CHAPTER 2
2.1 Introduction

Behavioural modification is one of the most sensitive indication of environmental stress and may directly affect survival. Many bivalves when exposed to lethal environmental stress, rely on behavioural mechanisms which enable them to avoid such condition. Mobile species move away from unfavourable condition where as for sedentary species survival depends upon behavioural mechanisms such as burrowing into the substratum, retracting into the existing burrows or closing of the valves.

Bivalve molluscs are able to isolate and protect themselves from a variety of adverse environmental conditions by closing the valves (Coleman and Trueman, 1971; Gilles, 1972; Bayne, 1973b; Nagabhushanam and Bidarkar, 1975; Akberali et al., 1977; 1981; Akberali, 1978; Davenport, 1979; Akberali and Davenport, 1981; 1982). Valve closure of bivalves prevents drastic changes in osmotic concentration of their body fluids when exposed to short term fluctuations in salinities (Shumway, 1977). Valve closing mechanism allows the animal a period of grace and thus prevents osmotic shock. Although altering the shell movement/closing may help the organism to withstand transient adverse changes in the environment it can not contribute to its long term survival in situations where the change in the environment may be of a permanent nature. This is because
during any period of valve closure the animal incurs penalties related to feeding, reproduction or exchange of gases and metabolites.

Davenport (1979) showed that the closure of the exhalent siphon, thus preventing pumping was the crucial event which largely isolates the mantle cavity of mussel from falling external salinities, the shell valve closure occurred at rather lower salinities to produce virtually a complete isolation. The same was also observed for *Katelysia opima* (Mane, 1974); *Scrobicularia plana* (Akberali and Davenport, 1981) and *Donax denticulatus* (Genovea et al., 1988).

The development of electronic and other analytical techniques has led to significant advances in our knowledge of bivalve behaviour. Galtsoff (1964) studied the valve movements of the American oyster *Crassostrea virginica* using slow motion kymograph which is very time consuming. Under ordinary circumstances for most of the molluscan species the opening and closing movements of shell are so small. For long term observations the speed of the kymograph drum should be adjusted, and very slow movement is not possible with the kymograph. Various authors used impedance technique to record heart beat and valve activity of bivalve molluscs and other marine invertebrates in relation to a variety of environmental changes such as tidal exposure (Coleman, 1974; Earll, 1975), feeding (Thompson and Bayne, 1972; Widdows, 1973a), temperature (Stone, 1980; Davenport and Carrion-Cotrina, 1981), oxygen (Brand and
Roberts, 1973), salinity (Davenport, 1977; Akberali, 1978; Akberali and Davenport, 1981) and pollution (Davenport, 1982; Manley, 1983). Davenport (1979) has used stress gauge for monitoring valve movement of *Mytilus edulis* exposed to falling sea water concentrations. It is not clear whether the stress gauge senses the valve movement without making any stress to the natural behaviour of the animal. For the present study an 'Oyster Activity Monitor' was constructed in collaboration with Central Institute of Fisheries Technology, Kochi for studying shell movements of *S. scripta*. The instrument will not cause any stress to the natural behaviour of the animal while recording the valve movement.

The major advantage of the system is the antenna of the sensor which makes only a feather contact with the shell. The force applied on the shell is less than 30 mg. The antenna can move through a distance of about 8 mm which is enough to record valve opening. The paper recordings have shown the movements in the order of 0.05 mm can be detected easily. The method also allows continuous long term measurements as the paper charts are normally 50 m in length. The electronic circuits and controls are able to work at 9 V DC supply obtainable either from a battery or a battery eliminator.

The main aim of this study is to relate behavioural adaptations of *S. scripta* to changes in external salinity by using 'Oyster Activity Monitor'.
2.2 Materials and methods

2.2.1 Acclimation of test animals

*Suinetta scripta* were collected from Fort Cochin area (between latitude 9°28' and 10°00' N and longitude 76°13' and 76°11' E) and brought to the laboratory in plastic bags containing sea water collected from the same site, cleaned and grouped into three size groups; small (1.5 ± 0.5 cm), medium (2.5 ± 0.5 cm) and large (3.5 ± 0.5 cm). They were kept in large plastic basins which are used as acclimation tanks containing sand for three days at 30 ppt sea water. The acclimation period of three days was fixed by estimating oxygen consumption of the animals which was found to be in a steady state. During acclimation they were fed on 3-4 days old blue green algae *Synechocystis salina* cultured in a metal free medium. The water in the acclimation tank was purified using biological filter (Spotte, 1970) (Fig. 1) and changed once in two days. After three days of acclimation 5 animals of each size group were taken for valve movement study. Then the salinity of the water was altered slowly by the addition of water of higher salinity obtained by the evaporation of sea water and deionised water so that salinity reached 40 ppt and 20 ppt respectively within three days. At the end of third day, the animals were used for valve movement study. The water of 20 ppt was further diluted with deionised water to obtain 10 ppt and 5 ppt, taking again three days for dilution at each step.
Fig. 1 Acclimation system with biological filter used for acclimating clams in different salinities
The animals thus acclimated were used for the experiment. During these periods they were fed on *S. salina* and gave aeration by biological filter.

Another set of animals of the above three size groups were acclimated at 5 ppt for three days. During acclimation, they were fed on *S. salina* and water was purified using biological filter. After three days, the records were taken continuously for six hours and after that the salinity of the medium was increased by the addition of sea water of higher salinity obtained by the evaporation of sea water, with an interval of 15 minutes. After each addition, salinity of the water was measured (Table 1) and noted the salinity at which the animal showed valve movement, siphon with drawl etc. The Table 1 shows the average salinity of four experiments. The salinity and temperature (28 ± 2°C) of the water samples were determined by using Salinity Temperature Probe developed by Central Institute of Fisheries Technology, Kochi.

### 2.2.2 Oyster Activity Monitor

The instrumental system consists of three essential parts as given in the schematic diagram (Fig. 2) namely sensor, electronic unit and paper chart recorder. The sensor consists of a light antenna made of 0.5 mm dia stainless steel wire and is capable of making both ends to move with the fulcrum at the middle. The lower end of the antenna rests on the upper shell
Table 1. Salinity variations during stepwise increase in salinity by addition of sea water (each step is the mean of four experiments)

<table>
<thead>
<tr>
<th>Salinity increase</th>
<th>Size Groups</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Small (1.5 cm)</td>
</tr>
<tr>
<td>Step 1</td>
<td>7.90 ± 0.4320</td>
</tr>
<tr>
<td>Step 2</td>
<td>9.73 ± 0.4272</td>
</tr>
<tr>
<td>Step 3</td>
<td>11.85 ± 0.6000</td>
</tr>
<tr>
<td>Step 4</td>
<td>13.30 ± 0.4163</td>
</tr>
<tr>
<td>Step 5</td>
<td>15.58 ± 0.5000</td>
</tr>
<tr>
<td>Step 6</td>
<td>15.73 ± 0.4924</td>
</tr>
</tbody>
</table>
Fig. 2 Schematic diagram of Oyster Activity Monitor

- Recorder gives qualitative features of the response.
- Recorder output.
- Meter indicates the shell movement.
- 6-Digit counter records total number of shell openings.
- 6-Digit counter records total duration of experiment.
- Movement sensor
- Sensor makes only a feather touch on the animal.
- Animal under test
of the animal while the upper end moves between an electro-optic sensing system producing electrical signals proportional to the obstruction caused on the optical path due to the movement of the shell. The electrical signals produced in proportional to the movements of the shell are transmitted to the electronic unit. The electronic unit converts the signals obtained from the sensor into DC voltage and fed to the paper chart recorder for recording.

2.2.3 Experimental setup

*S. scripta* acclimated at different salinities were placed on a tripod so that the animal is permanently fixed and kept in basin having enough water of desired salinity. The antenna is kept in contact with the upper shell of the animal from behind so that it moves freely upwards without making any stress. The position of the sensor is adjusted precisely such that the signal is properly set which is indicated in the meter of the instrument. Since the antenna is immersed in water, the variations in water level do not affect the recordings. The sensor is connected to the meter and the recorder pin is connected to the recorder port. Now the system is ready for observation.
2.3 Results

*S. scripta* belonging to three size groups acclimated at different salinities showed the following valve movements.

From the Fig. 3 it can be seen that at four different salinities 40, 30, 20 and 10 ppt, the smaller size group (1.5 cm) animals kept the valves in opened condition throughout the experiment. The animals acclimated at 40 ppt salinity showed regular valve movements with gradual opening and sudden partial closing. The *S. scripta* acclimated at 30 ppt and 20 ppt salinity sea water showed steady valve movements. The initial openings were found to be without much time lag followed by regular and rhythmic movement of valves at much shorter intervals and partial closure of the valves at more or less constant interval. At 10 ppt the magnitude of gradual openings and sudden partial closings was much less. At 5 ppt salinity they kept the valves closed with intermittent attempt to open.

The medium size group (2.5 cm) animals (Fig. 4.) acclimated at different salinities showed different types of valve movement. At 40, 30 and 20 ppt salinity the animals kept the valves in open condition throughout the period of experiment. A constant gaping and rhythmic partial closings of valves were observed only at 30 ppt. When comparing the valve movements at 40 ppt and 30 ppt, the partial closures at 40 ppt were found to be at wide intervals. When the animals were in 20 ppt, the partial openings and closings were much more frequent than in 40
Fig. 3 Shell valve movement of small size group (1.5 cm) acclimated in different salinities
a 40, b 30, c 20, d 10 and e 5 ppt
Fig. 4 Shell valve movement of medium size group (2.5 cm) acclimated in different salinities
a 40, b 30, c 20, d 10 and e 5 ppt
ppt though the magnitude is less than in 30 ppt. The intervals between partial closings were found to be greater than 30 ppt but less than 40 ppt. At 10 ppt salinity sea water during the initial stages almost regular openings and closings occurred but later the animal kept a prolonged closed condition with intermittent openings. At 5 ppt salinity sea water the animal remained with the valves closed.

When the larger size group (3.5 cm) animals (Fig. 5) were acclimated at different salinities the behaviours were found to be different from that of small and medium size groups animals. The animals kept the valves in open condition throughout the experiments at 40, 30 and 20 ppt salinity sea water. A constant gaping and partial closure of valves were observed at 30 ppt salinity sea water. In 40 ppt salinity a prolonged open condition was associated with partial closure at wide interval. In 20 ppt also the partial closure was at wide interval and also the width of the gap was much less than in 40 ppt. In both of the salinities (40 ppt and 20 ppt) the animals kept the exhalent siphon inside the valves. The animals kept in 10 ppt salinity sea water showed closed condition with regular attempt to open. At 5 ppt salinity a complete closure of valves occur.

When considering the three size groups together a regular and rhythmic movements of valves were observed at 20 ppt and 30 ppt salinities for smaller size group and 30 ppt salinity for medium and larger size groups animals. When the ambient medium alters from 30 ppt salinity the animals showed behavioural
Fig. 5 Shell valve movement of large size group (3.5 cm) acclimated in different salinities
a 40, b 30, c 20, d 10 and e 5 ppt
change by altering the pattern of valve movement and in extreme salinities by closing.

When the above three size groups animals acclimated at 5 ppt were subjected to gradual increase in salinity the smaller size group was found to open their valves at lower salinities compared to medium and larger size groups (Fig. 6). Salinity required to induce the valve opening in small, medium and larger size groups (average of four experiments) were 15.58, 17.48 and 20.33 ppt respectively (Table 1). The siphons came out at 15.73 ppt for smaller, 21.08 ppt for medium and 22.15 ppt for larger size groups. When observing the valve movement of the three size groups, the intervals between the closings were found to be decreasing as the size of the animal increases (Fig. 6). Valve closure was complete or nearly complete only in the case of large size group. But for small and medium size groups it was only partial (Fig.6).

2.4 Discussion

Since *S. scripta* tolerate a wide range of salinity, the animal must have adaptive capabilities at the behavioural levels. In extreme dilution of the ambient medium the animal closes the valves and isolated its tissues from the ambient conditions. Valve movements were found to be reduced in unfavourable salinities.
Fig. 6 Shell valve movement of three different size groups subjected to rising salinities from 5 ppt. a small b medium and c large 1,2,3,4,5,6 etc. steps of additions of increasing sea water salinity
Observations of Fig. 3.4 and 5 showed the valve movements varying in different salinities and complete closures were recorded only in 5 ppt which was reported as the most unfavourable salinity of the animal (Thampuran et al., 1982). In favourable salinities (20 and 30 ppt) for smaller size group (1.5 cm) animals the valve movements were rhythmic partial closures and openings after the initial gaps. Same valve movement was noticed at 30 ppt for medium sized (2.5 cm) animals. With slight variation in time component similar valve movement was noticed for larger sized (3.5 cm) animals in 30 ppt. This indicates that there is a rhythmic partial closure of valves in optimum salinity after the initial gap. The rhythmic partial closures and openings of the valves may be due to the muscular activity associated with water pumping and was exhibited as a regular process only in favourable salinities. In extreme of tolerance range or in resistance range the valve movement at greater intervals indicates reduced pumping activity. In extreme salinities (eg. 10 ppt) the pumping activity totally ceases for larger animals and much reduced for medium and smaller size groups. Previous studies (Thampuran et al., 1982) had showed greater tolerance of salinity variation by smaller animals and least tolerance by larger animals. The fact that even at 10 ppt salinity, the smaller animals are not totally cutting them off from the environment may be an important fact which enhances their survival in lower salinities.
The isolation of mantle cavity is not only produced by valve closure but also by closure of the exhalent siphon. In unfavourable salinities *S. scripta* showed closure of exhalent siphon which effectively ceases the irrigation of the mantle cavity. Same type of behaviour was also reported by Davenport (1979) and Akberali and Davenport (1981). In extreme conditions the intermittent testing of the external medium occurs and is found to be advantageous since it allows the *S. scripta* to monitor the situation. This type of testing behaviour was also reported for *Mytilus edulis* (Coleman and Trueman, 1971), *Modiolus* (Pierce, 1971) and *Donax denticulatus* (Trueman, 1983). These observations were cleared when the animals acclimated at very low salinity (5 ppt) were subjected to gradual increase in salinity (Table 1). Fig. 6 indicate that when the animals were subjected to very low salinity (5 ppt) they totally cut off from the ambient medium, as also observed in Fig. 3, 4 and 5. But once a near favourable or favourable salinity was obtained after a prolonged unfavourable condition they do active pumping most probably to repay the oxygen debt they had already built. Here also smaller animals resumed their ventilatory activity at lower salinities than the medium, and the medium sized one earlier than the large (Fig. 6). All the above observations clearly indicate that the behavioural component is playing an important role in the survival of *S. scripta* in varying salinities.