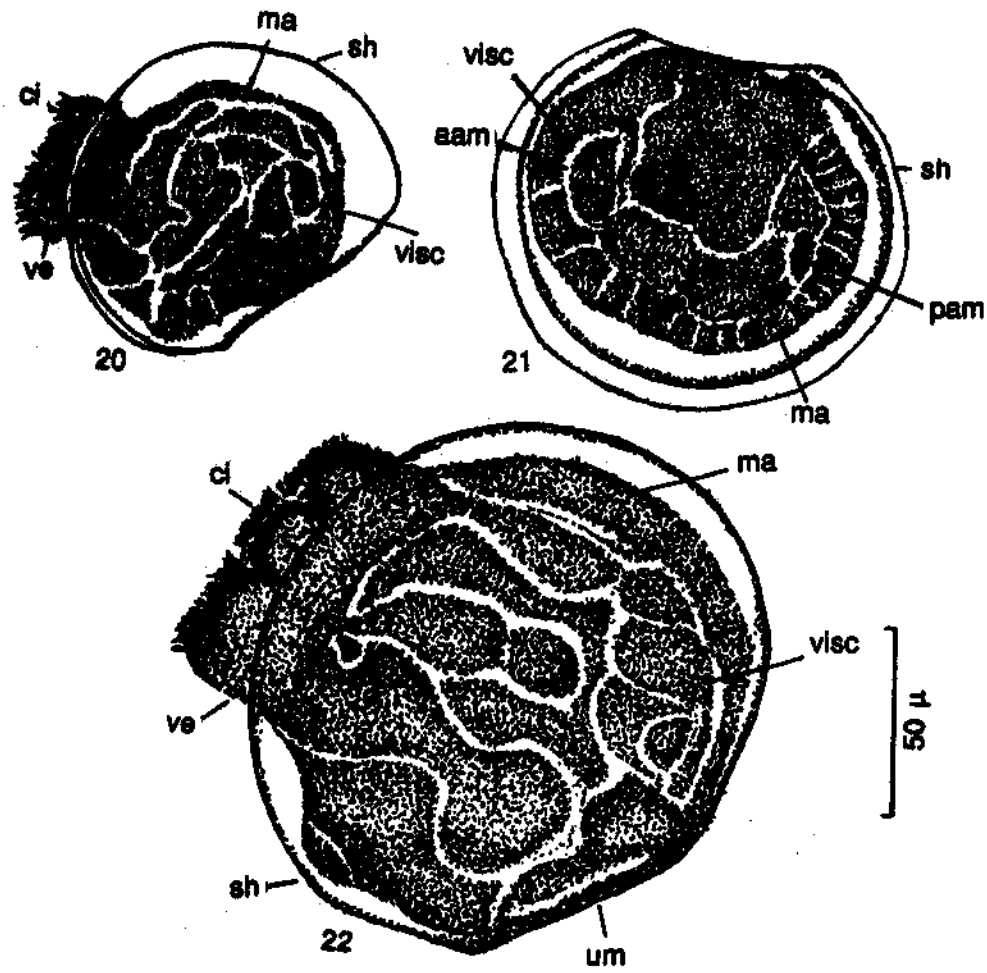
Figs 12-19. 12, Gastrula. 13, Formation of trochophore. 14, Trochophore larva. 15, Shell formation. 16, 17, Preveliger stages. 18, D-shaped larva (24 hr after spawning). 19, Second day veliger of *M. casta* (for abbreviations see Figs 1-11).

The larvae moved actively in group in the water column. They showed a tendency to congregate at the surface in the dark, but on the fall of light rays, they dispersed and moved towards the bottom of the tank indicating negative phototropism.

Further growth of the shell took place in the anteroposterior direction and the hinge was considerably shortened. Development of foot was noticed on sixth or seventh day after spawning. The velum functioned actively, but the larva also crawled slowly with the foot. The larva measured 0.203 mm × 0.176 mm at this pediveliger stage.

By the eighth or ninth day, the larva cast off the velar organ and settled as spat. The newly settled spat measured 0.216 mm × 0.201 mm. The settlement was completed by 11th day. The spat was oval and pale yellow. The ctenidia were well developed. The foot was protractile and measured twice the length of the spat. The spat moved briskly with the help of the foot on the bottom of the tank.

There were considerable variations in all stages of embryonic, larval and post-larval development leading to the presence of different stages and sizes on any single occasion. This feature is very common among the embryos, larvae and spat of bivalves. Chanley (1955) suggested that differences in the size of the larvae in same cultures from the same parents were due to inherited characters. He

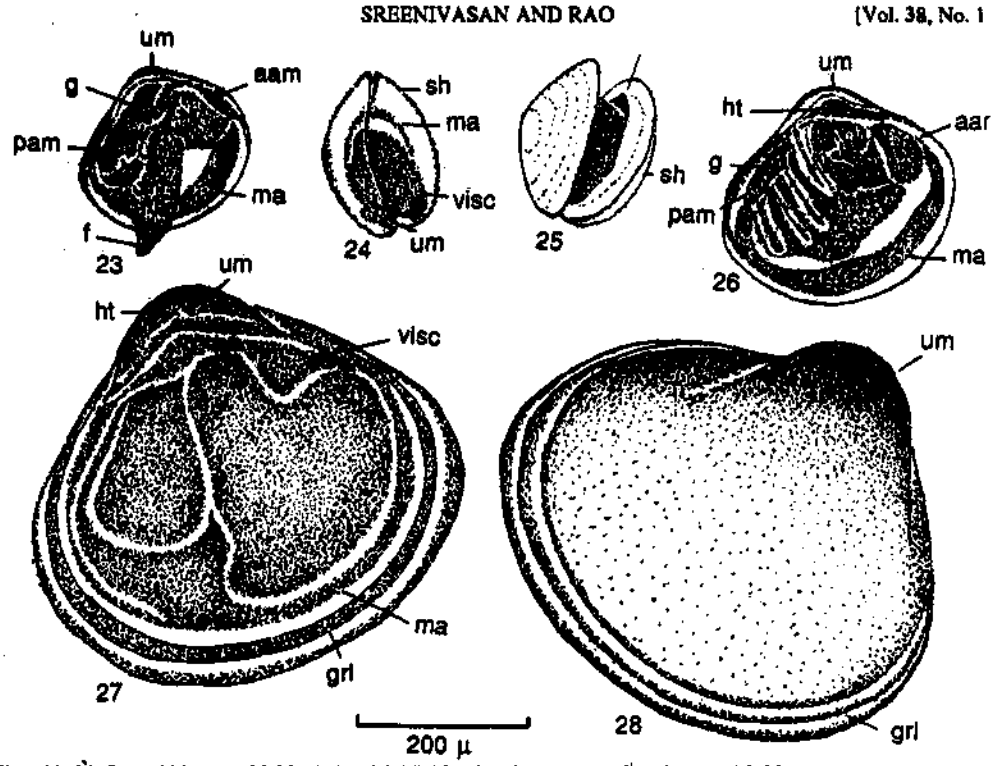


Figs 20–22. Veliger of *M. casta*. 20, Second day. 21, Third day. 22, Fifth day (umbo formation) (for abbreviations see Figs 1–11).

also opined that vitality of the eggs and larvae depended to some extent upon the position of the eggs in the ovaries and the amount of nutrient material stored in the individual eggs before they were released. Ecological factors like water temperature, availability of food etc., also played major role in determining the size and stage during development.

Spat

Settled spat attached themselves to the bottom of the tank by fine byssal threads, but the attachment appeared to be feeble. Washing with a little quantity of water was observed to dislodge the spat completely from the tank. Therefore, maximum care was taken while changing water in the tank. Mortality was



Figs 23–28. Spat of *M. casta*. 23, Ninth day. 24, 25, 12th day. 26, 14th day. 27, 19th day. 28, 20th day (for abbreviations see Figs 1-11).

maximum during spat settlement period.

In subsequent days, the shell got thickened and became opaque with calcium deposition. The periostracum became coloured with gradual development of chromatophores initially at the umbonal region and later widening along the shell towards the outer edge. The spat resembled the adult by 12th day acquiring the characteristic triangular shape (Figs 23–25). Visceral organs were well developed by the 14th day (Fig. 26). The foot became broader and thick by the 20th day. The shell became totally opaque making visceral organs invisible from outside. Growth lines also appeared on the outer edge of the shell (Figs 27, 28). Both anterior and posterior siphons were well developed and protruded frequently. The spat moved briskly with the help of the foot. By 30th day, the spat measured 1.0 mm × 0.9 mm (Fig. 29). By 40th day, the spat developed colouration on the shell. As in the case of the larvae, the spat also showed much variation in size. They had a tendency to congregate in batches at the bottom of the tank. These congregations had no uniformity either in size or in colour of the individuals.

Polymorphism in shell colouration was recorded among the spat of *M. casta*. The details relating to the different morphs are:

Pale white with red streaks : The shell was bright white with radiating red streaks. The streaks were more evident in smaller individuals. This formed 52% of the total spat.

Pale yellow with dark umbonal region: Periostracum was yellow with black or red streaks. Rows of radiating bands were evident. The posterior half of the shell was dark. Faded horizontal bands were also visible. This morph formed 31% of the total spat.

Bright yellow with red streaks: Posterior

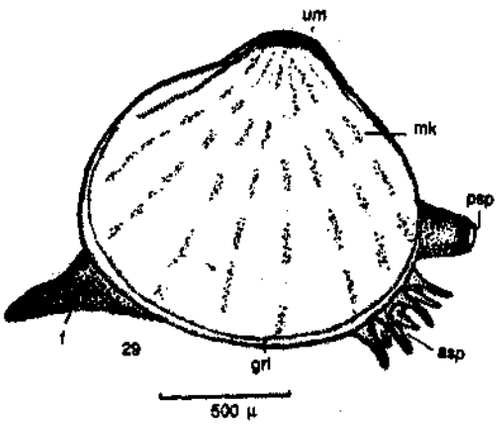


Fig. 29. 30th day spat of *M. casta* (for abbreviations see Figs 1–11).

region was bright yellow with radiating red streaks. There was no dark colouration near umbo. This variety formed 14% of the spat.

Pure dark brown: Periostracum was dark-brownish. This type formed 3% of the spat.

Pale brown with lip region dark: Periostracum was pale brown with outer edge of the shell black. From umbo to outer edge of the shell 2 radiating bands were present. Horizontal lines with alternating pale area were observed. Only 1% of the spat had this colouration.

There were also intermediate colour combinations of the above mentioned patterns. These colourations continued to be present even in the larger spat. Significance of this polymorphism could not be understood, but appeared to be genetically controlled.

Growth of the spat

By 11th day, when the settlement was complete, the spat attained a size of 0.245 mm × 0.232 mm (Fig. 30). Growth was faster from this stage onwards. The spat attained 0.478 mm × 0.435 mm size by 24th day. With the introduction of mixed algal food from 25th day, there was further increase in the growth rate. The spat measured 1.018 mm × 0.895 mm by 32nd day, 2.015 mm × 1.832 mm by 45th day and 2.5 mm × 2.35 mm by 50th day.

When the growth of the larvae and spat was plotted against days after spawning, the following regression equations were obtained:

Length : $\log e\ Y = -2.0904 + 0.619\ X$

Height : $\log e\ Y = -2.2081 + 0.0622\ X$

where Y, the variable and X, the days.

Correlation coefficients (r) for the above relationships were 0.9954 and 0.9951, respectively, which indicated high degree of correlation. The relationship between length and height of the spat and larvae was linear and was expressed by the equation: $H = -0.038 + 0.915\ L$ where, H and L indicated the height and length respectively. The correlation coefficient (r) for the relationship was 0.999.

Spat production

On the 11th day, in each of the experiments 50 000 spat were obtained. In the first experiment the total number of D-shaped larvae estimated on the second day was 489 000 and in the second 444 000. This indicated 10% survival until metamorphosis in the first experiment and 11% in the second. Mortality was recorded on all the days of rearing and by 50th day, the survival was about 4 or 5% of the initial larval concentration.

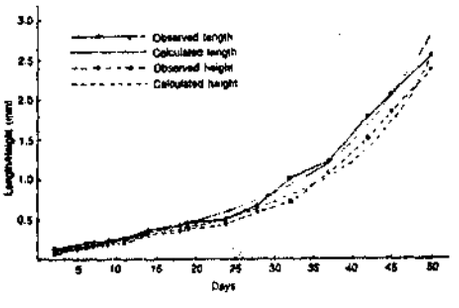


Fig. 30. Growth of larvae and spat of *M. casta*.

DISCUSSION

The development of embryo, shape and size of larvae, period of larval life, time of setting and growth of spat of *M. casta* showed similarity with those of *Meretrix meretrix* reported by Narasimham *et al.* (1988). The structural features of larvae and spat of *Meretrix* spp. also showed resemblances to those of related venerid clams, *Merecenaria mercenaria* and *M. campachiencis* studied by Loosanoff (1959) and Loosanoff and Davis (1963) respectively. In all these cases, eyed stage was not found prior to metamorphosis. The larval phase was of 7–11 days in *M. casta* and *M. meretrix* unlike 14–23 days observed in some bivalves like the pearl oyster *Pinctada fucata* (Alagarswami *et al.* 1983), the oyster *Crassostrea madrasensis* (Nayar *et al.* 1984) and the green mussel *Perna viridis* (Sreenivasan *et al.* 1988). From the point of view of hatchery production of seed, shorter duration of planktonic larval phase is advantageous in that it reduces the time of their maintanence within the hatchery which in turn reduces the inputs.

This study for the first time indicated the possibilities of hatchery production of the seed of *M. casta*. However, for taking up large-scale hatchery production of seed of *M. casta*, further investigations are required.

ACKNOWLEDGEMENTS

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