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Early development of *Cymatium (Monoplex) pileare* and *C. (Linatella) cutaceum* (Ranellidae : Gastropoda : Mollusca) in the laboratory

P. MUTHIAH* AND K. SAMPATH

Department of Zoology, V.O. Chidambaram College, Tuticorin-628 008, India

ABSTRACT

Early development of two species of ranellids, *Cymatium (Monoplex) pileare* and *C. (Linatella) cutaceum* were studied in the laboratory. Females incubated the egg mass for 12-25 days till fully developed larvae were hatched out through an apical orifice of the egg capsule. The mean length and height of the released larvae of *C.(M.) pileare* and *C.(L.) cutaceum* were 252 and 166 µm and 265 and 191 µm respectively. The percentage of hatching and larval development was 95.6 and 100 for *C.(M.) pileare* and 93.7 and 94.6 for *C.(L.) cutaceum* respectively. The larval development inside the egg capsule of these species are discussed.

Introduction

The egg masses of certain species of ranellids occurring in New Zealand (Laxton, 1969); Hawaii (Houbrick and Fretter, 1969) and India (Muthiah and Sampath, 1999) were studied earlier. Detailed reports on larval development of ranellids are not available. Scheltema (1961) studied the probable veligers of cymatiids collected in the plankton samples and Bandel (1975) described the veliger larvae of cymatiids. Thangavelu and Muthiah (1983) studied the larval stages of *C. cingulatum*, by rearing the teased out contents of the egg capsules. This was not in accordance with normal development taking place in situ the egg capsules. Ramon (1991) described the egg mass and development of *C. cutaceum*, a Mediterranean – West-African species *Cabestana cutacea* and *Cymatium corrugatum*. Govan (1995) reported the size of larvae released by *Cymatium* spp. This paper reports on the early development of larvae from the egg masses, to the juvenile ranellids in the laboratory.

Material and methods

Twenty two *C.(M.) pileare*, of length range 31.3-93 mm and 17 *C.(L.) cutaceum*, of length range 48-76 mm were collected from rearing cages in an experimental pearl culture farm at Tuticorin (Lat. 8°, 48' N; Long. 78° 11' E). These gastropods were maintained separately in FRP tanks of 100 l capacity (size: 75 x 50 x 50 cm). Sand-filtered sea water was used with mild aeration. The water was changed daily. The animals were fed with meat of edible oyster, *Crassostrea madrasensis*.

*Present address: Central Marine Fisheries Research Institute, 115, N.K. Chetty Street, Tuticorin-628 001, Tamil Nadu, India.

During January '92 to April '93, *C.(M.) pileare* deposited 20 egg masses and *C.(L.) cutaceum* 2 egg masses. Early developmental studies were conducted on the egg mass of *C.(L.) cutaceum* laid on 1.7.'92 and that of *C.(M.) pileare* on 23.7.'92. While laying eggs, the gastropod with its egg mass was maintained in the same tank it had originally lived in, while other animals were transferred to another tank.

Once in 2 days, an egg capsule was plucked from the egg mass lifting the gastropod slightly. The number of embryos was estimated by counting three samples each of 1 ml from the teased out contents of an egg capsule made upto 50 ml. The unfertilised eggs, if any, in the sample was counted and the percentage of development was calculated out of total number of embryos in the egg capsule. The length and height of 20 larvae fixed in 1% formalin, were measured as done by Ramon (1991). The percentage of hatching was calculated taking into account the number of unhatched egg capsules out of the total number of egg capsules in the egg mass. The released larvae were reared in the same tanks at the rate of 800-1,000 larvae/l. Water was changed on alternate days. The salinity ranged from 34.6 to 35.8 ppt and the temperature varied from 26.2 to 28°C. The nannoflagellate, *Isochrysis galbana*, commonly used as food for molluscan larvae (Davis and Guillard, 1958; Scheltema, 1961) was provided as food at the rate of 15,000 - 17,000 cells/larva/day.

Results

Cymatium (Monoplex) pileare

On laying, the egg mass (Fig. 1) was creamy white and it turned to yellow on the third day. It attained brown colour on the 10-12th day as the larvae gradually developed. The number of egg capsules in the egg mass was 180 and the average number of larvae per egg capsule was 2,880. Inside the egg capsules, there were no fertilised eggs that did not undergo larval development indicating 100% larval development. The period of incubation was 12 days. Hatching took place for two days. The larvae were released through an apical orifice of 1.8 mm diameter in the egg capsule (Fig. 2). Out of 180 egg capsules in the egg mass, 8 did not

Fig. 1. The egg mass of *C.(M.) pileare*. (Scale: bar = 1 cm in Fig. 1-11). The colour of egg capsule is creamy white.

Fig. 2. Hatched out egg mass indicating apical orifice.

release larvae. The percentage of hatching was 95.6.

The diameter of a fertilised egg (Fig. 3) ranged from 125-150 µm. The first cleavage divided it into two blastomeres (Fig. 4) and then protrusion of the vegetal pole led to a trefoil stage (Fig. 5). On the 2nd day, the four cell stage (Fig. 6) was attained. By a spiral pattern of cleavage, the morula stage (Fig. 7) and the solid blastula were formed. During gastrulation proceeded by epiboly, the micromeres multiplied and spread over the macromeres. For the next 3 days, the embryos elongated in the antero-posterior axis, with an ovoid anterior and a blunt posterior end with a tuft of cilia. The trochophore on the 7th day had a mean size of 173.9 x 163.2 µm (Fig. 8). On the 8th day, the formation of the visceral hump was completed after torsion. The larvae, measuring 207.9 µm in length developed a foot, a transverse antero-posteriorly flattened projection with short cilia, on the 10th day. On 11th day, larvae with a length of 219 µm moved vigorously with a bilobed velum (Fig. 9). The first shell whorl and the operculum were well formed with clear eye spot on the 12th day. At this stage, the larvae were released from egg capsules through an apical orifice.

The mean length and height of released larvae were 252 and 166 µm respectively. They were free-swimming and also were frequently moving along the bottom of the tank. The veliconch larvae (following the terminology of Fretter and Graham, 1962) were reared for 6 days. Though 95% of the larvae were dead, by further rearing, four juveniles of length ranging from 0.95 to 1.13 µm were obtained. Forty five days old *C.(M.) pileare,* attained a maximum length and height of 1.5 and 1.3 µm respectively registering a growth rate of 26.3 µm day^{-1}. Further rearing could not be continued because of mortality.

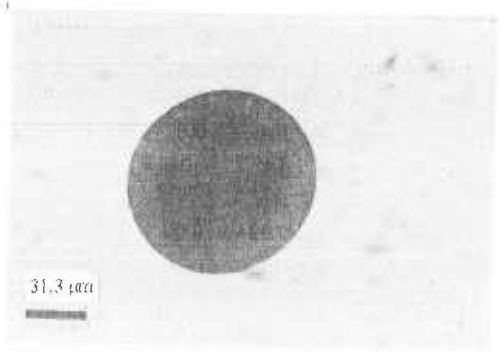

Fig. 3. Fertilised egg.

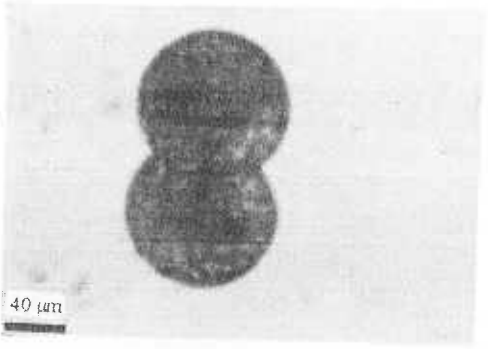

Fig. 4. Two cell stage.

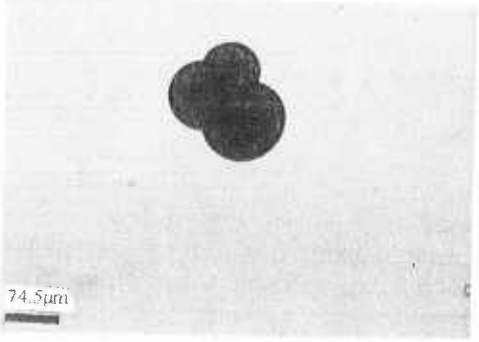

Fig. 5. Trefoil stage.

Out of 4 juveniles produced, one dead juvenile with the length of 1.12 mm was found to have a bore hold (114.6 µm in diameter) on the shell (Fig. 10).

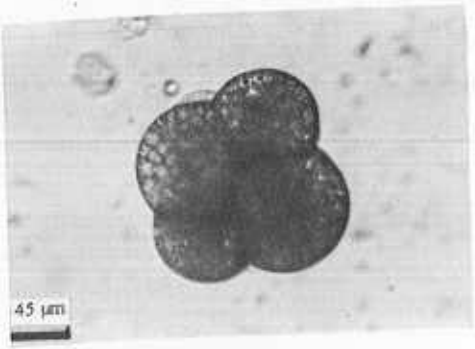

Fig. 6. Four cell stage.

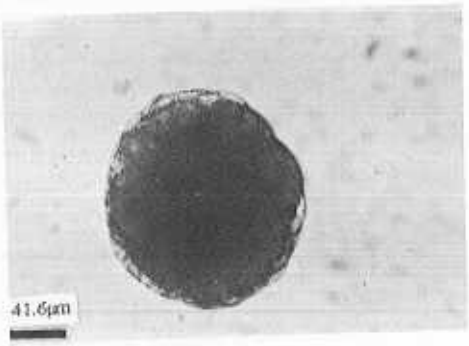

Fig. 7. Morula stage.

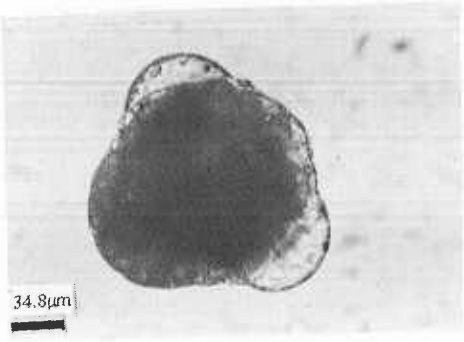

Fig. 8. Trochophore.

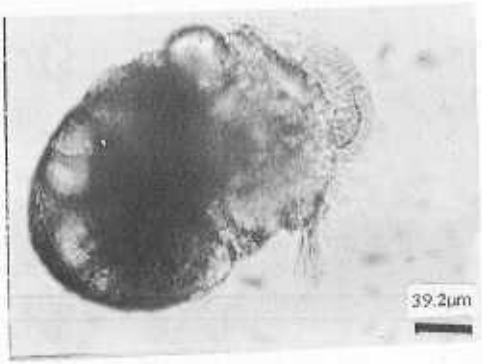

Fig. 9. Veliger.

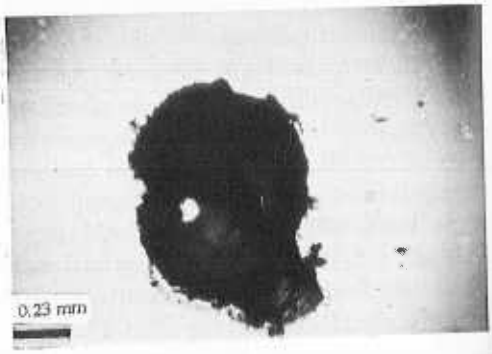

Fig. 10. Dead juvenile of *C.(M.) pileare* with a bore hole.

C.(L.) cutaceum

The colour change of egg mass during development was similar to those of *C.(M.) pileare*. The number of egg capsules in the egg mass was 175. Out of these, 11 did not release the larvae. The percentage of hatching was 93.7. The number of embryos in an egg capsule ranged from 840-3,070, with an average of 1,131. The egg capsules had 30-240 eggs that did not undergo embryonic development and the percentage of development averaged to 94.6.

The diameter of an egg was 95.2 µm and it increased to 136.5 mm on

fertilization. The early cleavage patterns were similar to those of *C.(M.) pileare*. On the 7th day, the trochophore stage was attained with a length of 175.8 µm and on the 9th day, after torsion, veliger larvae were produced; the length and height of the larvae being 239.7 and 171.0 µm respectively. By the 11th day, the foot became broad, the operculum larger and the eye spot conspicuous, and the size of the larva was 256.7 x 186.2 µm. On the 14th day, the larvae were released through an apical orifice in the egg capsule. The released larvae (size: 265 x 191 µm) exhibited bilobed velar movement and crawling on the bottom of the tank. They were reared for another 11 days to a maximum size of 352.8 x 252.0 µm (Fig. 11). Further rearing could not be carried out because of larval mortality.

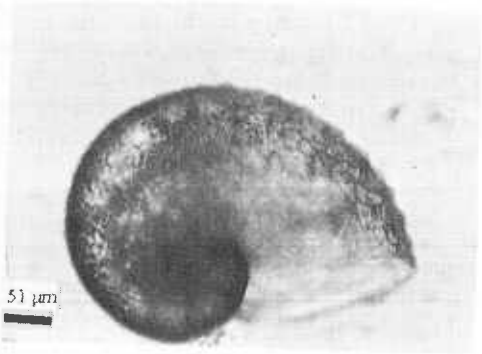

Fig. 11. Maximum larval size of *C.(L.) cutaceum* reared. (scale bar = 1 cm).

Discussion

The attainment of brown colour in the egg masses of ranellids indicate the development of the larval shell, as observed in *Eupleura caudata* (Mackenzie, 1961) and in *Cymatium corrugatum* (Ramon, 1991).

The developmental period of 12-14 days (at 27.2°C) observed in both the species in the present study is much shorter than other species and this may be attributed to the higher temperature. The incubation period for *C.(L.) cutaceum* was 14 days whereas it took 16 days at 27.2°C for *C.(M.) pileare* and at 24.8°C, the period extended to 25 days. Govan (1995) reported that the incubation period for *C. muricinum, C. nicobaricum* and *C. pileare* was 10-27 days. Hatching of *C. nicobaricum* took place 21 days after spawning (Purtymun, 1974). *C. corrugatum* took 18 days for development at a temperature of 20-23°C (Ramon, 1991). Similar observations of decrease in the incubation period with increasing temperature have been made in the oyster drill *Urosalpinx cinerea* (Ganaros, 1958) and in *Eupleura caudata* (Mackenzie, 1961).

The appearance of the prepodium is considered as a prerequisite for metamorphosis (Hadfield, 1978). The presence of well developed prepodium at a length of 214 µm in *C.(M.) pileare* and at 239 µm in *C.(L.) cutaceum* indicated their competence for metamorphosis. The larval length on hatching was 225 µm for *C.(M.) pileare* and 265 mm for *C.(L.) cutaceum*. The lengths of hatched out larvae of these ranellids were more close to 230-250 µm as reported by Govan (1995) for *Cymatium aquatile, C. muricinum, C. nicobaricum* and *C. pileare*.

It was observed that the larval growth in relation to duration was faster in *C.(L.) cutaceum* (length of larvae = 115.7178 µm x days0.304) (Fig. 12) compared with *C.(M.) pileare* (length of larvae = 129.3302 µm x days 0.2007) (Fig. 13). The surface sculpturing or ornamentation of protoconchs was similar to the observations of Ramon (1991) and Govan (1995) for the *Cymatium* species.

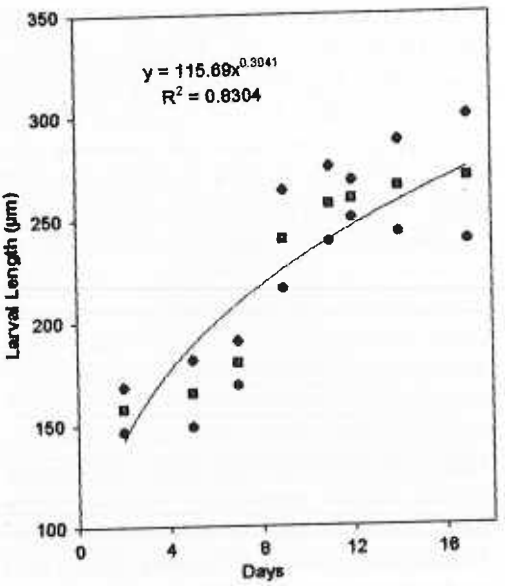

Fig. 12. *C.(L.) cutaceum* : Growth of larvae inside the egg capsule.

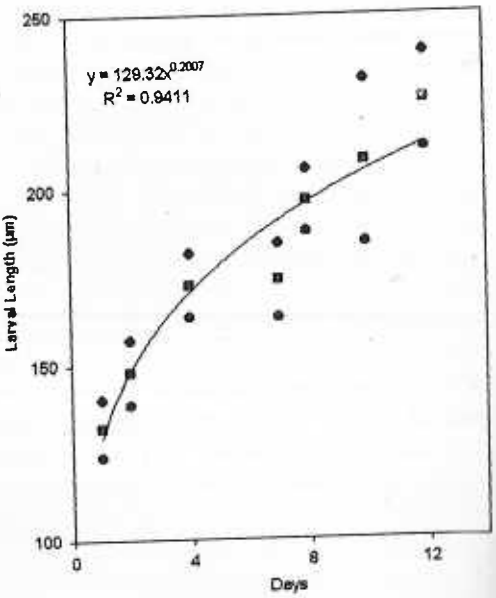

Fig. 13. *C.(M.) pileare*: Growth of larvae inside the egg capsule.

In this study the 'developmental period' of 12-15 days and the 'delay period' of 30 days are much lower than the estimated pelagic life of 320 days for *C. nicobaricum* and 293 for *C. parthenopeum* (Scheltema, 1971). Probably with the suitable food and favourable substratum, the 'obligatory developmental period' (ODP) (following the terminology of Scheltema, 1971) will be even much shorter. The growth rate of released larvae to juveniles of *C.(M.) pileare* ranging from 20.8 µm day^{-1} (during development inside the egg capsule) and 26.3 µm day^{-1} indicates that the ODP will be well below 1 month for the larvae of the ranellids, as Govan (1995) envisaged.

A bore hole found on the shell of a dead juvenile (Fig. 10) vouch for the cannibalistic behaviour of *C.(M.) pileare* even during juvenile stage, as no other animal was present that could produce the drill hole. It also revealed the fact that *C.(M.) pileare* could drill its prey, eventhough it is usually considered to be a non-boring predatory gastropod while feeding on edible oysters (Muthiah *et al.*, 1987) and pearl oysters (Chellam *et al.*, 1983).

Though large-scale larval mortality occurred, juveniles of *C.(M.) pileare* up to a length of 1.5 mm were successfully obtained in the laboratory. Ramon (1991) attributed absence of suitable substratum for the poor survival of larvae of *C. corrugatum* reared for 16 days after their release, similar to *Nassarius obsoletus* (Scheltema, 1961). Govan (1995) stated that due to lack of knowledge on appropriate food required for ranellid larvae, they could not be reared to settlement or even to the stage of protoconch II. Besides finding a suitable substratum, further study is needed to identify ideal food organisms for successful rearing of larvae and juvenile ranellids.

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