MATERIALS AND METHODS

The freshwater bivalve molluscs *Indonaia caeruleus* with specific size (small, medium and large), 46-51 mm, shell length, 55-56 mm shell length and 64-65 mm shell length during summer season; 49-52 mm shell length, 54-56 mm shell length and 66-69 mm shell length during pre-monsoon, 43-44 mm shell length, 52-54 mm, shell length and 68-69 mm shell length during post monsoon and 43-46 mm shell length, 52-54 mm shell length and 68-69 mm during winter season were collected from fixed site on bank of Godavari river at Paithan, 50 km away from city Aurangabad.

The animals were collected on full moon days of each season viz., summer (April-May), pre-monsoon (June-July), post-monsoon (September-October) and Winter (December-January), over a period of October 2002 to September 2004. Immediately after bringing to the laboratory, the shells of the bivalves were brushed and washed with freshwater in order to remove the algal biomass, mud and other waste materials. The cleaned animals were then allowed for defecation or depuration for 12 hr. in laboratory conditions under constant aeration. During this period the animals opened the shell valves and protruded the mantle edges and siphons outside the shells
to remove waste material from the viscera, thus cleaning the gills and mantle cavity. One of the primary endogenous factors affecting the oxygen uptake in the bivalves is shell valve movement and the factors which affect the degree to which the shell valves are remain closed will affect the rate of oxygen consumption (Shumway, 1982). Hence in the present study the animals with specific size were maintained in constant aeration and those which opened the valves and extended the organs to the maximum (as suggested by Galtsoff, 1964) were selected for studying the rate of oxygen consumption. The animals under constant valves opening could regulate the ventilation during this period, maintained normal respiratory rate.

The physico-chemical characteristics of water in different seasons were determined periodically. The temperature and pH of the water were recorded twice in a day, whereas hardness (in terms of carbonates) of the water and dissolved oxygen contents were determined time to time during different seasons. The rate of oxygen consumption was determined by alkaline-iodide-azide method of Winkler's modified technique (Golterman et al., 1959).

The rate of oxygen consumption of individual animal was determined in a specially prepared brown coloured respiratory jars of 500 ml capacity. The jars were fitted with rubber cork having an inlet
and outlet of glasstubes connected with rubbertubes and clips. Individual animal was placed in each jar and constant flow of water was given through the inlet to flow through the outlet for 2.0 to 3.0 minutes without disturbing the animal and slowly the flow of the water was shut down. After one hour, water from the respiratory jar was carefully siphoned out in a stoppered reagent bottle of 300 ml capacity and oxygen content was determined. The flesh of the individual animal was then taken out carefully from the shell and blotted on the filter paper to remove excess water. This flesh was then weighed to obtain the wet-weight of the individual. The oxygen consumed by each animal was then calculated and expressed as oxygen mgO₂ l/h wet-weight of the flesh. The rate of oxygen consumption of each group was measured between 3.00 to 4.00 pm in a day time twice in each season. The wet flesh weight of four individual animals were noted on each fortnight to get values and the size of specific data was collected over the period between October 2002 to September 2004 during all seasons.

The adult bivalves were placed individually in respiratory jars with 500 ml water. The rate of ammonia excretion was determined after 1 hr. by drawing water sample from each jar. The rate of ammonia excretion was measured according to phenol-hypochlorite
method of Solarzano (1969). Every 4 individual animals of each group were used and mean of triplicate water samples were estimated for each group. The statistical analysis was done, to express final data. The atomic equivalent values of oxygen and nitrogen were calculated on the basis of values of oxygen consumption and ammonia excretion obtained for the same individual. Finally the O:N ratio was established.

The mantle, hepatopancreas, gonad, foot, gills, anterior adductor muscles and posterior adductor muscles were dissected. – 100 mg of each tissue is taken for biochemical analysis. Protein was determined by the method of Lowery et al. (1951) using Bouvine Serum Albumin as standard. The glycogen content was estimated according to method proposed by De-Zwaan and Zandee (1972) using glucose as standard. The method used for determination of lipids was sulphophospho-vanilline method proposed by Barnes and Blackstock (1973) and Ascorbic acid was determined by Roe et al. (1967). The results are expressed as mg content per 100 mg wet tissue. Triplicate values of each biochemical constituents were subjected for statistical confirmation using student 't' test (Dowdeswell , 1957) percentage difference were also calculated in every season.
During the collection of animals from the site on every full moon days of different seasons, additional 15 (fifteen) individual animals of the similar size range from respective group were also collected for the histological study of hepatopancreas and gonad. Immediately after each collection, the animals were soaked carefully with the help filter paper and flesh of the animals was fixed in Bouin's Hollande fixative for 24 hrs. The fixative was renewed for next 24 hr to facilitate better fixation of the tissues. During each full moon period, hepatopancreas and gonad tissue were then removed and processed for preparation of Paraffin blocks. Dehydration of hepatopancreas and gonadal tissue was done through serial grades of ethyl alcohol and tertiary butanol while xylene was replaced by toluene during the process. The tissues were embedded in paraaffin-wax at 58° C and the sections of hepatopancreas and gonad were cut out at 6 to 7 µm thickness using spence-rotary-microtome. The hepatopancreas and gonad were stained with Mallary's Triple Stain. All the sections were observed under the research binocular microscope and wherever necessary, measurements were made before microphotography. Since the samples were collected during 3.00 to 4.00 pm of every full moon day over a period of two years cycle, the quantitative observations of digestive tubules of hepatopancreas and
gonadal follicles though to reveal homogenous cycling pattern to
correlated impact of ecological factors occurring in different seasons.

The experiments on determination of oxygen consumption,
ammonia excretion, biochemical composition of the whole body were
carried out on full moon days of summer (April-May), pre-monsoon
(July-July), post-monsoon (September-October) and winter
(December-January) seasons in the period of October 2002 to
September-2004. In each experiments using freshly collected animals
of 49-51 mm, 55-56 mm, and 64-65 mm shell length during summer;
49-52 mm, 54-56 mm, 66-69 mm shell length during pre-monsoon;
43-44 mm, 52-54 mm, 68-69 mm shell length during post-monsoon
and 43-46 mm, 52-54 mm, 68-69 mm shell length during winter
season.