CHAPTER II
STUDY AREA AND METHODOLOGY

DETAILS OF STUDY AREA:

For the present study, three stations were selected, two along Zuari estuary and one along the exposed seashore at Arambol. The Zuari estuary is located at lat 15° 25′N and long. 73° 45′E - 73° 55′E and the station Arambol is located at lat. 15° 41′N and long. 73° 42′E along the west coast of India. Zuari estuary is one of the largest estuaries along the west coast of India. The Zuari estuary finds its origin from the Western Ghats and after a prolonged flow of 65 km it joins the Arabian Sea. On one side of it is the Dona Paula point and on the other side is Marmagao. The mouth of the estuary is 5.5 km wide with an average depth of 6 m. This is classified as a microtidal estuary with average tide level below 2 m (Ahmad, 1972).

The Zuari estuary receives heavy monsoon rain fall and also the discharge from the catchment areas in western ghat. The average rain fall over Goa varies between 2600 - 3000 mm, of which major part of the rain fall occurs during the south west monsoon period (June - September).
The average runoff of Zuari estuary is 9 km /annum (NIO tech. Report, 1979). Studies on this estuary revealed that, it is a mixed water column from surface to bottom except during the monsoon season when it becomes clearly stratified. Penetration of seawater during the pre- and postmonsoon season is observed upto about 65km upstream but during the monsoon it gets reduced to about 20 km (NIO, tech. Report No., 02/79).

Two stations in this estuary were selected for the present study. They were Marmagao harbour and Dona Paula jetty. The third station selected for this study was Arambol rocky shore. It was due to two main reasons:

1) It represents an open sea shore, where the seawater is not directly diluted by the freshwater runoff through river drainage, and so represents a different environment than the above two stations.

2) Availability of abundant barnacle settlement on the rocky shore. Two species of barnacles, *Balanus tintinnabulum* and *Chthamalus* sp. were available in large numbers through out the year.

Except during the monsoon season, the movement of the water in the Zuari estuary as well as in the Arambol
The seashore is due to the semidiurnal tides. During the study, the tide along Marmagao (Goa coast) varied between -0.27 m to 2.48 m.

The two stations in the Zuari estuary viz. Marmagao Harbour and Dona Paula point are separated by about 5 km wide water column, but both are located very close to the mouth of the estuary. The distance from the Marmagao Harbour to Arambol is about 30 km (Fig: 1).

MATERIALS AND METHODS

The collection of samples (both animals and organic matter) for the calorific study were made from the above three stations as described below.

Harbour:

To get the fouling barnacles, three to four months before the commencement of the study, aluminium panels 3 admeasuring 15 x 10 x 0.3 cm were suspended in the subsurface water (~1 m) from the jetty No.7 with the help of polyethylene ropes. Sufficiently large number of panels were suspended so as to get a continuous supply of the live organisms corresponding to various sizes.
collection of these settled barnacles, mainly Balanus amphitrite and Balanus amaryllis were made by removing the panels. The settled barnacles were removed from the panels with the help of a sharp chisel. Number of barnacles with the percentage of mortality (by counting the shells without soft body) and the dominant size group was noted down at the time of collection. To get the sizes of the barnacles, measurement of Rostro Carinal Apex (RCA), Rostro Carinal Base (RCB), Latero Lateral Apex, (LLA) Latero Lateral Base (LLB) and Height (H) were noted down with the help of a Vernier callipers. Depending on the availability, barnacles belonging to the species B. amphitrite were divided into four size groups based on RCB i.e. below 5 mm, 5 - 10 mm, 10 - 20 mm and above 20 mm., while B. amaryllis were divided into three size groups like 10 - 15 mm, 15 - 20 mm and 20 - 30 mm. The Chthamalus sp. collected from Dona Paula and Arambol were divided into two size groups (RCB below 5 mm and 5 - 15 mm) and B. tintinnabulum from Arambol into four size groups like below 20 mm, 20 - 30 mm, 30 - 40 mm and above 40 mm. Barnacles were dissected open after washing them in distilled water to remove the soft parts and unwanted materials (Wu & Levings, 1978). They were dried in an oven at 70 C for 24 hrs. After this, the tissues were powdered in a mortar and stored in a desiccator till the final
analysis. Subsamples of these were taken for the measurement of water content (Wu & Levings, 1978). From each size group 10 to 20 organisms were taken for the observation and pooled together for the subsequent analysis.

The second division of the barnacles were made based on the development of ovary like mature and immature. This division of the barnacles were made according to the specifications given by Karande (1967). The specimens with immature ovary and without ovarian tissue were classified as immature and those bearing mature eggs and first stage of naupliii were classified as mature ones.

Measurement of calorific content of immature egg mass, mature eggs and first stage of naupliii of *B. amphitrite* were carried out as follows: The soft parts of barnacles after washing in distilled water were observed under a stereo microscope. The ovaries were removed and the eggs (both mature and immature) and naupliii were transferred with the help of a spatula and dropper into clean measuring cylinders. They were weighed before they were taken for the calorific content measurements. The methodology of calorific estimation is explained in detail in the latter part of this chapter.
Zooplankton samples from the subsurface water were collected from Harbour station with the help of a Heron Tranter net (Mesh size 300 μ). The samples were dried in an oven at about 70 °C for 24 hrs., before powdering in a mortar and the subsequent analysis. For measuring the calorific content of suspended matter in the seawater, 3 to 4 liters of seawater was filtered through pre-weighed millipore filters (4.7mm dia., 0.45 μ pore size). The filter papers along with the trapped matter were dried in an oven at 70 °C for 24 hrs. The dry weight of suspended matter was found out by weighing the above filters using a microbalance (Mettler, M - 3). Appropriate blanks were run along with the samples. The determination of calorific content of these samples was made after adding a known amount of standard benzoic acid.

Dona Paula:

Monthly collections of barnacles, B. amphitrite and Chthamalus sp. were made from the barnacle population on the concrete piles of the jetty. The barnacles were scraped out (approximately 300 Nos.) from the jetty piles with the help of a chisel and hammer. Extreme care was taken not to disturb the specimen during the collection. The groupings according to the size and maturity were
made. The soft parts were removed, dried and stored in a desiccator after powdering in a mortar as explained above. Due to the heavy churning up of sediment and less abundance of zooplankton, zooplankton sample collection could not be carried out at this station. Suspended matter samples for the measurement of calorific content were collected at the Harbour station.

Arambol:

The rocky shore of Arambol station inhabited a wide and well established barnacle population belonging to the species *B. tintinnabulum* and *Chthamalus* sp. Monthly collections by scraping them with the help of a chisel and hammer were made from a fixed area where the population number was monitored by counting the number of individuals in an area of 1 ft$^2$. Mortality was assessed in a similar way by counting the number of dead individuals and expressed as percentage of population. In the case of *B. tintinnabulum*, depending on their availability, they were divided into four size groups as explained earlier and were dissected open to separate the soft parts into body tissue, intestine, cirri and the whole body. These tissues were also dried and powdered and stored in desiccator for the subsequent analyses. Number
ranging from 5 - 10 were observed for each size group and pooled together for the sample preparation. The estimation of suspended matter for the calorific content measurement was also collected from this station. Zooplankton samples could not be collected from this station due to the difficulties in the operation of nets at the study area. Collections for June and July, 1986 and June, 1987 could not be made due to the bad weather conditions and heavy wave actions at the time of collection caused by the onset of monsoon.

**Calorimetry:**

Heat of combustion of a substance is measured in a bomb calorimeter. During the present study the Adiabatic Calorimeter (Model No.1241) was used for this purpose. This bomb calorimeter, employed for the measurement of heat of combustion required ≤ 1g of homogeneously powdered and dried biological samples. A weighed quantity of the sample (approximately 1 g) was then compacted into a small pellet by means of a pellet press. The pellet was again weighed and placed on a firing device (a capsule), held by the platinum wire through which an electric discharge was passed to ignite the pellet. The bomb was then filled with oxygen under pressure to the order of 25
to 30 atm. This oxygen filled bomb was then placed inside a steel bucket with water. The temperature of this water was made same as the temperature outside the bucket (the water in the jacket outside the bucket). After putting it on for 2 to 3 minutes so as to get it stabilized, the initial temperature was noted down with the help of a fine thermometer. The sample was ignited by passing electric current. The heat generated during the combustion transmitted to the water outside the bomb (bucket water). The change in temperature of the water in the bucket was noted down after stabilization (final temperature). From the difference in temperature and the fuse wire consumed (platinum), the calorific value was calculated using the formula

\[ \frac{t \cdot W - e_1 - e_2 - e_3}{m} = H_g \]

where

- \( H_g \) = gross heat of combustion or calorific content in cal.
- \( t \) = difference in temperature
- \( W \) = energy equivalent of calorimeter ie., calories required to rise the temperature by 1 C
el = correction in calories for heat of formation of nitric acid

e2 = correction in calories for heat of formation of sulphuric acid

e3 = correction in calories for heat of combustion of fuse wire.

The instrument was periodically standardised by using standard thermochemical quality benzoic acid pellets. Correction for the acid production was neglected in the present study, since the correction according to Paine (1964) was negligible and amounts less than 1% of the total calorific content.

The standard benzoic acid was added to those samples which were lesser in quantity at the time of combustion in the calorimeter. As recommended by Crisp (1984 a) millipore filters were used to measure the calorific content of larvae and eggs of the barnacles being lesser in quantity and smaller in size.

Ash content of the samples were estimated by ashing the pre-weighed dried samples in a muffle furnace by charring at 450 °C for 4 hrs. All calorific values were
corrected for ash contents and expressed as cal/g. dry wt. (ash free).

**Biochemical analysis:**

The important biochemical constituents such as protein, lipids, carbohydrates and total organic carbon were estimated for all the animal samples. The methods used for this purpose are described below: the specimens were dried in an oven at 70°C for 24 hrs. and powdered before taken for the analysis. Protein was estimated following the method suggested by Herbert et al. (1971). 0.5g of the dried sample was taken in a dried test tube and homogenized in 0.5 ml of 1N NaOH and 2.5 ml of distilled water. This was heated at 80°C in a water bath for 30 min. to extract the protein. After cooling, 0.5 ml of 1N HCl was added to neutralize it and subsamples were taken from this in other test tubes. 2.5 ml of mixed reagent (carbonate - tartarate - copper solution) was added followed by 0.5 ml of 1N Folins-Ciocalteu reagent. These solutions were mixed well, kept for 30 minutes and centrifuged before measuring the absorbance at 750 nm in a spectrophotometer. Appropriate blanks and standards (bovine serum albumin) was similarly treated to prepare standard curves. All concentrations were expressed in
Carbohydrates were estimated using phenol sulphuric acid method as described by Dubois et al (1956) and modified by Hitchcock (1977). To 1 mg of dried sample, 2 ml of 80% sulphuric acid was added and digested for 20 hrs at room temperature. To this 1 ml of phenol (5%) and 5 ml of concentrated sulphuric acid were added, the mixture was centrifuged before measuring the absorbance at 490 nm. Standard curve was drawn with the help of alar grade D-glucose. Finally concentrations were expressed in μg/mg.d.wt. of dried tissue.

Lipids were estimated following the method given by Parsons et al (1984). From the dried and powdered sample, 5 to 10 mg. was weighed and placed in a glass homogeniser with 8 ml of chloroform-methanol mixture (1:2:0.8 V/v of chloroform, methanol and distilled water respectively). This mixture after filtering through an ignited (450°C/4 hrs.) GF/C filter paper, was transferred to a separating funnel. Extraction of lipid was made by the addition of 2 ml of distilled chloroform and 2 ml of distilled water by continuous shaking for 10 minutes. The chloroform layer was separated and evaporated to dryness in vacuum. 2 ml of 0.15% potassium dichromate solution was added and the
tubes were kept in boiling water bath for 15 minutes. 4.5 ml of distilled water was added to this, after cooling the absorbance was measured at 440 nm. Blanks and standards (stearic acid) were treated similarly and standard graph was plotted. All concentrations were expressed as μg/mg.d.wt. of dried tissue.

Estimation of total organic carbon in the animal tissue was made by wet oxidation method as suggested by Parsons et al (1984). To a known quantity of the dried and powdered sample, 1 ml of phosphoric acid and distilled water was added in order to prevent the chloride interference and kept in a boiling water bath for 30 minutes. Sulphuric acid dichromate reagent was added (10 ml) to these tubes and were digested in a boiling water bath for a period of 1 hr. This was diluted with distilled water to 50 ml. Subsamples (approximately 10 ml) were centrifuged (4000 rpm) for 20 minutes and absorbance was measured on a spectrophotometer at 440 nm. Blanks and standards (D-glucose) were treated similarly and the standard curve was plotted. Concentrations were expressed as μg C/mg.d.wt. of animal tissues. All the above measurements of absorbance were made using a spectrophotometer.
Collection and Analysis of Hydrographic Data:

Hydrographic parameters such as temperature, salinity, dissolved oxygen, PH, total suspended matter, particulate organic carbon, chlorophyll a, phaeopigments of the surface waters at 3 stations were monitored at monthly intervals. Seawater samples for this purpose were collected from the surface water with the help of clean plastic bucket.

Temperature of the water samples was monitored with the help of a mercury thermometer immediately after the collection. These values were expressed as °C.

Salinity of the water samples were estimated following the method given by Strickland and Parsons (1968). This involved the determination of chlorinity by the Mohr Knudsen titration method with standard silver nitrate solution with potassium chromate as an indicator. Salinity was calculated and expressed as parts per thousand (‰).

Dissolved oxygen concentration of the water sample was monitored using Winkler method. This method involved the fixation of dissolved oxygen using Winkler A and B
followed by the titration against standard sodium thiosulphate solution using starch as indicator. The concentration was expressed in ml/lit of the sea water. The pH measurements were made with the help of a pH meter (Philips, PP 9045, digital pH meter).

The total suspended matter was determined by filtering 1 - 4 lts. of seawater through preweighed millipore membrane filter paper. The residue was dried in an oven at 70°C and weighed in a balance (Mettler M-3). The concentrations were expressed as mg/lit (dry weight) of seawater. Particulate organic carbon was estimated following the wet oxidation method using sulphuric acid - dichromate reagent as described by Parsons et al (1984).

Chlorophyll a estimation was done spectrophotometrically. After filtration of the seawater sample (500 ml to 1 lit.) through GF/C filter papers, they were extracted with 90% acetone in dark bottles below 5°C (Yentsch and Menzel, 1963). The values were expressed in mg/lit. of seawater. Phaeopigments were estimated by the acidification of the above sample (acetone extract) with two drops of 50% HCl and measuring the absorbance spectrophotometrically as suggested by Parsons et al (1984).
Fig. 1 Location of three study areas (*)