Chapter 1

Osmotic behaviour
Osmotic behavior

An estuary is "a semi-enclosed coastal body of water which has a full connection with the open sea and within which sea water is measurably diluted with fresh water derived from land drainage" (Pritchard, 1967). Many estuarine animals show a remarkable power of adaptation though they are exposed to dynamic environmental changes due to tidal oscillations, influx of river water, water pollution and many other factors.

Most marine animals are unable to maintain their normal activities in sea water salinities below 30% and are subject to a relatively constant environment with respect to salinity, temperature, pH etc. Blum (1922) observed the effect of low salinity on *Teredo navalis*. Atwood and Johnson (1924) obtained identical results with *Ranilia gouldi*. The adult California mussel according to Fox (1941) was unable to adjust itself to a considerable range of salinities. Jgabhushanam (1955) and Kotwani (1955) studied the adaptations of *Martesia striata* and *Lytilus edulis* to salinity fluctuations respectively.

Coming to the estuarine environment, much of the work has been done on the edible oysters by Amemiya (1928)
in Japanese oyster, Hopkins (1936) in Ostrea gigas, Nelson (1938) in Crassostrea sp. and Ostrea circumpicta, Loosanoff (1948, 1950), in Crassostrea virginica. Loosanoff (1952) showed that as long as the valve of Crassostrea virginica remained open the changes in salinity of their body fluids follow changes in salinity of outside water. Fingerman and Fairbanks (1956) studied the osmotic behaviour and bleeding in the American oyster, Crassostrea virginica. Chanley (1958) had studied survival of juvenile bivalves in waters of low salinities.

Recently the survival and behavioural study was made by Walne (1961) in Ostrea edulis, Brafield and Newell (1961) in Macoma balthica from J.K., Webster and Shaw (1968) in Crassostrea virginica and Tegelberg and Lagoon (1967) in Pacific razor clam. Boyden (1972) studied the behaviour and survival and Boyden and Russel '1972) studied the habitat and distribution of two species of the brackish water cockle Cerastoderma edule and C. glaucum.

The present study was undertaken with a two-fold purpose. The first aim was to study the salinity tolerance i.e. survival and behaviour of Paphia laterisulca
to low and high salinities and secondly to obtain a quantitative information concerning the weight changes and fluid losses that occur during the summer season (March, April and May). Investigations were conducted on the following lines.

(A) Survival and behaviour in relation to salinity

1. Determination of lethal salinity in the laboratory during the monsoon and summer.

2. Time taken to open the shell valves in relation to salinity gradient.

3. The exchange between the mantle water and surrounding medium in relation to environment.
   a. Exchange with shell valves naturally closed.
   b. Exchange with shell valves artificially closed.
   c. Exchange with the part of shell valve removed.
   d. Influence of desiccation on the mantle water salinity.

4. The nature of the stimulus which open the shell valves of clams.

(B) Osmoregulation by weight changes

1. Weight losses induced by draining the free fluid between the shell valves.
a. Due to evaporation of water from shell valves.
b. Due to forcibly kept apart shell valves in order to discard the accumulated free fluid prior to each weighing.
c. Kept a gape in air by means of a wedge.

2. Weight losses induced by draining the free fluid between the shell valves and subsequently return to the aquaria.

3. Weight changes of wedged open clams in several concentrations of sea water.

4. Fluid and weight losses due to the injury and non-injury to the mantle and underlying parts of the body.

Habitat and salinity range in Kalbadevi estuary.

The river Kalbadevi, Ratnagiri, India is a narrow stream communicating with the west coast by a single opening forming Kalbadevi estuary (Fig. 1) and the harbour of Mirya is situated at the mouth of the river. The clam bed is approximately 6 k.m. from the bay side laboratory at Ratnagiri.

The salinity of the Kalbadevi estuary varied from 4.1% to 36.0% during the year. In the upper estuary,
Fig. 1   Map showing the Kalbadevi estuary.
MAP SHOWING KALBADEVI ESTUARY
rapid dilutions as a result of rain and run off at river water are common, but concentration is generally a relatively slow process related to evaporation. An exception might be a rapid salinity increase to some extent due to storm tides. The lowest salinity was observed in July and August on account of rain and flooding. Fig. 2 shows that the variation of salinity and temperature over the clam bed within 12 hours. However, no large scale mortality of clams was observed.

_Paphia laterisulca_ lives buried into the sand and were collected by wet digging or by hauling the catch in a clam rake net with feet. Besides _Paphia laterisulca_, a number of other bivalves are also found in the clam beds, the commonest among them being _Katelysia opima_, _Meretrix meretrix_, _Cardium sp._, _Crassostrea gryphoides_, _Crassostrea cucullata_ (Ratnagiri rock oyster) and _Meretrix casta_. It seems that these clams could tolerate wide fluctuations in the salinity. Some of these clams were brought from the beds when the salinity was high (34.0%); on transfer to distilled water, were found to close their valves immediately and remained in this condition even for two days or more but when retransferred to the normal sea water, they opened their
Fig. 2 Fluctuations in the salinity and temperature over the clam bed within 12 hours on eleventh August, 1972.

A Salinity.

B Temperature.
shell valves within a short time. The closure of the shell valves becomes an adaptation to withstand unfavourable conditions in the environment, thereby keeping the mantle fluid unaffected by external changes. This adaptation is only a temporary measure so long as the animals are able to live without opening the valves for both respiration and feeding. It was observed by Deshmukh (1972) and Mane (1974) that the salinity has a great influence on the growth and breeding of *Meretrix meretrix* and *Katelysia opima* respectively.

**Material and Methods**

*Paphia laterisulca* ranging from 30 mm. to 45 mm. were collected during the low tides. The clams were immediately brought to the bay side laboratory, washed with sea water and were kept into large troughs containing fresh unfiltered sea water which was changed twice a day. No extra food and aeration were given to specimens during the acclimation period (24 hours) and the experiments. Loss by evaporation through the surface of stored sea water was adjusted by adding distilled water.

All experiments were conducted from March 1972 to May 1972 except few experiments which were conducted in
the monsoon (August 1972). Only active clams with fully extended siphons and feet were used in experiments.

Distilled water was used for dilutions of normal sea water and the whole sea salt was added to sea water for obtaining higher concentrations in all experiments described. During the experimental period daily records of temperature and salinity were maintained. The salinity was determined by simple titration of sea water against silver nitrate (AgNO₃) using potassium chromate as an indicator.

Results

A. Survival and behaviour in relation to salinity.

1. Determination of lethal salinity in the laboratory during the monsoon and summer.

Mortality of Paphia laterisulca was tested in monsoon and summer at various salinities ranging from tap water to 31.8 %. The requisite salinities were changed twice a day. Aeration was given to clams for an hour during every 24 hours. The clam was considered dead only when it did not respond to mechanical touch by a glass rod.
and did not show any activity when returned to normal sea water. The dead clams were immediately replaced by new one and the time of immersion was recorded. The mortality was observed for a period of ten days. The dilution wherein survival was 50 percent during this period was considered as lethal salinity.

From Fig. 3 it is seen that two different lethal salinities were obtained for *Paphia laterisulca* during the summer and monsoon seasons. On the basis of 50% mortality the lethal salinity during the summer season was 19.2 % and for monsoon season it was 12.2 %. This shifting of lethal salinity from 19.2 % to 12.2 % in the monsoon season revealed that the clams get adjusted themselves to the drastic conditions of monsoon season over the clam beds.

The general observations showed that there was differentiated behaviour of the clams with regard to the length of siphons and faecal accumulation in response to salinity gradations. The clams remained closed in salinities below the lethal salinity and died without opening the shell valves while they behaved in different manner in higher salinities than lethal salinity. The length of
Fig. 3  Lower tolerance limit of *Paphia lateralisulca*

in low salinities of sea water during different seasons.

A  March to May (summer season).
B  July to August (Monsoon season).
foot and siphons were varied and the accumulation of
faeces and pseudofaeces was progressively increased in
salinities above 26.2 % and 20.6 % in which only 10 %
mortality was observed.

100 % mortality was occurred from fresh water to
10.5 % salinity in summer within eight days. In 12.2 %
14.9 % and 16.7 % salinities there was 90 %, 80 % and
70 % mortality was observed respectively. At 19.2 %
salinity exact 50 % mortality was occurred. In 23.0 %
and 26.0 % salinities, 30 % and 10 % mortality was obser-
ved respectively, where the clams were active and accumu-
lation of pseudofaeces was seen. Above 26.2 % salinity
i.e., no mortality occurred and the clams were active with
fully extended siphons and feet and the faeces and pseudofaeces in large quantities were accumulated.

In monsoon from tap water to 4.1 % salinity there
was 100 % mortality within eight days and 80 % and 70 %
mortality was observed in 6.7 % and 9.8 % salinities
within seven days. At 12.2 % salinity exact 50 % morta-
ality was occurred and in 13.3 %, 15.0 %, 17.4 % and
20.6 % salinities 40 %, 30 %, 20 % and 10 % mortality
occurred respectively. Above 20.6 % salinity no morta-
lity was observed and hence these points are not included
in Fig. 3.
2. *Time taken to open the shell valves in relation to salinity gradient.*

While studying the salinity tolerance, it was observed that the clams did not open their shell valves or they opened after a lapse of hours in low salinities whereas they did so immediately in higher salinities than their lethal salinities. The experiments were conducted to study in detail the relation of valve opening of clams to the external sea water gradient. Ten clams of equal size were used in each set of various gradations of sea water dilutions or concentrations and the time taken for opening of valves was noted. The experiments were divided into four sets.

A. Progressive (step by step) dilution of sea water
   a. Progressive concentration of sea water.
B. Sudden dilution of sea water
   b. Sudden concentration of sea water.

The clams were acclimated for 24 hours in glass troughs and were so arranged as to be distinguishable for subsequent observations. During the experiments it was observed that the clams showed individual variation in opening of the shell valves in different salinities.

In progressive dilutions, the observations were
Fig. 4 Time taken by *Paphia lateralisulca* to open their shell valves in various dilutions and concentrations of sea water.

A Concentrated sea water.
B Diluted sea water.

Progressive change.
Sudden change.
made on the same clams in increasing dilutions of salinities such as 33.0 \%, 30.0 \%, 28.0 \%, 24.3 \%, 20.5 \%, 15.0 \% and 12.5 \% and then in tap water, with 24 hours of acclimation in normal sea water between each transfer. The time of immersion for each step was immediately noted and the time taken by clams to part their valves and for full opening of shell valves was also recorded individually.

The same procedure was followed for the experiments on progressive concentrations of sea water such as 33.0 \%, 35.5 \%, 38.5 \%, 42.0 \% and 44 \% with 24 hours acclimation period in normal sea water between each transfer.

The observations were made for sudden dilutions and sudden concentrations following same method as above in which the clams were acclimated to the respective salinities instead of reimmersing them in normal sea water between each transfer. The laboratory temperature varied from 29 \(^{\circ}\)C to 31 \(^{\circ}\)C during the experimental period. The variations were observed in behaviour and response of individual clam to each transfer. Hence the means of all valves have been calculated and plotted in Fig. 4.

From Fig. 4 it is seen that there was a progressive increase in the time of opening the shell valves with the increasing change in dilution or concentration of sea water.
whether the change was sudden or progressive. It is interesting to note that the time lapse by clams to begin to open after immersion was correspondingly increased with the salinity of the medium which departs from the normal sea water salinity. The interval between these two stages was also correspondingly greater.

The experimental observations apart from Fig. 4 showed that the clams behaved in erratic manner and there was a permanent closure of shell valves of some individuals in the salinities which are mentioned as follows:

1. Dilutions
   (a) Progressive 15.0% to 12.5%
   (b) Sudden 20.5% and below

2. Concentrations
   (a) Progressive 42.0% and above.
   (b) Sudden 42.0% to 44.0%.

Some mortality was observed in various salinities. Maximum mortality was observed in 12.5%. In 15.0% and 20.0% salinities the death rate was slow.

From the above it would appear that the mortality in sudden dilutions was rapid as soon as the clams reached
closer to the lethal salinity they died. In progressive dilution step by step mortality was observed and it was the same as was found in salinity tolerance experiments.

From the general shape of the two pairs of curves (Fig. 4) it is revealed that when the clams were subjected to the same salinity gradient but in opposite directions, the time taken for opening of the shell valves was almost the same except in very low salinities (15.0 % and 12.5 %). The two curves are close together in each case before the critical salinities (12.5 % to 15.0 % and 42.0 % to 44.0 %) were reached. Beyond this range the curves begin to diverge more sharply in the case of sudden transfers in various dilutions of sea water. It is further observed from Fig. 4 that the time for opening the shell valves was greater in progressive transfers than in sudden transfers. This may be due to the mantle water salinity gradient in the sudden transfers being greater than the progressive changed series.

3. Exchange between mantle water and surrounding medium
in relation to environment.

Being a poikilotherm, the mantle water of a clam would set an equilibrium with varying external media. It
is, therefore, possible that when clams are transferred to dilute or concentrated sea water, some inhibitory stimulus from the environment must have forced them to remain closed, since the time lapse for reopening of the shell valves varied in different concentrations of sea water. This stimulus may possibly result from a slight leakage (even when the valves were apparently closed) between the external medium and the mantle water resulting in some change in concentration of the latter or from the stimulus which acts directly from environment on the external edges of the mantle.

When the clams were subjected to very dilute sea water, they did not part their shell valves as far as could be seen by constant observations. With a view to find whether any exchange of mantle water took place while the valves remained apparently closed, or artificially closed the following experiments were conducted in the laboratory.

a. Exchange with shell valves naturally closed.

Twenty equal sized clams were selected from the aquaria of which 5 were shucked. The mantle water was collected and titrated against silver nitrate (AgNO₃) using potassium chromate as an indicator. Thus the mantle
water salinity was obtained. The remaining 15 clams were transferred to low salinity (10.6%) and were examined for mantle water salinity in three batches so that five animals were examined after one hour; next five after two hours and the last batch was examined after three hours. The second batch of twenty clams were subjected to 34.85% salinity in the same manner as described above. The experiments were repeated twice and the mean values are shown in Table 1.

It is seen from Table 1 that no marked change was observed in the mantle water salinity (31.7%) except at the end of three hours. It was observed that the mantle water salinity was lower than the original. Hence it appears that in the clams with open shell valves in high salinity there was a diffusion between mantle water and outside medium.

b. Exchange with shell valves artificially closed.

In order to rule out the possibility of diffusion taking place in above experiment due to small leakage or the shell valves not being as tight as they appeared, following experiments were conducted with shell valves artificially closed.

A batch of 40 clams was selected from the aquaria
### Table 1

Exchange between mantle water and surrounding medium when shell valves are naturally closed in *Paphia lateralisulca*

Temperature: \(33.0^\circ C \pm 1.0^\circ C\)

<table>
<thead>
<tr>
<th>Original mantle water salinity</th>
<th>Changes in the mantle water salinity</th>
</tr>
</thead>
<tbody>
<tr>
<td>%o</td>
<td>%o</td>
</tr>
<tr>
<td>Outside salinity</td>
<td>1 hr.</td>
</tr>
<tr>
<td>%o</td>
<td></td>
</tr>
</tbody>
</table>

| 31.7 | 10.6 | 30.15 | 28.05 | 27.20 |

*20 clams were used for each set of experiment.*
and the original mantle water salinity was determined by draining the mantle water sample from 5 clams which was sufficient for three to four titrations. Remaining 35 clams were tightly clamped by means of a thin wire without injuring the mantle edges between the valves. These clamped clams were immersed in 24.0% salinity. The mantle water salinity of 5 clamped clams was determined after every four hours for a total duration of 28 hours after immersion. The temperature was recorded for every four hours which was $32.5^\circ C \pm 1.0^\circ C$.

The values tabulated in Table 2 show that the forcibly closed clams did not show marked diffusion within first 8 hours. The original salinity of the mantle water was 33.80% which was reduced to 33.86% by the end of 12 hours, again no marked change was found up to 16 hours. By the end of 20 hours it was only reduced to 33.06% ; at 24 hours it was found to be 32.32% and after 28 hours it was reduced to 31.09%. This shows that no appreciable change occurred in the original mantle water salinity. Therefore, it can be concluded that the shell valves which appear closed were not so tightly closed as to ensure complete isolation, though the apparently closed shell valves provide an adequate protection against diffusion of water.
Table 2

Exchange between mantle water and surrounding medium when shell valves are artificially closed in *Paphia lateralisulca*

Temperature 32.5°C ± 0.5°C.

<table>
<thead>
<tr>
<th>Original Salinity mantle of the water</th>
<th>Changes in the mantle water salinity outside salinity medium</th>
<th>8 hr.</th>
<th>12 hr.</th>
<th>16 hr.</th>
<th>20 hr.</th>
<th>24 hr.</th>
<th>28 hr.</th>
</tr>
</thead>
<tbody>
<tr>
<td>%o</td>
<td>%o</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>33.80</td>
<td>24.00</td>
<td>33.79</td>
<td>33.36</td>
<td>33.34</td>
<td>33.06</td>
<td>32.32</td>
<td>31.09</td>
</tr>
</tbody>
</table>

*40 clams were used for each set of experiment.*
r. Exchange of mantle water with the part of shell valve removed.

During the monsoon season clam beds meet with drastic conditions in the Kalbadevi estuary. Many clams get injured by damage to their shell valves, in the fast streams of water coming down hill. To find out the effect of shell damage on diffusion, the following experiments were conducted.

A large number of acclimated clams were taken from aquaria and a portion of shell valve on one side (middle part) of each clam was removed to expose approximately 5 to 7 mm. square area without disturbing the mantle membrane of the animal. The clams were then tightly clamped with wire and divided into two batches, each of which including about 45 to 50 clams. One batch was immersed in high salinity of 42% and the other one in low salinity of 20.2%. At the time of immersion the original mantle water salinity was determined by draining the fluid from five clams from the aquaria which was 31.5%. Samples of mantle water from a batch of five clams from each set of experiments were collected at one hour intervals for seven hours after immersion and the salinities were
determined. The experiments were repeated thrice and the mean values are plotted in Fig. 5.

From Fig. 5 it can be observed that the clams showed diffusion in concentrated sea water and the salinity of mantle water increased by 2.35% whereas in dilute medium diffusion caused decrease in the salinity of mantle water by 5.58%; the mean diffusion rate per hour in concentrated sea water was 0.33% while it was 0.78% in dilute medium. The greater permeability in dilute medium than in concentrated medium might be due to the deleterious effects of dilute sea water on survival. The low salinity (20.2‰) used in the present experiment is close to the lethal salinity during summer season (Fig. 3). So it can be concluded that the salinity of the mantle water is sufficiently strong to prevent drastic alterations in the concentration of the mantle water salinity inspite of the shell damage.

d. Influence of desiccation on the mantle water salinity.

Mostly during the low tide the clam beds get exposed to the drying conditions and are subjected to desiccation effect. To study how the clams overcome this drastic
Fig. 5 Changes in the mantle water salinity of tightly closed *Paphia lateralisulca* of which a part of shell valve removed.

A Diluted 12.5%.

Salinity exposed

B Concentrated 42.0%.
effect of desiccation the following experiments were conducted.

One large enamel tray was filled with mud (5 cm. thick layer) brought from Kalbadevi estuary and made wet with sea water. Another similar tray was filled with dry sand brought from the same beach of estuary. About 40 to 50 clams at a time were kept in each tray and exposed to the atmospheric air on a sunny and windy day. The wet mud was used to provide natural conditions encountered during the low tide in the estuary while dry sand represented rather drastic condition that frequently occurs in nature over the clam beds. Laboratory temperature was $23^\circ C \pm 1^\circ C$ at the time of experiments.

Samples of mantle water from five specimens from each tray were taken and the mantle water salinity was determined for every hour for six hours. The original mantle water salinity was determined at the beginning of the experiment. The results are graphically represented in Fig. 6.

In the period of six hours the mantle water salinity showed little difference. In the mud the change in the mantle water salinity after six hours was 3.35% giving
Fig. 6  Changes in the mantle water salinity of *Paphia laterisulca* subjected to various drying conditions.

A  Dry sand.

B  Wet sand.
mean rate of evaporation 0.55% per hour. When subjected to dry sand the change in mantle water salinity after six hours was 5.97% giving mean rate of evaporation of 0.99% per hour. The original mantle water salinity was 32.0%. Thus it is clear that when the clams are exposed to low tide or to any drastic change in the environment they significantly try to restrict the rate of evaporation of mantle fluid and protect themselves by closing their shell valves until they meet with the favourable conditions in the estuary.

4. The nature of the stimulus which open the shell valves of clams.

The initial responses to environmental changes are usually behavioural. It appears that the sense organs are sensitive to specific stimuli. While studying the habitat it was observed that when the clams were subjected to distilled water or to low salinities, they remained close for more than 2 to 3 days and it was interesting to note that when such clams were retransferred to the normal sea water they opened their shell valves within few minutes, extended their siphons and started circulating the sea water freely through their mantle cavities. The results obtained from experiments on tightly clamped clams and progressive
dilutions confirmed this. The question now arises whether this sensitivity to open their shell valves is primarily due to external change in the ionic concentration (Na⁺ or Cl⁻) or due to change in osmotic pressure. To confirm this the following experiment was conducted, which was divided into two parts as follows.

a. Effect of ionic concentration (Na⁺ or Cl⁻).

Since sodium chloride (NaCl) is the major constituent of the sea water, it was considered that either Na or Cl ions may have effect on the opening of the shell valves.

Taking distilled water as base, only the Na⁺ ions were raised in the solution to about normal sea water (33.0%) by adding sodium sulphate (Na₂SO₄). The clams were subjected to this solution. It was observed that the clams did not open their shell valves indefinitely in the above solution. Hence it may be concluded that the Na⁺ ions had no effect on the opening of the shell valves.

In second set of experiment taking distilled water as base only Cl⁻ ions were raised to about normal sea
water content by the addition of magnesium chloride ($\text{MgCl}_2$). Few clams were subjected to this solution. It was observed that in this case also they did not respond and thus it was inferred that $\text{Cl}^-$ ions as well as $\text{Na}^+$ ions are not responsible stimuli to compel the clams to keep apart their shell valves. Thus it becomes evident that the ionic concentrations of either of the major ions are not responsible as a stimulus.

b. Effect of osmotic pressure.

Distilled water was taken as a base and its osmotic pressure was increased by adding a nonelectrolyte. A stock solution of glycerine, isotonic to the full strength sea water (34.8%) was prepared by taking 76.4 ml. of glycerine to make one litre with distilled water. Further isotonic sea water solutions such as 70%, 50%, 40%, 30%, 20%, 10% and 5% were prepared by adding full strength sea water to stock isotonic glycerine solution. Ten glass troughs were arranged, first containing normal sea water, second 70% normal sea water and third one containing isotonic glycerine stalk solution and rest of the troughs contained the above gradations from 70% isotonic to 5% isotonic solutions. A batch of 10 clams was immersed in
each glass trough and the observations were made for parting of the shell valves.

The results are given in Table 3. It is seen from the table that the time taken to open the shell valves in 50% and 40% isotonic sea water was almost same as the time taken in normal sea water but differed from that in 70% normal isotonic sea water. The time gradually increased as the dilutions of the isotonic sea water were increased. 20% mortality was observed in 5% isotonic sea water solution. The clams did not open at all in 5% isotonic sea water solution.

The isotonic sea water concentrations such as 6%, 7%, 8%, 9% were prepared and 10 clams were subjected to each of these grades and observations were made which revealed that clams opened in 7% isotonic sea water while remained closed in 6% isotonic sea water.

The results (Table 4) of the above experiments revealed that only osmotic pressure is not a sufficient stimulus to open the shell valves fully and some salt should be present to open the shell valves fully.
Table 3

Average values of time taken by *Paphia lateralisulca* to part shell valves in various dilutions of isotonic sea water

<table>
<thead>
<tr>
<th>S. Type of solution No.</th>
<th>Average values for the time taken to part shell valves (in minutes)</th>
<th>Mean time taken to part shell valves in normal sea water (in minutes)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>B</td>
<td>F</td>
</tr>
<tr>
<td>1 Normal 70 % sea water</td>
<td>1.04</td>
<td>2.15</td>
</tr>
<tr>
<td>2 Isotonic 70 % sea water</td>
<td>3.46</td>
<td>3.21</td>
</tr>
<tr>
<td>3 Isotonic 50 % sea water</td>
<td>3.18</td>
<td>3.45</td>
</tr>
<tr>
<td>4 Isotonic 40 % sea water</td>
<td>3.33</td>
<td>3.58</td>
</tr>
<tr>
<td>5 Isotonic 30 % sea water</td>
<td>5.40</td>
<td>6.07</td>
</tr>
<tr>
<td>6 Isotonic 20 % sea water</td>
<td>7.24</td>
<td>7.42</td>
</tr>
<tr>
<td>7 Isotonic 10 % sea water</td>
<td></td>
<td>13.50</td>
</tr>
<tr>
<td>8 Isotonic 5 % sea water</td>
<td></td>
<td></td>
</tr>
<tr>
<td>9 Isotonic glycerine stalk solution</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

B : Beginning to open stage of shell valves.
F : Fully open stage of shell valves.
Table 4

Condition of the shell valves of *Paphia laterialis*ca below 10 percent isotonic sea water concentrations

Temperature 30.0°C. ± 0.5°C.

<table>
<thead>
<tr>
<th>No.</th>
<th>Isotonic sea water concentrations %</th>
<th>Condition of the shell valves of clams</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>5</td>
<td>All closed</td>
</tr>
<tr>
<td>2</td>
<td>6</td>
<td>All closed</td>
</tr>
<tr>
<td>3</td>
<td>7</td>
<td>Nine open</td>
</tr>
<tr>
<td>4</td>
<td>8</td>
<td>All open</td>
</tr>
<tr>
<td>5</td>
<td>9</td>
<td>All open</td>
</tr>
</tbody>
</table>

*10 clams were used for each set of experiment.*
B. Osmoregulation by weight changes.

1. weight losses induced by draining the free fluid between the shell valves.

This experiment was conducted in three phases one after another.

a. Control phase: Keeping the clams for evaporation exposed to atmospheric air.

Five equal sized clams were selected from the aquaria to serve as controls. These clams were dabbed on blotting paper to remove moisture and were kept on the table top in atmospheric air for two hours. Clams were weighed at 0, 15, 30, 45, 60, 90 and 120 minutes after blotting. After final weighing the body weights including the fluids were determined. The mean values are presented by calculating the percent weight loss by each clam (Fig. 7a).

b. First phase: The clams kept for evaporation but before weighing forced apart to drain off the accumulated free fluid between the shell valves.

A second group of five equal sized clams were taken from the aquaria, blotted and kept on the table for
evaporation. These clams were weighed at the same time intervals as in the control phase. Prior to each weighing the shell valves were forced apart slightly and the accumulated free fluid was drained off by shaking. These weights were treated in the same manner as did in the above experiments and the results are plotted in Fig. 7C.

c. Second phase: The clams with wedges between their shell valves to prevent complete closer were kept for evaporation and before each weighing the accumulated free fluid between the shell valves was drained off.

Wedges were placed between the shell valves of each of five clams while the shells were a gape in the aquaria. These clams were allowed to close on the wedges which prevented complete closer of the shell valves. These clams were removed from the aquaria and free fluid between the shells was drained off, blotted and were kept on the table top for 120 minutes and were weighed at the same time intervals as in the controls. Prior to each weighing the accumulated free fluid between the shell valves was drained off by shaking. The results are shown in Fig. 7B.

The summarised results plotted in Fig. 7 show that the percent loss of weight is much greater in the animals
Fig. 7  weight loss in *Paphia laterisulca* when kept in air for 120 minutes.

A  Control.
B  Wedged open.
C  Forced open.
which were forced open than those in which the valves were kept slightly opened with a mechanical wedge. Thus it may be concluded that the clams must be free to open and close their shell valves in the normal fashion in order to regulate their body weights and volume. It appears that the clams were stimulated to secrete more fluids after draining the accumulated free fluid between their shell valves which caused the decrease in body weights.

2. *weight loss induced by draining the free fluid between the shell valves and subsequently returned to the aquaria.*

This experiment consisted of two phases: first with wedges between shells of clams and exposing to the atmospheric air and second without wedges and kept for exposure. In both these phases 60 minutes after the start of experiment and weighing, the clams were returned to the normal sea water for the next 60 minutes and weighing was done. A batch of 10 clams was allowed to close on the wedges which prevented complete closure of the shell valves. These clams were removed from the aquaria and the free fluid between the shell was then drained off and shells were blotted. The clams were kept on the table top and exposed to air and weighed at 0, 15, 30, 45 and 60 minutes after
the free fluid had been drained off from the shells. After
the experiment the body weight was determined individually
in the same fashion as was done in the previous experiments.
The mean results are shown in Fig. 8A.

The second group of ten clams of equal size was
taken out from the aquaria, blotted and exposed to atmos-
pheric air. At the intervals of 0, 15, 30, 45, 60 minutes,
these clams were forced apart; free fluid between the shells
was drained and their weights were determined. After 60
minutes weighing these clams were transferred back to nor-
mal sea water and weighed at the intervals of 15 minutes
for the next 60 minutes. Before each weighing free fluid
between the shell valves was discarded. The mean values
are plotted in Fig. 8B.

From Fig. 8 it is seen that the loss of weight in
wedged clams was greater than forced open clams. This is
because the clams were unable to regulate their weights
when they were not free to open and close their shell
valves for normal rhythmic activities. Forcing the shells
to open at regular intervals or keeping them a gape by
means of wedges prevented the clams from regulating their
weights and consequently their volume, both in air as well
Fig. 8  Weight loss in *Paphia laterisulca* due to draining free fluid between the shell valves in air and in sea water.

A  Wedged open.
B  Forced open.

Arrow indicates the time when the clams were returned to aquaria after 60 minutes.
as in normal sea water. While control clams would open their shells and pump sea water in the aquaria in the normal rhythm.

3. **Weight changes of wedged open clams in several sea water concentrations.**

Thirty clams from the aquaria were wedged open as demonstrated in previous experiments. The free fluid between the shells was drained off and weighed. Various concentrations of normal sea water of 23.8‰ salinity were prepared by adding distilled water and sea salt such as 120 ‰, 110 ‰, normal sea water (100 ‰), 90 ‰, 80 ‰ and 70 ‰. A batch of 5 wedged clams were kept in each of above sea water concentrations for a period of 120 minutes. Prior to each weighing the free fluid between the shell valves was drained off. After 120 minutes the clams were sacrificed and the loss or gain in weights were calculated to their original weights and summarised values are presented in Fig. 9 which show that in all sea water gradations the clams lost their weights. The decrease in weight was progressive with the dilution of sea water. Decrease in weight observed in higher concentration was progressively small. This experiment also
Fig. 9  Weight changes in *Paphia laterisulca* with a wedge between their shell valves.

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<td>A</td>
<td>120 %</td>
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<td>B</td>
<td>100 %</td>
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<td>C</td>
<td>110 %</td>
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<td>D</td>
<td>90 %</td>
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<td>E</td>
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demonstrate that the clams were unable to regulate their weights in various sea water concentrations because they were not free to open and close their shell valves in normal rhythmic manner.

4. Fluid and weight loss due to injury to the mantle and underlying body parts.

When the clams get ruptured due to the great changes in the physical environment in the estuary over the beds in the monsoon season they lose some body fluids. The following experiments were conducted to see the effect of injury to the mantle and underlying body parts on the body weights of clams.

Ten clams of equal size were removed from the aquaria, blotted and weighed. Then the clams were shucked, the mantle and underlying body parts were ruptured during the removal of flesh. After shucking the bodies were kept in covered petridishes and the initial body weight including the fluids were immediately determined. The weight of the body alone was determined at 0, 15, 30, 45, 60, 90 and 120 minutes. Prior to each weighing the flesh was blotted.

The weight changes of clams shucked with injury have been expressed as the percentage of original body weight of
the initial weight including the body fluids. The average values are plotted in Fig. 10A.

A batch of another 10 clams of equal size were removed from the aquaria, blotted and weighed. Then the clams were shucked and were kept in covered petridishes without injuring the mantle and the underlying body parts. Prior to each weighing the clam body was blotted. The body weights were taken at 0, 15, 30, 45, 60, 90 and 120 minutes. The results were based on the mean values of 10 clams as the original body weight of the intact clam including body fluids. The summarised values are presented in Fig. 10B.

It is seen from Fig. 10 that there was a greater loss of body weight in both the cases during the first 15 minutes and then gradually decreases with the increase in time. The total weight loss after 120 minute in injured clams was 64.5% while it was 62.5% in the noninjured clams. The difference between the total loss of body weight was 1.75%. The weight loss in injured clams was observed to be due to fluid losses from the sinuses in the mantle and from the cut surfaces of the adductor muscles.
Fig. 10 weight changes of the body of *Paphia laterisulca* shucked without injury and injury to the mantle and underlying body parts.

A  Without injury.

B  With injury.
Discussion

If the environmental change is abrupt or great, the animal shows an initial overshoot or undershoot to a new stabilized rate. The net effect of adaptive changes in an altered environment is 'homeokinesis' which refers to constancy of life fluctuations such as locomotor activity and energy liberation, all of which permit survival in an altered environment, sometime even when internal state varies. Time period in the response of an animal to an environmental alterations may be minutes or hours, days or weeks. The magnitude of the physiological adjustment varies with the amount of environmental changes. Changes associated with wet and dry seasons, low salinity shifts in estuaries and seasonal changes in temperature and variable food may develop the compensatory alternations gradually.

Marine molluscs are osmoconformers with varying degrees of stenohalinity. Behavioural responses are made to salinity change. According to Beaudant (1916) some marine molluscs (Patella vulgata, Cardium edule, Lytilus edulis etc.) were acclimatized to fresh water by very gradual lowering of salinity within six months and
could live in healthy state with fresh water forms like *Lymnea* and *Planorbis*. Fox (1941) on *Mytilus californianus* has studied the survival period in various sea water concentrations. Loosanoff (1948, 1952) reported that *Crassostrea virginica* remained open till the changes from higher to lower salinity of the surrounding water did not occur.

The results of the present investigation indicate that *Paphia* is osmoconformer and apparently unique among marine and estuarine bivalves in its osmotic ability. Robertson (1964) states that 'Unequivocal evidence of osmotic control in brackish water bivalves is absent'. The initial responses to environmental changes are usually, behavioural, and many sense organs are equitably sensitive to specific stimuli. *Paphia laterisulca* has been shown to be sensitive to environmental changes even when apparently closed. The powerful adductor muscle is capable to close the shell valves hermatically in *Paphia* and to remain in a closed condition for two to three days without expending energy to withstand its periodically changing and generally unstable estuarine environment. This ability to separate itself from the outside environment is remarkable safety device. Milne (1940) states
that in the estuary of Aberdeenshine Dee, the valves of the mussels found at lower salinity (3.45%) were closed, thus isolating themselves and their mantle water from the unfavourable environment.

The results obtained in the present investigation generally support the findings of Dodgson (1928) on *Mytilus edulis* from Conway (North Wales), Motwani (1955) on *Mytilus edulis* from Blyth estuary, Fingerman and Fairbanks (1956) on American oyster, *Crassostrea virginica* from Barataria Bay and Mane (1974) on *Katelysia onima* from Kalbadevi estuary, Ratnagiri. There is no information available regarding the salinity tolerance range of other marine and brackish water bivalves which do not possess any osmoregulatory mechanism.

The low tolerance limit of this euryhaline clam to the changes in the concentrations of salt in the sea water likewise extends over from 12.2% salinity in the monsoon season to 19.2% salinity in the summer season respectively. *Paphia* is sensitive to dilutions of sea water and the valves opening is progressively delayed with increasing dilutions. In waters more dilute than 12.2%, there is considerable retardation in time of
the shell valves, when the clams are subjected to progres-
sive salinity changes. This salinity almost corresponds
with the lethal salinity. The critical salinity in sea
water concentrations for valves opening is found to be
42.0 %. The experiments involving exposure of the mantle
by removal of a portion of shell, showed that the mantle
is in fact somewhat permeable.

There may be some exchange of salts through a
leakage between the edges of the mantle. The animal may
react to a slight change in the concentration of the
mantle water. Bouxin (1931) stated that there was no
oxygen uptake in Mytilus sp. for first two hours, after
closing the shell valves in low salinity. It was obser-
ved that when the shell valves were closed apparently
very small area of edges of the mantle was likely to be
exposed to surrounding medium. It might cause an
increase or decrease of hydrostatic pressure developed
on the mantle edges by slight shell valve movements.
Thus Paphia may be sensitive to these pressure changes
though at least 7% sea salt is necessary for full opening
of the shell valves. (Table 3). This is generally supp-
ported by the results obtained by Motwani on Mytilus
edulis and Man (1974) on Katelysia onima.
when the clams are exposed to normal and concentrated or dilute sea water with permanent wedge in the valves they lose weight as a result of continuous loss of salts or loss of water or loss of both salts and water.

The greater weight loss due to injury to the mantle and underlying body parts was observed because of the more body fluid lost from the sinuses in the mantle and from the cut surfaces of the adductor muscles. The clams prevented from the complete closing of their shell valves lost weight both in and out of the sea water. The clams were unable to regulate their weights in wedged open experiments; thus showing the necessity of the natural opening and closing of the shell valves in rhythmic manner. Frederik (1961) studied the reaction of injury in the oyster, *Crassostrea virginica*. Lange and Mostad (1967) found that in *Mytilus edulis* the large adductor muscle regulates volume only incompletely and Lange (1968) found that the gill tissue in *Mytilus edulis* regulates volume completely.
Summary

1. *Paphia laterisulca* is an estuarine bivalve mollusc from the Kalbadevi estuary, Ratnagiri, subjected to a wide range of salinity fluctuations (4.1% to 36.0%) during different seasons.

2. A study on the survival and behaviour of the clam, *Paphia*, in different environmental fluctuations was made. Under experimental conditions, in the summer season (March to May), the clam found to tolerate the salinity up to 19.2%. Continued submergence in low salinities appeared to have marked effect to the clams in getting themselves acclimatized to low salinity resulting in the reduction of tolerating capacity down to 12.2%. This acclimatization thus has a survival value during the monsoon (July to September), when the salinity of the estuary reduces due to influx of flooded river drainage.

3. *Paphia* when transferred from normal sea water to the salinity ranges between 30.0% to 38.5% there was very little change in the mean time taken by the clams to open their shell valves irrespective of whether the transfer was sudden or progressive. A comparison between
the effects of sudden and progressive transfer would suggest that the time of opening of the valves is determined by the salinity of the outside medium rather than the differences between the nature of the transfer and that of mantle water.

4. With closed valves, exchange between mantle water and outside water was very small in *Paphia*. Shell valve closing, therefore, constitute a very effective insulating mechanism to protect the clams against unfavourable salinity fluctuations in the Kalbadevi estuary during the rainy season.

5. With naturally closed shell valves in high salinity there was a diffusion between mantle water and outside medium but when the clams artificially clamped with the help of wire no appreciable change occurred in the original mantle water salinity. It was concluded that the shell valves which appear closed were not so tightly closed to ensure complete isolation, though the apparently closed shell valves provide an adequate protection against diffusion of water.

6. When a portion of the mantle was exposed, it allowed some permeability which was greater in dilute than concentrated sea water in *Paphia*. 
7. When exposed to the atmospheric air, the changes in the concentration of mantle water, due to the evaporation was measurable and therefore, it is inferred that this change is insignificant as a factor in the normal life of clams.

8. When the shell valves were closed apparently very small area of the mantle was exposed to surrounding medium. According to the results the small area was directly sensitive to the external salinity.

9. Experiments conducted with nonelectrolyte appeared to show that the inhibitory stimulus on the clams in opening the valves results from the change of osmotic pressure of the outside medium with at least 7% sea salt rather than a change in the ionic concentration.

10. The clams must be free to open and close their shell valves for weight and volume regulation. The clams forcibly prevented from complete closing of their shell valves lost their weights due to the secretion of body fluids by evaporation and osmosis.

11. The clams shucked from their shells with no injury to the mantle and underlying body parts lost fluids
of 62.5% of their original body weight after 120 minutes. Further observations from the experiment showed that most of the fluid loss occurred within first 15 minutes after shucking. The injury to the mantle and underlying body parts of the shucked clams resulted in a 64.5% weight loss.

12. Clams have a limited ability to osmoregulate. They showed no weight and volume regulation when subjected to different dilutions and concentrations of normal sea water.

13. The weights get reduced progressively with increase in the period of desiccation.
References


Bouxin, H. 1931 Influence des variations rapides de la salinité sur la consommation d’oxygène chez Mytilus edulis var galloprovincialis.

Boyden, C.R. 1972 The behaviour, survival and respiration of the cockle Cerastoderma edule and C. glaucum in air.


Fredrik, B.B. 1961 Reaction to injury in the oyster *Crassostrea virginica*.

Hopkins, A.E. 1936 Adaptation of feeding mechanism of the oyster (*Ostrea gigas*) to changes in salinity.


Loosanoff, V.L. 1950 On behaviour of oysters transferred from low to high salinities.
Ibid., 108 : 91.

Loosanoff, V.L. 1952 Behaviour of oyster in water of low salinity.

Lange, R. 1968 The relation between the oxygen consumption of isolated gill tissue of the common mussel, *Mytilus edulis* and salinity.


<table>
<thead>
<tr>
<th>Author(s)</th>
<th>Year</th>
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<tr>
<td>Robertson, J.D.</td>
<td>1964</td>
<td>Osmotic and ionic regulation.</td>
</tr>
<tr>
<td>Walne, P.R.</td>
<td>1961</td>
<td>Observations on the mortality of Ostrea edulis.</td>
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