6. REPRODUCTION
6.1. SPAWNING AND FECUNDITY

6.1.1. Introduction

Gastropods show diversified reproductive systems. The variations pertain to protection of eggs and degree of provision of food to the developing embryos. In primitive gastropods, fertilization is external and occurs at random in sea water. In higher prosobranchs, internally fertilized eggs are enclosed in tough capsules or oothecae. In predatory gastropods, many oothecae are clustered together to form an egg case. Lebour (1937) initiated studies on the oothecae of oyster predator *Ocenebra erinacea*. Later attempts were focussed on egg cases of oyster drills *Urosalpinx cinerea* (Hancock, 1956), *U. perrugata* (D'Asaro, 1986), *Eupleura caudata* (Mackenzie, 1961), *E. sulcidentata* (D'Asaro, 1986) and *Purpura clavigera* (Lin and Hsu, 1979).

Anderson (1959) described egg case of cymatiid *Cymatilesta spengleri* which was later considered as *Cabestana spengleri* (Riedel, 1992). Beu (personal communication) stated that the specimen illustrated by Ganapati and Sastry (1973) was *Bursa granularis* (Roding) and not *C. pileare*. Thangavelu and Muthiah (1983) described an egg case of *C. cingulatum* but dimensions of oothecae and their relationship with gastropods were not mentioned. Though Ramón (1991) gave an account on morphological
dimensions of Cymatium cutaceum and C. corrugatum, he did not mention about fecundity. Since cymatiids cause considerable damage to tended stock in bivalve culture farms, studies on their spawning and fecundity assume vital significance. The reproductive strategies of C.(M)pileare and C.(L)cutaceum are reported in this chapter.

6.1.2. Material and methods

The collection and maintenance of C.(M)pileare and C.(L)cutaceum in the aquarium were described in the chapter 3.

During the period between January 1992 and April 1993, C.(M)pileare deposited 20 egg cases and C.(L)cutaceum laid 2 egg cases. The morphometric measurements of the gastropods and the diameter of the egg cases were obtained using a vernier caliper. The wet weight of the animal, egg case and capsule were taken in Sartorius (Type 1712) electronic balance to an accuracy of 0.1 mg. The length and width of oothecae were measured through a microscope having precalibrated ocular micrometer.

To estimate the number of embryos, 20 oothecae were collected at random from an egg case, weighed and the contents were teased out in a petridish. The weight of empty ootheca after wiping out in a filter paper was also recorded. The embryos from the petridish were transferred into an measuring
cylinder and made upto 250 ml. After thorough mixing, 1 ml of sub-sample was taken in a counting chamber for enumeration of embryos. Likewise estimation was carried out thrice for a particular egg case. The remaining oothecae in that egg case were counted by sacrificing the case. From the average number of embryos per ootheca and total number of oothecae per egg case, the fecundity was estimated.

For correlating the oothecae length to number of embryos, 5-12 oothecae of different length in the egg cases were measured separately and the embryos contained in each ootheca were made upto 50 ml by adding sea water. Average number of embryo in 3 subsamples of 1 ml was recorded.

6.1.3. Results

6.1.3.1. Egg case

The egg cases of both C. (M) pileare and C. (L) cutaceum were cup shaped with an array of spirally arranged conical oothecae. The outer surface of the egg case was made of thin horny and transparent plates of 0.6 mm thickness. The closely packed oothecae were attached firmly to the inner surface of the egg case with a hollow centre. Both the species took 2 days to complete the deposition of an egg case. There were no nurse eggs in the oothecae.
The colour of the egg case initially was creamy white (Plate 6.1.1). On 3rd day, it turned yellow (Plate 6.1.2) and on 10-12th day, the oothecae attained brown colour (Plate 6.1.3).

### 6.1.3.2 Spawning period

Of the 20 egg cases laid by C. (M)pileare, 14 were deposited during July to November indicating peak spawning period and a minor season during February-May. C. (L)cutaceum laid 2 egg cases during July-August of this study period (Table 6.1.1).

### 6.1.3.3 Repeated spawning

During the intensive spawning period, it was observed that a C. (M)pileare of 93 mm length deposited 2 egg cases successively on 1.7.92 and 27.8.92 at an interval of 58 days. And another gastropod of 85.4 mm laid 2 egg cases (23.7.92 and 9.9.92) at 48 days interval (Table 6.1.1).

### 6.1.3.4 Spawners aggregation

It was observed that at the time of spawning, the female C. (M)pileare settled in the corner of aquarium tank and 2-3 males were found to aggregate gregariously with that female till it started laying the egg case. In contrast to the mass congregation among C. (M)pileare, pairing of individuals was observed among C. (L)cutaceum, where a male was found mounted on the right side of the female.
Plate 6.1.1. *C. (M) pileare* incubating the egg case. The colour of ootheca is creamy white. Scale: 1 cm = 7 mm.

Plate 6.1.2. *C. (M) pileare* egg case. The colour of oothecae turned yellow. Scale: 1 cm = 6.4 mm.
Plate 6.1.3. *C. (M) pileare* egg case. The colour of oothecae turned brown.

Scale: 1 cm = 10.1 mm.
Table 6.1.1: Dimensions of egg cases and fecundity of Cymatiids.

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<tr>
<th>S.No</th>
<th>Date of egg case deposition</th>
<th>Gastropod</th>
<th>Egg case</th>
<th>No. of embryos in egg case</th>
<th>Fecundity (no. of eggs)</th>
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<tbody>
<tr>
<td></td>
<td></td>
<td>Length (mm)</td>
<td>Width (mm)</td>
<td>Diameter (mm)</td>
<td>Wet weight (g)</td>
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<th>Gastropod Width (mm)</th>
<th>Gastropod Weight (g)</th>
<th>Egg Case Diameter (mm)</th>
<th>Egg Case Wet weight (g)</th>
<th>No. of oothecae in egg case</th>
<th>No. of embryos in egg case</th>
<th>Fecundity (No. of eggs)</th>
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C. (L) cutaceum

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<th>Date of egg case deposition</th>
<th>Gastropod Length (mm)</th>
<th>Gastropod Width (mm)</th>
<th>Gastropod Weight (g)</th>
<th>Egg Case Diameter (mm)</th>
<th>Egg Case Wet weight (g)</th>
<th>No. of oothecae in egg case</th>
<th>No. of embryos in egg case</th>
<th>Fecundity (No. of eggs)</th>
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<td>10.6</td>
<td>8255.5</td>
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* Repeated spawning: combined spawnings dt 1.7.92 and 27.8.92

** Combining the spawnings of 23.7.92 and 9.9.82

*** For average fecundity, repeated spawning was not included.
6.1.3.5. **Incubation**

The female cymatiids brood their embryos by remaining on and completely covering the egg case (Plates 6.1.1 & 6.1.4). They left the egg case only after larvae were released from the oothecae. The duration of incubation of *C. (M)pileare* extended from 12 to 25 days, with an average of 18 days; incubation duration of *C. (L)cutaceum* was 14 days. The brooding gastropods did not feed during the incubation period.

6.1.4. **C. (M)pileare egg case**

6.1.4.1. **Diameter of the egg case**

The diameter of the egg cases of *C. (M)pileare* ranged from 28.2 to 47.0 mm with average of 38.9 mm. Egg cases of 28.2 and 47.0 mm diameter were laid by *C. (M)pileare* of length 53.0 and 92.8 mm respectively (Table 6.1.1). The values of correlation coefficient and regression equation between diameter of egg cases and length and width of the gastropod (Fig 6.1.1) were highly significant (Table 6.1.3).

6.1.4.2. **Wet weight of egg case**

The wet weight of the egg case varied from 2.6 to 16.8 g for gastropods weighing from 11.8 to 49.7 g respectively (Table 6.1.1). The relationship between these two parameters was found to be significant (*r* = 0.6506; *p* < 0.05; *ts* = 4.315) (Table 6.1.3; Fig 6.1.2).
Plate 6.1.4. *C. (L) cutaceum* incubating the egg case.

Scale: 1 cm = 6.9 mm.
FIG. 6.1.1

Egg Case Diameter (mm) vs. Gastropod Width (mm)
FIG. 6.1.2

Egg case weight (g) vs. gastropod weight (g)
6.1.4.3. **Number of oothecae per egg case**

On an average, an egg case contained 200 oothecae. Maximum of 272 oothecae was observed in the egg case laid by a *C. (M) pileare* measuring 85.6 mm length. A gastropod with 53.0 mm length laid the egg case having minimum of 133 oothecae in its egg case (Fig 6.1.3). In the repeated spawning, a female of 85.4 mm laid 2 egg cases having 180 and 230 oothecae (Table 6.1.1). There was no significant relationship between the number of oothecae in the egg case and the length of the gastropod (*r*=0.4212; *p*>0.05; *ts*=1.609) (Table 6.1.3).

6.1.4.4. **Oothecae dimensions: Length of oothecae**

Oothecae were tubular, cone shaped with 2 projections on the side facing centre of the egg case (Plate 6.1.5) and had broader base with an average width of 3.9 mm. The average width at middle and top regions were 2.4 and 1.9 mm respectively (Table 6.1.2). The wall of oothecae was transparent. The length of oothecae depended on the size of the female. A small 53.0 mm gastropod laid oothecae of length ranging from 4.5 to 7.3 mm with an average of 5.9 mm; whereas a female measuring 85.6 mm had oothecae varying from 8.2 to 10.1 mm with an average of 9.5 mm in length. The average length of oothecae of *C. (M) pileare* was 7.3 mm (Table 6.1.2). The relationship between the length of oothecae and gastropod was highly significant (*r*=0.7813; *p*<0.01; *ts*=9.468) (Fig 6.1.4; Table 6.1.3).

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Fig. 6.1.3: Relationship between the length of *C. (M) pileare* and number of oothecae in egg cases.

\( r=0.4212; \ p < 0.05; \ ts=1.609 \).
FIG. 6.1.3

NO. OF OOTHECAE/EGG CASE

GASTROPOD LENGTH (mm)

- Data points and curve indicating a relationship between gastropod length and the number of oothecae/egg cases.
Plate 6.1.5.

Left: Ootheca of C. (M) pileare with 2 lateral projections. Scale: 1 cm = 2.2 mm.

Right: Ootheca of C. (L) cutaceum. Scale: 1 cm = 1.9 mm.
Table 6.1.2: Dimensions of the oothecae and number of embryos of C. (M) pileare and C. (L) cutaceum.

Each value is the average (x ± SD) of number of observations indicated in parantheses.

<table>
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<tr>
<th>Particulars</th>
<th>C. (M) pileare</th>
<th>C. (L) cutaceum</th>
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</thead>
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<tr>
<td>Oothecae dimensions (mm)</td>
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</tr>
<tr>
<td>Length</td>
<td>7.3 ± 1.3</td>
<td>7.4 ± 1.0</td>
</tr>
<tr>
<td>Width Base</td>
<td>3.9 ± 0.7</td>
<td>3.9 ± 0.2</td>
</tr>
<tr>
<td>Middle</td>
<td>2.4 ± 0.4</td>
<td>2.5 ± 0.2</td>
</tr>
<tr>
<td>Top</td>
<td>1.9 ± 0.3</td>
<td>2.5 ± 0.2</td>
</tr>
<tr>
<td>Number of embryos per ootheca</td>
<td>2985 ± 1184(69)</td>
<td>1502 ± 636(13)</td>
</tr>
<tr>
<td>Wet weight of an ootheca (mg)</td>
<td>33.2 ± 12.3(19)</td>
<td>22.0 (1)</td>
</tr>
<tr>
<td>Wet weight of an embryo (µg)</td>
<td>7.6 ± 2.4</td>
<td>10.6 (1)</td>
</tr>
</tbody>
</table>
Fig. 6.1.4: Relationship between the length of *C.(M) pileare* and ootheca length.

\( n = 68; \ a = -0.7097; \ b = 0.8400; \ r = 0.7813; \ p < 0.01; \ ts = 9.468 \).
Table 6.1.3: The regression analyses and significance of regression coefficients between length and weight of *C. (M) pileare* and different reproductive parameters.

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<th>No.</th>
<th>Parameters</th>
<th>n</th>
<th>Regression equation</th>
<th>r</th>
<th>p</th>
<th>Significance of regression coefficient</th>
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<tr>
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<td>$Y = -0.1870 + 0.7202x$</td>
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<td>&lt; 0.05</td>
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<td>Gastropod length vs No. of oothecae in egg case</td>
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<td>$Y = 1.3098 + 0.5172x$</td>
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<td>Oothecae length vs No. of eggs per ootheca</td>
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<td>$Y = 1.6776 + 2.1229x$</td>
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<td>&lt; 0.01</td>
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<td>$Y = -0.1265 + 1.8969x$</td>
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<td>&lt; 0.01</td>
<td>22.718</td>
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6.1.4.5. Weight of oothecae and embryo

The wet weight of an ootheca with embryos ranged from 19.2 to 58.0 mg with an average of 33.2 mg and the empty ootheca from 9.7 to 18.1 mg with an average of 10.6 mg. The mean weight of an embryo was 7.6 μg though it ranged from 3.9-13.1 μg (Table 6.1.2).

6.1.4.6. Number of embryos per ootheca

The number of embryos in an ootheca depending on its length, varied from 1150-6275. The average number of embryos per ootheca was 2985. The minimum of 1150 embryos was obtained from an ootheca of 5.3 mm length and maximum of 6275 from ootheca of length 10.0 mm. The relationship between number of embryos to ootheca length (ranging from 4.5-10.0 mm) obtained randomly from different egg cases showed high significance (r=0.7809; p<0.01; ts=8.839) (Table 6.1.3; Fig 6.1.5).

The length of oothecae was previously observed significantly related to length of the gastropod. It was also observed that number of embryos in oothecae depended on the length of oothecae. When number of embryos per ootheca was compared to length of gastropod the correlation was highly significant (r=0.9099; p<0.01; ts=5.802) (Table 6.1.3; Fig 6.1.6).
Fig. 6.1.5: Relationship between oothecae length and number of embryos per ootheca in C. (M) pileare

(n=52; a=1.6776; b=2.1229; r=0.7809; p < 0.01; ts=8.839)
FIG. 6.1.5

Graph showing the relationship between the number of eggs per ootheca and the ootheca length (mm). The data points form a linear trend, indicating a positive correlation. The x-axis represents the ootheca length in millimeters, while the y-axis represents the number of eggs per ootheca.

Key:
- 0: 1 4 0
- L: 5 6 7 8 9 10
- F1: (i.E1
- 700 L,Ji1i
- 5000
- 4000
- 3000
- 2000
- 1000

Scale:
- Y-axis: 0 to 8000
- X-axis: 0 to 11
Fig. 6.1.6: Relational between length of C. (M) pileare and number of embryos per ootheca.

\[(n=9; a=-0.1265; b=1.8969; r=0.9099; p < 0.01; ts=5.802)\].
FIG. 6.1.6

No. of Embryos / Ootheca

Gastropod Length (mm)
6.1.4.7. Number of embryos per egg case and fecundity

The number of embryos in an egg case deposited by 53 mm C. (M) pileare (shell-on weight: 11.8 g) was 1,56,275. Maximum number of embryos 10,68,960 was observed in the egg case laid by 85.6 mm gastropod with 50.4 g in weight. The average number of embryos per egg case was 6,02,520. The correlation coefficient obtained between number of embryos in egg case and length of gastropod was highly significant (r=0.6894;p<0.01;ts=3.297) (Table 6.1.3; Fig 6.1.7). The relationship between number of embryos and weight of gastropod was also highly significant (Table 6.1.3; Fig 6.1.8).

Of the spawns deposited, 88.9% of C. (M) pileare laid single egg case. The average fecundity was estimated to 5,85,048 eggs. By repeated spawning, 2 gastropods produced 1.6 to 1.9 times more number of eggs than the average fecundity (Table 6.1.1; Fig 6.1.9).

6.1.5. C. (L) cutaceum egg case

The diameter of 2 egg cases of C. (L) cutaceum was 37.6 and 40.6 mm. The height of the egg case was 11.6 mm. The weight of ootheca (with embryos) was 22.0 mg and the weight of empty ootheca was 8.1 mg (Table 6.1.2). The ootheca were tubular with a broad base. There were no projections on the side walls of ootheca. The length ranged from 6-9 mm containing 840-3070
Fig. 6.1.7: Number of embryos in egg cases in relation to length of C. (M) pileare.

\( n=14; \quad a=1.8092; \quad b=2.0593; \quad r=0.6894; \quad p < 0.01; \quad ts=3.297 \)
FIG. 6.1.7

![Graph showing the relationship between gastropod length (mm) and number of embryos/egg case (x10^5).](image-url)
Fig. 6.1.8: The relationship between weight of *C. (M) pileare* and number of embryos per egg case.

(n=12; a=4.4588; b=0.8095; r=0.8333; p < 0.01; ts=5.516).
embryos with an average of 1502 (Table 6.1.2). The number of oothecae per egg case varied from 160-175 (Table 6.1.1).

6.1.6. Discussion

For the first time mass deposition of 20 egg cases by C. (M)pileare was observed under laboratory conditions. The colour of egg cases of these cymatiids changed from initial creamy white to yellow on 3rd day; on 10-12th day, it changed to brown. Similar change in colour was reported for Cymatium cutaceum and C. corrugatum (Ramón, 1991). The attainment of brown colour was mainly due to the development of larval shell as observed in Eupleura caudata (Mackenzie, 1961).

Studies on incubation period will be useful in developing measures to destroy egg case. The incubation period for C. (L)cutaceum was 14 days, whereas it ranged from 12-19 days for C. (M)pileare (at the mean temperature of 27.2°C). At lower temperature of 24.8°C, the period extended upto 25 days. Incubation period decreases with increasing temperature in oyster drill Urosalpinx cinerea (Ganaros, 1958). The incubation period for Eupleura caudata ranged from 24 to 31 days at 21.1°C and with increase of temperature to 26.5°C the period was reduced to 15-17 days (Mackenzie, 1961). The incubation period of these cymatiids was 2 to 6 days shorter than E. caudata. Ramón (1991) reported 27
days as incubation period for \textit{C. cutaceum} at 16-19°C and 18 days for \textit{C. corrugatum} at 20-23°C. Among the cymatiids, \textit{C. (M) pileare} and \textit{C. (L) cutaceum} showed shorter incubation period probably because of high temperature (24.8-27.2°C).

Anderson (1959) and Mackenzie (1961) suggested that diameter of the egg case of predatory gastropods might correspond to the diameter of shell mouth. In \textit{C. (M) pileare} it was observed that diameter of the egg case was significantly related to length and width of the gastropod.

The dimensions of oothecae of the two species did not differ much (Table 6.1.2). The size of oothecae of \textit{C. (L) cutaceum} (6-9 mm in length; 3.6-4.1 mm in width) was comparable to that of \textit{C. cutaceum} (6.10-7.84 mm in length; 3.42-4.44 mm in width) and \textit{C. corrugatum} (6.89-8.71 mm in length; 3.38-4.98 mm in width) (Ramón, 1991). The ootheca of \textit{C. (M) pileare} (having two lateral projections) was distinctly different from that of \textit{C. (L) cutaceum} (Plate 6.1.5). D'Asaro (1986) reported on the taxonomic importance of the shape of oothecae, since it is species specific. The difference in the shape of oothecae noticed among these sympatric species of cymatium may be due to pedal moulding mechanism. Fretter and Graham (1962) opined that pedal moulding mechanism of higher prosobranchs could produce specific differences in the shape of ootheca.
The correlation coefficients obtained showed that length of oothecae \( r=0.7813; \ p<0.01 \) and the number of eggs per ootheca \( r=0.9099; \ p<0.01 \) were highly significant to the length of \( C.(M)pileare \). Carriker (1955) observed that older and larger \( Urosalpinx \) cinerea produced oothecae with more eggs than smaller individuals. Thus the number of eggs in the oothecae is dependent on adult length and environment (Purchon, 1977; Webber, 1977). \( C.(L)cutaceum \) did not show any such relationship.

The observation on 2 \( C.(M)pileare \), depositing 2 egg cases successively within 48-58 days revealed the intermittent spawning nature. Repeated spawning has been reported in \( Sepia \) officinalis (Boletzky, 1987) and in \( Littorina littorea \) (Daguzan, 1976). The tendency of repeated functioning of ovary and successive egg laying may be important for maintaining the population level (Mac Arthur and Wilson, 1967).

On an average, \( C.(M)pileare \) produced 200 oothecae with 2985 eggs each and \( C.(L)cutaceum \) 168 oothecae with 1502 eggs each. This is comparatively higher than that of \( Cymatilesta spengleri \) which produced 100 oothecae with 400 eggs each (Anderson, 1959); 45 oothecae each with 12.5 eggs of \( U.cinerea \) (Galtsoff et al., 1937); 55 oothecae with 14 eggs each of \( E.caudata \) (Mackenzie, 1961); and 92 oothecae each having 250 eggs of \( Purpura clavigera \) (Lin and Hsu, 1979).
Extended and repeated spawning, high fecundity, laying eggs in capsules and brooding the embryos are the reproductive strategies for successful and wide distribution of these predatory gastropods.
6.2. EARLY DEVELOPMENT

6.2.1. Introduction

Information on early development and life history of predators is essential for their control. Anderson (1959) described the larval development of Cymatilesta spengleri which Riedel (1992) considered it as ranellid Cabestana spengleri. D'Asaro (1966) and Galtsoff (1964) reported the early development of Thais haemastoma. Ramón (1991) studied the larval development of Cymatium cutaceum and C.corrugatum. Thangavelu and Muthiah (1983) reported the larval stages of C.cingulatum rearing the teased out contents of the oothecae. This had not been in accordance with the normal development in situ in the oothecae. Further no information is available on larval development of C.(M)pileare. This chapter gives a detailed account on the early development of C.(M)pileare and C.(L)cutaceum.

6.2.2. Material and methods

Larval samples for developmental studies were made from the egg case of C.(M)pileare and C.(L)cutaceum deposited in the laboratory on 23.7.92 and 1.7.92 respectively (Chapter 6.1.2). The base of the egg case was cemented to the bottom corner of the aquarium and the female incubated. Other gastropods and oysters
were removed from the aquarium. Filtered sea water was changed daily. Once in 2 to 3 days an ootheca was plugged out from the egg case by slightly lifting the gastropod. The embryos in the ootheca were teased out in a petri dish. The contents were made upto 50 ml using a measuring cylinder. Out of this, 1 ml sample was taken in a counting chamber and the number of larvae were counted. Average of 3 samples was considered for estimating number of larvae per ootheca. The number of fertilized eggs which had not undergone development was also noted. The percentage of development was calculated from the number of developing larvae and the number of undeveloped fertilized eggs. After larval counting, 20 larvae fixed in 1% formalin were measured for length and height under a microscope having precalibrated ocular micrometer and the mean size of larvae on different days of incubation were calculated.

After larval release, the egg case was removed from the aquarium. The number of oothecae that had not released their contents, if any, were counted. The percentage of hatching was calculated from the total number of oothecae in the case to the number of oothecae that had not released larvae.

The larvae released from the oothecae were collected in a 50 μm sieve and transferred to an aquarium (Size: 70 X 50 X 50 cm) having 105 litres of filtered sea water. Sand filtered sea water used for larval rearing was neither sterilized nor treated with
any antibiotics. Water was changed on alternate days. The water temperature ranged from 26.2-28.0°C and the salinity from 34.6-35.8 ppt. Gentle aeration was provided. The larvae of these two species were reared separately. The larvae of C. (M)pileare and C. (L)cutaceum were reared at the concentration of 1000 and 800 larvae/l respectively.

**Isochrysis galbana** was provided as larval food. This phytoflagellate measuring 7-8 µ and having 26-38% protein by body weight is ideal food for molluscan larvae (Nayar et al. 1987 a). Davis and Guillard (1958) considered *I. galbana* as the best larval food among the ten microalgae tested. *I. galbana* was cultured in the laboratory using Walne's medium. Following serial dilution techniques and employing sub-culturing method, pure stock cultures were maintained in 3 l conical flask. The flasks were placed in front of fluorescent lights fitted in wooden rack (Plate 6.2.2.1). Using this stock inoculum, mass culturing of this algae was carried out. From this pure culture, *I. galbana* was provided at the rate of 15,000-17,000 cells/larvae/day as food for the larvae.

### 6.2.3. Result

#### 6.2.3.1. C. (M)pileare development

The fertilized egg of *C. (M)pileare* was spherical; diameter of egg ranged from 125-150 µm with an average of 131.8 µm
Plate 6.2.2.1. Culture of *Isochrysis galbana*.
The yolk was uniformly distributed in the egg. The first cleavage divided the egg into two equal blastomeres (Plate 6.2.3.2). This was accompanied by formation of a protrusion in the vegetal pole of the egg (Plate 6.2.3.3) leading to trifoil stage. There were 2 nucleolated blastomeres and an anucleolated polar lobe. On 2nd day four cell stage of average length of 131.8 μm was observed (Plate 6.2.3.4). Then the cleavage followed spiral pattern leading to solid blastula. Gastrulation proceeded by epiboly, the micromeres multiplied and spread over macromeres (Plate 6.2.3.5). During next 3 days, the embryo elongated in the antero-posterior axis and became ovoid with pointed anterior and blunt posterior end, with a tuft of short cilia; this stage was the early trochophore stage. The larvae beginning to increase in length had a small cap-shell at the posterior end. On 7th day the mean size of the larvae was 173.9 X 163.2 μm (Plate 6.2.3.6).

On 8th day after torsion, the formation of visceral hump was complete. The shell attained a globular shape with a wide mouth from where velum was projecting (Plate 6.2.3.7). On 10th day the oothecae had larvae of mean length of 207.9 μm. The foot, a transverse antero-posteriorly flattened projection was covered with short cilia. The posterior face of foot had a thin operculum. On 11th day, the shell attained a spiral form and the foot became broad. The larvae moved inside the oothecae with
Plate 6.2.3.1. C. (M) pileare fertilized egg.
Scale: 1 cm = 26.6 μm.

Plate 6.2.3.2. Two cell stage. Scale: 1 cm = 27.8 μm.
Plate 6.2.3.3. Early trifoil stage. Scale: 1 cm = 25.2 \mu m.

Plate 6.2.3.4. Four cell stage. Scale: 1 cm = 31.5 \mu m.
Plate 6.2.3.5. Gastrula. Scale: 1 cm = 50.3μm.

Plate 6.2.3.6. Trochophore. Scale: 1 cm = 27.2μm.
Plate 6.2.3.7.  Veliger. Scale: 1 cm = 30 μm.
vigorous activity of the enlarged bilobed velum. The mean size of larva was 219 μm in length and 164 μm in height.

Shell whorls were formed on 12th day and very active muscular movement was observed. The anterior larval end could be withdrawn into the shell and the operculum was well developed, with the ability to close the shell. Eye spots were clear. The locomotion was effected by velar movements. At this stage, the veliconcha larvae (according to terminology used by Fretter & Graham, 1962) were released from oothecae. The hatching took two days and the larvae were released through an apical orifice of 1.8 mm diameter. The mean length of larvae released was 225.2 μm and mean height was 166 μm (Table 6.2.1; Plate 6.2.3.8).

Out of 180 oothecae in the egg case, 8 oothecae did not release larvae. The percentage of hatching was 95.6%. All the fertilized eggs in the remaining oothecae underwent further development. The percentage of development was 100% (Table 6.2.1).

Though there was wide variation in larval size, there existed a significant relationship ($r = 0.7971; p<0.01$) ($t=12.999$) between length and height of larvae of C. (M)pilear from egg case deposited to larval release (Table 6.2.2; Plate 6.2.3.1).

The larvae released from the oothecae was free-swimming and was frequently moving along the bottom of the aquarium tank.
### Table 6.2.1: Sizes of larval stages, percentage of larval hatching and development in *C. (M) pileare* and *C. (L) cutaceum*.

<table>
<thead>
<tr>
<th>Particulars</th>
<th><em>C. (M) pileare</em></th>
<th><em>C. (L) cutaceum</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>Diameter of egg (µm)</td>
<td>-</td>
<td>95.2 ± 0.8</td>
</tr>
<tr>
<td>Diameter of fertilized egg (µm)</td>
<td>131.8 ± 8.2</td>
<td>136.5 ± 6.5</td>
</tr>
<tr>
<td>Length of trophophore larva (µm)</td>
<td>173.9 ± 10.8</td>
<td>175.8 ± 10.4</td>
</tr>
<tr>
<td>Length of Veliger larva (µm)</td>
<td>214.2 ± -</td>
<td>239.7 ± 23.9</td>
</tr>
<tr>
<td>Length of larva at hatching (µm)</td>
<td>225 ± 13.8</td>
<td>265.1 ± 22.3</td>
</tr>
<tr>
<td>Percentage of development upto larval release</td>
<td>100 ± -</td>
<td>94.6 ± 3.4</td>
</tr>
<tr>
<td>Time taken for hatching of larva after spawning (days)</td>
<td>12</td>
<td>14</td>
</tr>
<tr>
<td>Percentage of oothecae hatching</td>
<td>95.6</td>
<td>93.7</td>
</tr>
<tr>
<td>Juvenile length (mm)</td>
<td>1.18 ± 0.24</td>
<td>-</td>
</tr>
</tbody>
</table>
Plate 6.2.3.8. Released veliconcha larva. Scale: 1 cm = 50μm.
Table 6.2.2  Mean larval length of *C.(M) pileare* and *C.(L) cutaceum* during their development inside oothecae.

<table>
<thead>
<tr>
<th>Days</th>
<th><em>C.(M) pileare (μm)</em></th>
<th><em>C.(L) cutaceum (μm)</em></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>± 8.2</td>
<td>± 6.5</td>
</tr>
<tr>
<td>1</td>
<td>131.8</td>
<td>136.5</td>
</tr>
<tr>
<td>2</td>
<td>147.7 ± 9.4</td>
<td>157.5 ± 10.9</td>
</tr>
<tr>
<td>4</td>
<td>172.6 ± 9.2</td>
<td>-</td>
</tr>
<tr>
<td>5</td>
<td>-</td>
<td>164.9 ± 16.4</td>
</tr>
<tr>
<td>7</td>
<td>173.9 ± 10.8</td>
<td>179.6 ± 10.8</td>
</tr>
<tr>
<td>8</td>
<td>196.7 ± 9.0</td>
<td>-</td>
</tr>
<tr>
<td>9</td>
<td>-</td>
<td>239.7 ± 23.9</td>
</tr>
<tr>
<td>10</td>
<td>207.9 ± 23.4</td>
<td>-</td>
</tr>
<tr>
<td>11</td>
<td>-</td>
<td>256.4 ± 18.2</td>
</tr>
<tr>
<td>12</td>
<td>225.2 ± 13.8 *</td>
<td>259.2 ± 9.2</td>
</tr>
<tr>
<td>14</td>
<td>-</td>
<td>265.1 ± 22.3 *</td>
</tr>
<tr>
<td>17</td>
<td>-</td>
<td>269.3 ± 30.6</td>
</tr>
</tbody>
</table>

Larval length at hatching
Fig. 6.2.3.1: Relationship between length and height of *C. (M) pileare* larvae.

\[ n=99; \ a=80.7467; \ b=0.4106; \ r=0.7971; \]
\[ p < 0.01; \ ts=12.999. \]
Above 95% of the larvae survived for maximum of 6 days rearing when the size of the protoconchs was 249.2 μm in length. Remaining live larvae were reared further and on 14th day, four larvae reached juvenile stage with length ranging from 0.95 to 1.13 mm. Other larvae were dead. The live young ones of C(M)pileare survived for another 25 days. Out of 4 juveniles, one gastropod of 1.115 mm length had a bore hole of 114.6 μm diameter (Plate 6.2.3.9). The maximum length and height of the young C.(M)pileare was 1.523 and 1.380 mm which were attained in 45 days after larval release and 57th day after egg case deposition (Plate 6.2.3.10). Further rearing could not be continued due to death of juveniles.

6.2.4. C.(L)cubaceum development

The egg was spherical; the average diameter of unfertilized and fertilized eggs were 95.2 μm and 136.5 μm respectively (Plate 6.2.4.1; Tables 6.2.1; 6.2.2). The yolk was uniformly distributed which was covered by a thin membrane. On 2nd day, first cleavage divided the egg into two equal blastomeres (Plate 6.2.4.2). After a brief trifoil stage (Plate 6.2.4.3) cleavage started in spiral pattern leading to four cell stage (Plate 6.2.4.4). On 5th day, morula had an average diameter of 151.5 μm (Plate 6.2.4.5). Embryos retained spherical shape after blastulation and gastrulation. By peripheral ciliary motion, embryos moved inside the ootheca.
Plate 6.2.3.9. Juvenile C.(M) pileare having bore hole. Scale: 1 cm = 161.5μm.

Plate 6.2.3.10. Young C.(M) pileare. Scale: 1 cm = 160μm.
Plate 6.2.4.1. Fertilized egg of C. (L) cutaceum.

Scale: 1 cm = 30.2 µm.

Plate 6.2.4.2. Two cell stage. Scale: 1 cm = 24.4 µm.
Plate 6.2.4.3. Trifoil stage. Scale: 1 cm = 54.6μm.

Plate 6.2.4.4. Four cell stage. Scale: 1 cm = 26.3μm.
Plate 6.2.4.5. Morula stage. Scale: 1 cm = 28.5 μm.
On 6th day, the embryo elongated and the posterior end had short cilia. This stage corresponded to trochophore which measured 175.8 μm in length. On 7th day, trochophore larvae attained a maximum length of 201.6 μm and height of 170 μm. The mean size was 179.6 X 143.6 μm in length and height respectively (Plate 6.2.4.6). During the next two days, the shell covered the posterior end of the embryo as a broad cap. After torsion, on 9th day the veliger measured 239.7 μm in length and 171.0 μm in height. The larvae had visceral mass covered by the shell (Plate 6.2.4.7).

On 11th day, the foot became broad and the operculum larger with strongly beating cilia in bilobed velum. The head and foot were withdrawn into the shell. The colour of the larval shell was brown. Eye spots were conspicuous. The average size of larvae was 256.7 X 186.2 μm in length and height respectively (Plate 6.2.4.8).

On 14th day, the larvae were released and their mean length and height was 265.1 μm and 191 μm respectively. The released larvae, though crawled on the bottom of the aquarium had always planktonic existence with active movement of bilobed velum. The maximum size of larva was 321 X 227 μm in length and height. In this veliconcha stage, the anterior end was able to retract into the shell and shell mouth closed by operculum (Plate 6.2.4.9).
Plate 6.2.4.6. Trochophore. Scale: 1 cm = 27.5μm.

Plate 6.2.4.7. Veliger larva. Scale: 1 cm = 27.1μm.
Plate 6.2.4.8. Released veliconcha larva. 
Scale: 1 cm = 31.5μm.

Plate 6.2.4.9. Maximum size of C.(L) cutaceum larva reared. Scale: 1 cm = 35.7μm.
Out of 175 oothecae, 11 did not release larvae. The percentage of hatching was 93.7. In an ootheca, out of 1000 fertilized eggs, 30 did not undergo further development. In another ootheca, there were 240 undeveloped fertilized eggs out of 3070 embryos. The percentage of development ranged from 92.2 to 97.0% with a mean of 94.6% (Table 6.2.1).

The larvae of same brood of \( C. (L) \) cutaceum showed wide variations in size during development (Table 6.2.2). On 14th day, when the larvae were released, the larval length ranged from 239.4 to 321.3 µm. There was significant correlation between the length and height of \( C. (L) \) cutaceum larvae \( (r = 0.8755; \ p<0.01; \ ts=22.035; \) Fig 6.2.3.2).

The released larvae on 3rd day of rearing attained mean length and height of 269.3X195.0 µm respectively. The larvae survived for 11 days after their release. During this period, they led free-swimming as well as crawling existence. They attained a maximum length and height of 352.8 and 252.0 µm on 25th day after egg case deposition. Further rearing could not be carried out due to total larval mortality.

6.2.5. Discussion

Both \( C. (M) \) pileare and \( C. (L) \) cutaceum were devoid of nurse eggs. There was no nurse eggs in the oothecae of Urosalpinx
Fig. 6.2.3.2: The relationship between larval length and height in C.(L) cutaceum.

(n=150; a=67.4407; b=0.4764; r=0.8755
p < 0.01; ts= 22.035).
*U. cinerea* (Hancock, 1956). In the absence of specialised nurse eggs, embryos are said to derive nourishment from albumen apart from yolk (Webber, 1977). Albumen in the oothecae has protein, free lipid and neutral polysaccharides (Bayne, 1968). Besides a source of nourishment to larvae, albumen is also involved in raising the osmotic pressure of egg capsular fluid which is responsible for bursting the oothecae in facilitating larval release.

The larvae were released through an apical oval orifice of oothecae in both species (Plate 6.2.5). Similar mode of larval release without damaging the sides of oothecae wall was observed in *U. cinerea* (Hancock, 1956), *Nassarius obsoletus* (Scheltema, 1962), *N. corrugata* (D'Asaro, 1969) and *Cymatium cutaceum* and *C. corrugatum* (Ramón, 1991).

In the present study, the hatching of larvae occurred 12-14 days after deposition of egg cases at the temperature range of 26-28°C. At lower temperatures, the development required 27 days for *C. cutaceum* (16-19°C) and 18 days for *C. corrugatum* (20-23°C) (Ramón, 1991). In the present study, *C. (M)pileare* and *C. (L)cutaceum* were observed to release larvae 4-5 days earlier than the cymatiids in other studies. This difference in the incubation period may be due to temperature difference. It is well known that higher temperature accelerates the rate of development.
Plate 6.2.5. Hatched out egg case indicating apical orifice. Scale: 1 cm = 5.5 mm.
The length increment of C. (M)pileare larvae in relation to duration of development inside the oothecae was fitted by the following exponential equation:

\[
\text{Length of larvae} = 129.3302 \times \text{days}^{0.2007}
\]

and significant correlation \( (r = 0.9701; p < 0.01; t_s = 9.124) \) existed between these two parameters (Fig 6.2.5.1). The length of C. (L)cultaceum larvae and the duration of development could be described as

\[
\text{Length of larvae} = 115.7178 \times \text{days}^{0.3039}
\]

and the parameters were significantly correlated \( (r = 0.9114; p < 0.01; t_s = 7.479) \) (Fig. 6.2.5.2). In C. (L)cultaceum the larval growth in length increased at higher rate in relation to days when compared to C. (M)pileare.

Eventhough there was considerable variation in larval lengths (Table 6.2.2) of the same brood, there existed a highly significant correlation between length and height of the larvae (Figs. 6.2.3.1; 6.2.3.2). The variation in larval sizes may be due to some endogenous factors. The larval length to height relationship of other cymatiids and other gastropods have to be studied. The regression lines drawn for various species are to be compared and the differences, if any, will help to identify the larvae. Loosanoff et al. (1966) observed that the differences in regression line of larval length and width of oyster Crassostrea gigas to that of Ostrea lurida were
Fig. 6.2.5.1: Growth of larvae of C. (M) pileare in the ootheca. Mean and SE are shown. The curve obtained by fitting growth equation.

Length of larvae = 129.3302 X days $^{0.2007}$

($r=0.9701; p < 0.01; ts=9.124$).
FIG. 6.2.5.1

DEVELOPMENTAL PERIOD (DAYS)

LARVAL LENGTH (μm)
Fig. 6.2.5.2 Growth of larvae of *C. (L) cutaceum* in the ootheca. Means and SE are indicated.

The growth curve obtained by fitting the following exponential equation.

\[
\text{Length of larvae} = 115.7178 \times \text{days}^{0.3039}
\]

\(r=0.9114; \ p < 0.01; \ ts=7.479\).
statistically significant. They stated that this difference could be used in distinguishing bivalve larvae of different genera.

The larvae of these two cymatiids, though mainly planktonic existence after release from oothecae, remain on the bottom of the aquarium for short durations. Anderson (1959) stated that larvae of *Cymatilesta spengleri* were benthic dwellers. Ramón (1991) reported that larvae of *Cymatium cutaceum* and *C. corrugatum* had only planktonic existence when reared in the laboratory. Larvae of cymatiids are known for a long pelagic phase. Larvae of *Cymatium parthenopeum* collected from plankton samples remained planktonic for more than two months (Scheltema, 1966). The planktonic duration of larvae of *C. (M) pileare* and *C. (L) cutaceum* could not be determined because of their survival only for a few days after hatching. The presence of velum even after well developed foot and high velar activity enable them to drift to long distances facilitating wider distribution.

Appearance of prepodium is considered usually as a prerequisite for metamorphic competence (Hadfield, 1978). The presence of well developed prepodium at a size of 214 µm in *C. (M) pileare* larvae and at 239 µm in *C. (L) cutaceum* larvae suggest their competence for metamorphosis. However, large scale mortality occurred a few days after larval release. Ramón (1991)
observed that the larvae of C. cutaceum and C. corrugatum survived for 7 and 16 days respectively after release from oothecae. Poor survival of the larvae in the laboratory may be attributed to the absence of suitable substratum as experienced in larval rearing of Nassarius obsoletus (Scheltema, 1961). Success has not been achieved so far in rearing the cymatiid larvae to adult stage.

This is the first instance of producing juvenile C. (M) pileare in the laboratory. A bore hole was observed on a juvenile (Plate 6.2.3.9) which indicated the cannibalistic behaviour of C. (M) pileare. Also it revealed the capacity of C. (M) pileare to adopt to boring method for feeding gastropods since cymatiids could not easily introduce the proboscis as the gastropod retracts inside the shell and operculum closing the shell aperture. This early carnivorous nature exhibited among juveniles indicated the necessity to evolve suitable food for successful rearing of cymatiids.

The provision of albumen as larval nourishment, high percentage of larval hatching and development, and planktonic cum crawling existence of released larvae are some of the developmental strategies for successful larval establishment and distribution of C. (M) pileare and C. (L) cutaceum.
Information on predatory gastropods are essential for evolving suitable control measures. The salient findings of this study on \textit{C. (M)pileare} and \textit{C. (L)cutaceum} along with possible control methods are discussed.

In the prey preference study, it was observed that \textit{C. (M)pileare} was able to prey upon more than one species of bivalves. The bivalves, edible oyster \textit{Crassostrea madrasensis}, pearl oyster \textit{Pinctada fucata}, mussel \textit{Perna indica} and clam \textit{Mesodesma glabratum} were consumed by non-boring method. Surprisingly, in the early development studies (Chapter 6.2) juvenile \textit{C. (M)pileare} was found to drill their counterpart. This observation documents the carnivorous and cannibalistic behaviour of juveniles. The bore-hole on a young \textit{C. (M)pileare} (Plate 6.2.3.9) showed the ability of cymatium to drill the shell.

\textit{Cymatium} spp. were reported to feed by non-boring method on bivalves and gastropods (Houbrick and Fretter,1969), on edible oyster (Thangavelu and Muthiah,1983; Littlewood,1991), on pearl oysters (Chellam et al.,1983), and on tridacnid clams (Perron et al.,1985; Govan,1990). Another cymatiid \textit{Monoplex australasiae} feeds on bivalve prey by non-boring method (Laxton, 1971). Hughes and Hughes (1981) stated that drilling of molluscan shells by tonnaceans (cymatiids belong to this group) has never been recorded with certainty.
Whereas Morton and Miller (1968) reported that cymatiids bore European edible mussels. Day (1969) opined that cymatiids have the ability to bore the shell with their CaCO$_3$ dissolving acid secretion. But he observed that cymatiid *Argobuccinum argus* feeds on tubicolous polychaetes *Gunnarea capensis* by non-boring method.

The histochemical studies of proboscis gland of *C. (M)pileare* indicated the presence of organic sulfuric esters and toxin secreting cells. These secretions along with radula facilitated the cymatiids to bore the molluscan shell and to paralyse the prey.

This study, therefore, provides clear evidence to show that cymatiids feed by employing both the methods (ie. non-boring and boring or drilling). Further it was interesting to note that cymatiids judiciously employ non-boring method for easily accessible prey (ie. bivalves) and for in-accessible gastropods, they adopt boring method.

Having shown the capability of cymatiids to bore the molluscan shells, further investigation on the mode of shell penetration have to be attempted. Carriker and Williams (1978) in their hypothesis on drilling of muricid and naticid gastropods stated that the combined action of acid, a chelating agent and enzymes was responsible for shell dissolution. Day (1969)
concluded that secretion of proboscis gland of cymatiid *A. argus* eroded the mineral fraction of shell in contrast to accessory boring organ in muricids and naticids which attack organic matrix prior to dissolution of mineral fraction. This study thus focusses on the imperative need for further studies on shell dissolution mechanism of cymatiids.

Another interesting observation was the cannibalistic behaviour among juvenile *C. (M) pileare*. Muthiah et al. (1987) briefed on the cannibalism of cymatiids occurring in the oyster rearing cages. Manzi (1970) observed the cannibalistic behaviour of drills *Urosalpinx cinerea* and *Eupleura caudata* in laboratory experiments.

Prey density appears to remarkably influence the predation rate of cymatiids. As the prey density increased from 5 to 20 oysters/400 cm² the feeding rate increased from 4.3 to 8.3 oysters/month/gastropod. Muthiah et al. (1987) observed that the number of cymatiids increased 14 times as the farm stock was doubled from 3 to 6 lakh oysters. Govan (1990) stated that the incidence of cymatiids was apparent when more than 5000 juvenile tridacnid clams were stocked in the nursery. These observations and the findings of the present experiment suggest avoidance of overstocking as the possible means of reducing the predation of cymatiids in bivalve farms.
Species specificity of jaws and radula, the organs of feeding, seem to have taxonomic importance. The jaws of \( C.\) \( (M) \) pileare had 9 platelets less than in \( C.\) \( (L) \) cutaceum. The taenioglossan radula in both species are similar. The radular length and width were significantly related to the size of the gastropods. While observing radular uniformity among various species of cymatiids, Clench and Turner (1957) considered that jaws may have much taxonomic importance. Beu and Kay (1988) observed difference in the number of platelets in \( C.\) \( (M) \) pileare (18 - 22) and \( C.\) \( aquatile \) (26).

For the first time, the regeneration of proboscis was observed in cymatiids. Proboscisectomy experiments showed that \( C.\) \( (M) \) pileare was able to regenerate amputated portion of proboscis in 20 days whereas \( C.\) \( (L) \) cutaceum took 34 days. Regeneration of amputated portion of proboscis in \( Thais \) haemastoma (Demoran and Gunter, 1956), \( U.\) \( cinerea \) and \( E.\) \( caudata \) (Carriker et al., 1972) have been reported. Even the \( C.\) \( (M) \) pileare in which whole proboscis (13 mm length) was removed, resumed feeding in 18 days.

Along with proboscis, jaws and radula also regenerated. Person and Philpott (1969) reported that regeneration of jaws and radula will be helpful in the study of cartilage and skeletal regeneration. Hence the chemical constituents of collagen and odontophoral cartilages of cymatiids warrant attention in future studies.
Proboscis gland of C. (M) pileare possesses toxin secreting cells and organic sulfuric esters. Acrylylcholine and a neurotoxin-tetramine were secreted by cymatiids (Carriker, 1981). Acrylylcholine is considered as a neuro-muscular blocking agent. Whittaker (1960) had identified Urocanycholine as the pharmacologically active choline esters in muricid drills U. cinerea, Ocenebra erinacea and Murex spp.. Further Urocanycholine is used in clinics as muscular relaxant. Thus these biologically active compounds could be useful in developing therapeutic drugs. Functions of these bioactive compounds in the gastropods that secrete are lacking. Further it is not known whether mantle tissues and mucus of predatory gastropods contain these valuable bioactive compounds. This is a vital area for pharmacologists for future research.

Judiciously employing boring and non-boring method of feeding, feeding on mixed prey organisms, regenerating proboscis, jaws and radula, and secreting acidic choline esters with a toxin from proboscis gland, and adopting cannibalism are the salient predatory feeding behaviours of cymatiids.

Repeated spawning of cymatiids under laboratory conditions was observed for the first time. By successively laying 2 egg cases at an interval of 48-58 days, two C. (M) pileare produced 1.6-1.9 times more eggs than normal gastropod which spawned once.
This interesting reproductive behaviour enhances normal gonadal capacity for egg production. As Boletzky (1987) considered for Sepia officinalis, this successive egg laying may be due to the animal's tendency to maintain their population level.

Fecundity studies have shown that C. (M) pileare produced more eggs than C. (L) cutaceum. The average fecundity of C. (M) pileare was 5.85 lakh eggs whereas in C. (L) cutaceum it was 2.04 lakh eggs. The information on spawning season and incubation period (12-14 days) are of use for constant inspection to remove egg cases from oyster farm.

This study established that the fecundity of C. (M) pileare was significantly related to the length of gastropod, to the length of oothecae and eggs contained in it.

The number of eggs in the oothecae (Y) was significantly (p<0.01) related to the length of oothecae (X)

\[ Y = -3830.8125 + 1006.2707 \times X \]  \hspace{1cm} 1.

and the high significance (p<0.01) between length of oothecae to length of C. (M) pileare (Z) was expressed as

\[ X = 0.8060 + 0.0879 \times Z \]  \hspace{1cm} 2.

By substituting the right hand side values of 2nd equation to X of the first equation, the relationship could be as

\[ Y = -3019.7583 + 89.25 \times X \]
The slope of this linear regression indicated an increase of 89 eggs per ootheca for 1 mm increase in length of gastropod. With an average of 200 oothecae per egg case, the total increase in number of eggs works out to 17800.

The equation relating fecundity (Y) to length of \textit{C. (M)pileare} (X)

\[ Y = -512570.155 + 13959.278 \times X \]

has also shown an increase of 13959 eggs for 1 mm increment in length of \textit{C. (M)pileare}. The estimated increase in fecundity ranging from 13959-17800 per 1 mm increase length of \textit{C. (M)pileare} indicated its higher reproductive potential than \textit{Purpura clavigera} where Lin and Hsu (1979) observed an increase of 2926 eggs for 1 mm increment in length.

The shape of oothecae was species specific. The oothecae of \textit{C. (L)cutaceum} had 2 lateral projections which were lacking in the oothecae of \textit{C. (M)pileare} (Plate 6.1.5). D'Asaro (1986) noted structural differences in egg capsules of predatory muricids and buccinids. The shape of oothecae offers additional criteria for species separation apart from the external shell characters considered at present. Previously it was shown that jaw platelets are also taxonomically important. The shape of oothecae and number of jaw platelets in sympatric and allopatric species of \textit{cymatium} have to be given importance in future studies.
Larvae of *C. (M)pileare* were successfully reared in the laboratory up to juvenile stage. Nevertheless, *C. (L)cutaceum* larvae survived for 11 days only. Ramón (1991) reared the larvae of *C. cutaceum* for 7 days after hatching. The poor survival rate may be attributed to the non-availability of suitable food for carnivorous juveniles. There is imperative need to identify suitable food and to standardize the rearing technique for successful production of young cymatiids in the laboratory. This will alleviate the problem of availability of these gastropods for experimental studies as also experienced by Hughes and Hughes (1981) for study on *Cassis tuberosa*, a tonnacean predatory gastropod.

The length of *C. (L)cutaceum* larvae was 40 µm more than that of *C. (M)pileare*. To find out the significance of the difference in the larval sizes, length-height relationships for released larvae of both species were analysed. Their relationships were significant at 1% level in both species (*r* = 0.8649; *ts* = 9.118 for *C. (M)pileare*; *r* = 0.5840; *ts* = 4.608 for *C. (L)cutaceum*). The regression equation obtained for *C. (M)pileare* was

\[ H = 36.7707 + 0.60038 L \]

and for *C. (L)cutaceum* was

\[ H = 124.0791 + 0.2628 L. \]
The difference between the above two regression coefficients was highly significant ($F_s=9.074$; df 1, 69; $p<0.01$). Studying the length-width (term equal to height) relationship of bivalve larvae, Loosanoff et al. (1966) observed significant difference in the regression equations for larvae of different genera in bivalves. This difference was considered helpful in distinguishing the bivalve larvae of different genera. However, they also suggested that this may be of little use to distinguish the larvae within genus. Ramón (1991) observed quite similar surface ornamentations of protoconchs of *C. cutaceum* and *C. corrugatum*. Comparing this with *C. caribbaeum*, *C. labiosum*, *C. parthenopeum*, *C. nicobaricum* and *C. pileare*, it was concluded that surface ornamentation could not serve as a distinctive character for distinguishing protoconchs of cymatiids (Ramón, 1991). The significant difference in the length-height of protoconchs could help to distinguish them. To establish the usefulness of larval length-height data, observations on other species of Cymatium have to be undertaken in future.

The released larvae of both cymatiids were able to swim and crawl. The incidence of cymatiids in the farm could be controlled by dispensing with rearing cages which act as suitable enclosures for the swimming larvae for their settlement and growth. By adopting suspension method of oyster culture with strings well above the bottom, the crawling larvae could be prevented from climbing on the strings and their subsequent predation.
Various methods have been suggested for controlling the drills *U. cinerea* and *E. caudata*. Loosanoff (1957) stated that immersion of egg cases in saturated salt solution for 3-5 minutes caused mortality of embryos. Loosanoff and Nomejko (1958) found that drills buried at 6 cm depth of muddy and sandy bottom could be killed by turning the bottom using agriculture blows or oyster dredges. Creation of chemical barriers using 95% sand with 5% orthodichlorobenzene around the farm boundaries effectively stops entry of drills (Loosanoff et al., 1960).

Divergent views exist on the use of polystream (a mixture of polychlorinated benzene) for controlling the drills. Haven et al. (1966) reported that the application of polystream and sevin mixture did not reduce the drill population. They found that the treatment adversely affected the benthic organisms such as polychaetes, shrimps, crabs and razor clams. Mackenzie (1970) stated that spraying 1.9 Kl of polystream with 9.5 Kl of sand/ha was effective in the areas where current velocity was less than 2.7 km/hr. They stated that polystream treatment killed only a small percentage of benthic organisms. Haven et al. (1966) were of the view that experimental chemical treatment could not be applicable in the field.

In the absence of effective control measures, manual searching in rearing cages and removal of cymatiids was resorted to control the predation (Perron et al., 1985; Muthiah et al., 1987; Littlewood, 1991).
The cost of control measures accounted to 9% of annual operational cost for manual removal method (Nayar et al., 1987b) and $ 40 per year for spraying chemicals (Mackenzie, 1970). The predation and predator control measures cause considerable financial implications in the economics of bivalve culture.

These control measures are labour-intensive and cost effective and practically difficult to follow in large scale commercial farming. And based on the observations made on feeding and reproductive strategies of cymatiids in this study, effective steps have to be undertaken to prevent or reduce the occurrence in the culture areas rather than controlling them. Predator preventive or reduction measures could be attempted by i) manipulating the density of bivalves in the rearing areas thereby avoiding over stocking the farm, ii) by adopting suspension method of culture.

These preventive measures may be more effective economically. This will probably be useful in maximizing return on investment in farming the bivalves.
Besides exploiting the natural resources, oyster farming (both edible and pearl oyster) is gaining momentum recently to augment their production. Nevertheless, few species of gastropods remain unchallenged as predators causing huge mortality and economic loss impending the development of oyster farming. *Cymatium (M)* pileare and *C.* (L) cutaceum are the two important predators among the gastropods. With a view to control these predators, a detailed study has been made on the feeding behaviour, reproductive performance and larval development of these two species.

The predatory gastropods, *C.* (M) pileare and *C.* (L) cutaceum were collected from a pearl oyster farm situated in Tuticorin Bay and maintained separately in FRP aquaria having filtered sea water. Edible oysters were provided as food for the stock gastropods. *C.* (M) pileare generally adopt non-boring method of feeding ie introducing proboscis in the gap between the shell valves and paralysing the prey.

Field experiment was conducted to study the prey preference of the gastropod, *C.* (M) pileare. The test gastropods (length : 65 ± 0.9 mm) were provided with edible oyster, pearl oyster, mussel and clam each 2 animals. Out of the total food consumed, edible oyster *Crassostrea madrasensis* was the most preferred (47.4%) prey, the mussel *Perna indica* formed 26.3% followed by
pearl oyster *Pinctada fucata* (15.8%) and clam *Mesodesma glabratum* (10.5%).

The effect of temperature (24, 28, 31 and 34°C) on predation rate of *C. (M) pileare* was determined. The predation rate increased progressively with increasing temperature. At 24°C, the rate was 2 oysters/gastropod/month; it was 3.3 or 5.3 oysters/gastropod/month at 28 or 31°C. Maximum rate of predation (5.3 oysters/gastropod/month) was recorded in 31°C. The upper tolerance limit for feeding was 34°C.

Apart from environmental factors, prey density influences the predation of *C. (M) pileare*. Influence of prey densities on predation rate was studied in a field experiment conducted in a cage maintaining 5, 10, 20 and 30 oysters per gastropod. At the lowest density of 5 oysters/quadrat, the predation rate was 4.3 oysters/gastropod/month. It increased to 7.3 or 8.3 oysters at prey densities of 10 or 20 oysters/quadrat. However, at the maximum density of 30 oysters/quadrat, the rate decreased to 1 oyster/gastropod/month.

For studying the shape and dimensions of the feeding organs, namely, jaws and radulae, the tip portion of proboscis was excised and soaked in alkali solution. After disintegration of tissue, jaws and radulae were collected and measured. The jaws were subtriangular in shape. The jaw of *C. (L) cutaceum* had 29
platelets whereas C. (M) pileare had only 20 platelets. This difference in the number of platelets in jaws is of taxonomic importance.

The radula was taenioglossate in both species. The length of radula ranged from 2.3-7.7 mm with an average of 66 rows of teeth in C. (M) pileare (length 43.6-98.4 mm). In C. (L) cutaceum (length 5.4-73.0 mm), radular length ranged from 0.85-6.12 mm with 51 rows of teeth. The length and width of radula were significantly correlated to the size of gastropods. A significant relationship existed between length and width of radulae of C. (M) pileare (r=0.8165; p<0.01) and C. (L) cutaceum (r=0.9650; p<0.01).

Proboscisectomy experiments were conducted by amputating a portion (5-13 mm length) of proboscis from C. (M) pileare and C. (L) cutaceum. Proboscisectomized C. (M) pileare resumed feeding after 20 days and C. (L) cutaceum took 34 days to resume feeding. The rate of proboscis regeneration was faster in C. (M) pileare (0.45 mm/day) than C. (L) cutaceum (0.15 mm/day). The rate of regeneration was significantly correlated to the amputated length of proboscis. This is the first report on the regeneration of proboscis in cymatiids.

Secretions of proboscis gland assist in feeding. Histochemical properties of the proboscis gland were observed by
staining the gland sections in Mallory's triple stain and in Aldehyde fuschin. Acidophil and basiphil cells were found in the proboscis gland. The staining properties in these metachromatic stains showed the presence of organic sulfuric esters. Toxin secreting cells were described.

Reproductive studies are vital to control predatory gastropods. Detailed studies were made on the reproductive performance and larval development. Mass deposition of 20 egg cases by C. (M) pileare (length: 53-93 mm) and 2 egg cases by C. (L) cutaceum (length: 72.5 mm) were observed in the laboratory. One of the salient observations was the repeated spawning by two C. (M) pileare laying successive egg cases in 48-58 days interval. The females of both the species incubated their egg cases.

Peak spawning period was from July-November and a minor spawning period was during February-May in C. (M) pileare. Spawning occurred during July-August in C. (L) cutaceum.

The egg cases of both the species were hemispherical in shape and consisted of oothecae. An egg case of C. (M) pileare had 200 oothecae with 2985 embryos each. An egg case of C. (L) cutaceum had 168 oothecae with 1502 embryos each.

The oothecae of C. (M) pileare differed from the oothecae of C. (L) cutaceum by having two lateral projections.
The dimensions of egg cases, number of oothecae, length of oothecae and the number of embryos in the oothecae recorded were correlated with the size of \( \text{C.}(\text{M})\text{pileare} \). The diameter of egg case was significantly related \( (r=0.9656; p<0.01) \) to width of the gastropod. Length of oothecae was significantly correlated to the length of \( \text{C.}(\text{M})\text{pileare} \) \( (r=0.7813; p<0.01) \) and to the number of embryos in the oothecae \( (r=0.7809; p<0.01) \).

Fecundity was estimated from the number of oothecae in an egg case and number of embryos contained in an ootheca. The average fecundity of \( \text{C.}(\text{M})\text{pileare} \) was 5.85 lakh eggs. Significant relationships were observed between the number of embryos in the egg case to the length and weight (shell-on) of \( \text{C.}(\text{M})\text{pileare} \). It was predicted that for every 1 mm increase in length of \( \text{C.}(\text{M})\text{pileare} \), it was capable of producing 17,800 eggs.

By repeated spawning gastropods produced 1.6-1.9 times more eggs than the normal \( \text{C.}(\text{M})\text{pileare} \) which spawned once.

\( \text{C.}(\text{L})\text{cutaceum} \) produced 2.03 lakh eggs which is 65% less than the fecundity of \( \text{C.}(\text{M})\text{pileare} \).

Early larval development occurred in the oothecae. Larval development and growth studies were carried out on the larvae in the oothecae taken from an egg case of \( \text{C.}(\text{M})\text{pileare} \) and \( \text{C.}(\text{L})\text{cutaceum} \).
The average diameter of fertilized egg of *C. (M) pileare* was 131.8 μm. Trochophore larval stage was reached on 7th day and larvae were released on the 12th day through apical orifice. The percentage of hatching was 95.6% and all the fertilized eggs developed into larvae. The released larvae were reared in the laboratory providing *Isochrysis galbana* as food. After 57 days, four juveniles were produced. The maximum length of juvenile *C. (M) pileare* was 1.5 mm. The bore-hole on the shell of juvenile indicated the cannibalistic behaviour and also its capability to bore the shell for feeding.

The egg of *C. (L) cutaceum* was spherical. The average diameter of fertilized egg was 136.5 μm. The trochophore stage was reached on 7th day and the larvae were released on 14th day. About 94% of the oothecae in the egg case released the larvae. The swimming - crawling stage of released larvae survived for 11 days of rearing. Of the fertilized eggs, 94.6% developed into larvae.

The regression equation developed for larval growth in the oothecae of *C. (M) pileare* was

\[
\text{Length of larvae} = 129.3302 \times \text{days}^{0.2007}
\]

and for *C. (L) cutaceum*,

\[
\text{length of larvae} = 115.7178 \times \text{days}^{0.3039}
\]
The larvae of *C.* (L.) *cutaceum* grew faster than that of *C.* (M) *pileare*. Significant relationship between larval length and height have been observed in both species.

Feeding on variety of bivalves, ability to feed both by drilling and non-boring methods, regeneration capability of proboscis (with its associated jaws and radula) and proboscis gland having toxin secreting cells and presence of sulfuric esters are the important feeding strategies of these cymatiids.

Encapsulation of eggs, laying egg cases, incubation, presence of albumen in the oothecae as nourishment for developing larvae, high fecundity, repeated spawning and planktonic-crawling behaviour of released larvae are the reproductive strategies of these gastropods that ensure them to maintain their population and wide dispersal.