3. GENERAL MATERIALS AND METHODS
3.1. **Collection**

For this study, the gastropods were collected from a pearl oyster farm situated in the coastal region of Tuticorin Bay (Lat 8°.48’N; Long 78° 11’E). For this farm, pearl oysters were collected from pearl oyster beds or 'parrs' off Gulf of Mannar. The oysters collected by the divers are stocked in cages of size 40 X 40 X 10 cm (6 mm M.S. welded and webbed with 2.5 mm synthetic twine). The cages are suspended from the raft moored in the coastal waters, Hare Island near Tuticorin. Pearl culture division of Tamil Nadu Fisheries Development Corporation maintains these stock oysters until being transported to their farm at Krusadai (Lat 9° 40’N; Long 79°35’E) where implantation and further rearing are carried out for production of culture pearls. During this interim period of rearing, the pearl oysters and cages were cleaned at frequent intervals so as to remove the foulers and predators. During the cleaning operation, *Cymatium* spp. present inside the cages were collected and brought to the laboratory (22 *C. (Monoplex) pileare* (length: 31.3-93.0 mm); and 17 *C. (Linatella) cutaceum* (length: 48-76 mm).

3.2. **Feeding and water change**

The gastropods collected were maintained separately in FRP aquarium of size 75 X 50 X 50 cm. The aquaria were filled with
filtered sea water upto 38 cm height. For supply of filtered sea water, raw sea water drawn gravitationally through PVC pipe was collected in a closed well dug adjacent to the shore. This water was pumped to sedimentation tanks and a filter bed consisting of layers of coal, granite stones and river sand. The water got filtered, stored in sump, from where it was pumped to an overhead tank. Through pipe-line and supply gate-valve, the filtered water was supplied to the laboratory. For the stock snails maintained, edible oysters of 25-57 mm length were provided as food ad libitum. The edible oysters were collected from natural beds occurring in the nearby creeks. Sea water was changed daily. Dead oysters, if any, were replaced with oysters of equal size. Gentle aeration was provided by a 5 H.P. air compressor. From this stock, individual healthy gastropods required for the experimental studies were used.

3.3. **Experimental design**

Laboratory as well as field experiments were carried out on two species of cymatiids. Detailed investigations were made on the predation rate of *C.(M) pileare* in relation to prey density and prey preference. Influence of temperature on feeding rate of *C.(M) pileare* was also investigated. Radular dimensions and regeneration of proboscis were studied. Histochemistry of proboscis gland was carried out.
A detailed study was made on the fecundity and development from 20 spawns of *C. (M)pileare* and 2 spawns of *C. (L)cutaceum*.

3.4. **Estimation of hydrological conditions**

For the laboratory experiments, the water temperature after water change and the surface water temperature on the days of observation for field experiments were recorded. Weekly samples of sea water during experimental period were analysed for dissolved oxygen content by Winkler's method, the salinity by Harvey's titration method and pH was recorded by pH meter following the procedure given by Strickland and Parsons (1972).

3.5. **Statistical treatments**

The data collected were subjected to the following statistical analyses:

i) Standard deviation

ii) Standard error

iii) Simple regression

iv) Simple correlation

v) Analysis of variance

i) **Standard deviation:**

\[ SD = \sqrt{\frac{\sum d^2}{N - 1}} \]
where \( d \) refers to the deviation of each score from mean and \( N \) the total number of observation.

ii) **Standard error**

\[
\text{SE} = \frac{SD}{\sqrt{N - 1}}
\]

iii) **Simple regression**

The regression equation was computed using the least square method. The basic formula followed was:

\[ Y = a + bX \]

where \( Y \) is the dependent variable, \( X \) the independent variable, \( a \) the intercept on \( Y \) and \( b \) the slope. The formulae used to derive the values of \( a \) and \( b \) are:

\[
\begin{align*}
    b &= \frac{\Sigma xy}{\Sigma x^2} \\
    a &= \bar{Y} - b \bar{X}
\end{align*}
\]

where \( \bar{Y} \) and \( \bar{X} \) denote the means of \( Y \) and \( X \), \( \Sigma xy \) and \( \Sigma x^2 \) are derived as follows:
\[ \Sigma xy = \Sigma xy - \frac{(\Sigma x)(\Sigma y)}{N} \]

\[ \Sigma x^2 = \Sigma x^2 - \frac{(\Sigma x)^2}{N} \]

The capital X and Y denote the raw scores and the small x and y deviation scores.

iv) **Simple correlation coefficient \( r \)**

The simple correlation coefficient \( r \) was determined from the formula

\[ r = \frac{\Sigma xy}{\sqrt{\Sigma x^2 \Sigma y^2}} \]

v) **Analysis of variance:**

One-way analysis of variance:

Difference between different means of a single variable was tested following one way classification of variance described by Zar (1974). Sum of X for all the values was squared and correction factor \( 'C' \) was obtained

\[ (\Sigma x)^2 \]

\[ C = \frac{(\Sigma x)^2}{N} \]
Total sum of squares \[ = \sum X^2 - C \]
where \( X^2 \) represents the sum of squared values.

\[ (\sum X)^2 \]

Group sum of squares \[ = \frac{\text{---} - C}{N} \]
where \( N \) = number of observations in each sample.

Error sum of squares \[ = \text{Total SS} - \text{Group SS} \]

Considering the degrees of freedom for each source of variance, mean square was calculated.

**Degrees of freedom (DF)**

- Total SS \[ = \text{Number of values in the Table-1} \]
- Group SS \[ = \text{Number of groups} - 1 \]
- Error SS \[ = \text{Df of total SS} - \text{Df of group SS} \]

**Two - way analysis of variance**

Partitioning of total variance into variance due to the different experimental conditions (e.g. prey densities and prey choice among mixed prey organisms) was carried out following the procedure described by Zar (1974). Various values obtained for different experimental conditions were tabulated in columns. For each column \( \sum X \) and \( \sum X^2 \) were calculated. Sum of \( X \) for all columns was squared and divided by the number of tabulated values and a correction factor was obtained.
Correction factor (C) = \frac{\sum (\sum X)^2 - C}{N}

Total SS = \sum \sum X^2 - C

\text{Between column SS} = \frac{\sum \sum (\sum X)^2}{\text{Number of values in a column}} - C

\text{Between row SS} = \frac{\sum \sum (\sum X)^2}{\text{Number of values in a row}} - C

\text{Remainder SS} = \text{Total SS} - \text{Between column SS} - \text{Between row SS}

Considering the degrees of freedom for each source of variance, mean square (MS) was calculated.

\textbf{Degrees of freedom (DF)}

Total SS = \text{Number of values in the Table} - 1
\text{Between column SS} = \text{Number of columns} - 1
\text{Between row SS} = \text{Number of rows} - 1
\text{Remainder SS} = \text{Total SS Df} - \left( \text{Between column Df} + \text{Between rows Df} \right)
F value for the variance between columns = \frac{\text{MS between columns}}{\text{Remainder MS}}

F value for the variance between rows = \frac{\text{MS between rows}}{\text{Remainder MS}}

Significant level at the corresponding DF was read from Table D.11 given by Zar (1974).