# THE RESPONSES OF *SCROBICULARIA PLANA* (DA COSTA) TO OSMOTIC PRESSURE CHANGES

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(Text-figs. 1–5)

*Scrobicularia plana* is an estuarine lamellibranch often numerous in intertidal, brackish muds. The animal normally lies in a burrow, 6–10 in. below the surface of the mud, and possesses long, extensible siphons through which it makes contact with the overlying water. Therefore it is probable that, as far as the salinity relations of the animal are concerned, the external medium is represented by the water above the mud.

From studies of its distribution *S. plana* is evidently tolerant of wide salinity variations in the external medium. In the Tamar estuary the species is distributed mainly in the upper half of the tidal zone down to just below mid-tide level (Spooner & Moore, 1940), and at Whitstable it is most common at mid-tide level along seaward parts of the 'bound shingle zone' (Newell, 1954). Similarly, in the extensive mud flats of the Thames estuary at Chalkwell in Essex it occurs most abundantly at mid-tide level and is completely absent from the more sandy deposits lower down the shore. This concentration of the population near mid-tide level means that the animals are not exposed to the great changes in salinity during each tidal cycle experienced by those animals living near low-water mark (Milne, 1938). However, Green (1957) in a study of a population of *S. plana* in the Gwendraeth estuary in South Wales has shown that here they are subjected to a salinity as low as two parts per thousand for a short period before being uncovered by the retreating tide.

In the Tamar, Spooner & Moore record the species along the estuary from St John's Lake, where there are 'almost marine conditions in drier parts of summer', to North Hooe, with a salinity of 20·2‰ at high water during a dry summer (Percival, 1929). In the Thames estuary the variation in salinity during that part of the tidal cycle to which an animal living near mid-tide level would be subjected is not very great. There are, however, considerable seasonal variations depending on the extent of dilution of the river by fresh water from land sources, and at Chalkwell the mid-tide salinity ranges from 25·2‰ in a wet winter to 32·4‰ in a dry summer.[1] At Whitstable the salinity does not differ greatly from that of the Channel and southern North Sea, the

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[1] All information concerning salinities at Chalkwell has been obtained from the Water Pollution Research Laboratory, and we are grateful to the Director for permission to quote unpublished figures.

maximum variation at high water recorded during the years 1953–4 being from 29·58 to 34·52‰ (El-Maghraby, London Ph.D. Thesis, 1955).

These considerations make it clear that *S. plana* can tolerate almost marine conditions, salinities of about 20‰, and, for short periods at least, salinities as low as two parts per thousand.

Since *S. plana* normally experiences variations in external salinity, and since observations made during a study of rhythmic activities of this animal (Freeman, unpublished) showed that they were affected by dilution of the external medium, a study has been made of the effect of changes of external salinity on the concentration of the blood and on some aspects of the behaviour of *S. plana*.

## MATERIALS AND METHODS

Animals used in these experiments were collected from the mud flats at Chalkwell in Essex. They were allowed to burrow in mud from the same situation and kept under sea-water circulation at the Plymouth Laboratory.

Blood samples were obtained from the heart, which was exposed by cutting each valve of the shell along a line between the insertions of the anterior and posterior adductor muscles, and removing that part of the shell which, freed from the restraint of the adductor muscles, was raised by the elasticity of the hinge ligament. Samples of 4–6 mg of blood for measurements of depression of the freezing-point were taken directly into capillary tubes which were immediately sealed at both ends by injection of silicone stopcock grease from a hypodermic syringe. These operations were carried out in a moist chamber saturated with respect to sea water. Depression of the freezing-point was estimated by a method similar to that of Jones (1941) and Gross (1954). The blood samples and a series of NaCl standards of appropriate concentrations, similarly sealed in uniform capillary tubes, were fastened to a glass frame with silicone stopcock grease and frozen by immersion in alcohol cooled to the temperature of solid carbon dioxide. They were immersed in a vessel of cold, 5 M-NaCl, which was kept continuously stirred, and allowed to warm slowly in a lagged box. A rate of warming of 1° C in 45 min was obtained by keeping the box in a constant temperature room at 2° C. The time taken for the complete disappearance of the crystals was noted, this being facilitated by viewing the crystals through crossed polaroids. The depression of the freezing-point of the blood samples was calculated from the curve relating melting time to the freezing-point of the standards.

Specimens used for measurement of opening rates were removed from the mud or acclimation vessel and left in air for 5 min. At the end of this time they exhibited what may be termed the normal degree of closure. In this state the shell valves are sufficiently apposed to prevent the animal protruding its foot or siphons, but the free edges of the mantle lobes are still visible between the lower edges of the shell. Here the lobes of the two sides are closely applied

to form a pallial curtain that, in the absence of complete fusion, probably functionally simulates that condition. Twenty animals were placed in each experimental vessel, in which they were covered to a depth of about 2 in, and spaced widely so that the activity of any specimen would be unlikely to disturb those adjacent to it. They were observed at 5 or 10 min intervals and recorded as open if the inhalant siphon was clearly visible beyond the margin of the shell. If the foot alone was protruded or just the closed tip of the siphon was visible the animal was recorded as half open. Chapman & Newell (1956) observed that the inhalant siphon was protruded soon after an initial gaping of the shell valves and that the siphon was open at its tip during extension. The protrusion of the inhalant siphon therefore provided a convenient indication that the animal was open, in the sense of being exposed to the external medium.

Diluted sea water for all experiments was made by adding distilled water to Plymouth circulation water, which is referred to throughout as 100% sea water.

## EXPERIMENTS AND RESULTS

### THE RELATION BETWEEN OSMOTIC PRESSURE OF THE BLOOD AND THAT OF THE EXTERNAL MEDIUM

Although the estuarine and marine lamellibranchs that have been studied exhibit little or no osmoregulatory ability (Prosser, 1950), experiments were first carried out to determine whether *S. plana* conformed to this general picture. Animals were equilibrated at 15° C for 48 h in 100% sea water and in sea water diluted to 80, 70 and 60%. Others were equilibrated in 50% sea water for 48 h when some of them were transferred to 30% for a further 60 h, the rest remaining for the same length of time in 50%. Measurements of freezing-point depression of the blood and of the external medium are shown in Table 1.

These results show that the osmotic pressure of the blood agrees closely with that of the external medium down to dilutions of 50%, but in 30% sea water the osmotic pressure of the blood is significantly higher than that of the external medium.

The agreement of the osmotic pressure of the blood with that of the external medium means that, if, as suggested above, the external medium of the animal in its natural habitat is represented by the water above the mud, the osmotic pressure of the blood of animals taken from the mud should agree with that of the overlying water rather than that of water contained in the mud. To examine this the following experiment was carried out. Blood samples were taken from animals collected from the upper limit of their distribution at Chalkwell immediately after being uncovered by the tide, on a day (13 September 1956) when there had been a sudden decrease in flow of fresh water over Teddington weir. In Table 2 the depression of the freezing-point

of the blood is compared with that of samples of water taken from above the mud 15 min before the tide receded.

These figures show that there was no significant difference between the osmotic pressure of the blood and that of the overlying water. In his study of the salinity of intertidal muds, Smith (1956) comments that although the quantitative relationship between salinity of water contained in the mud and the varying salinity of the overlying water is not known, the interstitial

TABLE 1. RELATION BETWEEN DEPRESSION OF THE FREEZING-POINT OF THE EXTERNAL MEDIUM AND OF THE BLOOD OF *SCROBICULARIA PLANA*.

The figures in the right-hand column are calculated values of Student's $t$, applying Bessel's correction for small samples.

| | No. of observa-tions | Mean freezing-point depression (° C) | Standard deviation | $t$ |
|---|---|---|---|---|
| 100 % sea water | 13 | 1·90 | 0·048 | |
| Blood | 11 | 1·89 | 0·011 | 0·291 |
| 80 % sea water | 6 | 1·55 | 0·009 | |
| Blood | 9 | 1·60 | 0·073 | 1·06 |
| 70 % sea water | 7 | 1·37 | 0·077 | |
| Blood | 10 | 1·35 | 0·059 | 0·572 |
| 60 % sea water | 6 | 1·12 | 0·079 | |
| Blood | 9 | 1·17 | 0·041 | 1·49 |
| 50 % sea water | 5 | 1·052 | 0·016 | |
| Blood | 5 | 1·054 | 0·012 | 0·316 |
| 30 % sea water | 7 | 0·592 | 0·0104 | |
| Blood | 5 | 0·745 | 0·0382 | 13·6 |

TABLE 2. DEPRESSION OF THE FREEZING-POINT OF THE BLOOD OF *SCROBICULARIA PLANA* AT CHALKWELL

| | No. of observa-tions | Mean freezing-point depression (° C) | Standard deviation | $t$ |
|---|---|---|---|---|
| Overlying water | 9 | 1·756 | 0·025 | |
| Blood | 16 | 1·762 | 0·028 | 0·51 |

salinities give a picture of changes within past weeks. The immediate past history of the Chalkwell muds was of exposure to lower salinities than that given in Table 2. The records of the Thames Conservancy show that the dilution of the river by fresh water flowing over Teddington weir was less on the day when the samples were taken than on any of the preceding 10 days, and the survey of the Water Pollution Research Laboratory, which correlates freshwater flow at Teddington with chlorinity of the water along the Thames estuary, indicates that the average chlorinity at high water at Chalkwell over these preceding 10 days was about 16·5‰, and the average over the preceding 2 weeks was only 16·8‰. On the day the samples were taken the flow at Teddington was 656·2 million gallons, corresponding to a chlorinity at high

water at Chalkwell of about 17·5‰. This agrees fairly well with a calculated chlorinity of 17·85‰ for sea water with the depression of the freezing-point given in Table 2 (Robinson, 1954). The blood of the animals, therefore, agreed with the overlying water rather than with any average picture of changes within the preceding 2 weeks. Moreover, the normal situation of this deposit-feeding lamellibranch is with its siphons protruded into the overlying water, and experiments reported later in this paper show that the blood of specimens that do not protrude their siphons is almost unaffected by the salinity of the water that surrounds the shell and mantle margin. This lends support to the view stated earlier that the salinity with which the animal comes into equilibrium is that of the overlying water rather than water contained in the mud.

### RATE OF EQUILIBRATION TO DILUTED SEA WATER

Although it has been shown that, except when *S. plana* is subjected to very low external salinities, its blood attains the same osmotic pressure as the external medium, no indication has been given of the time necessary for equilibration. In order to study the rate of equilibration several animals were transferred from the mud under sea-water circulation to 80 and 60 % sea water at 15° C. At intervals specimens were taken at random from those in the experimental vessels and measurements made of the depression of the freezing-point of their blood. The results are shown in Fig. 1.

The time taken for equilibration was approximately 8 h in 80 % sea water and 25 h in 60 % sea water. Within the limits imposed by small sample size these figures give a picture of the effect on a population of *S. plana* of a sudden change in the salinity of the external medium. Most noticeable is the considerable scatter of the freezing-point of the blood of animals in 80 % sea water up to 4 h, and of animals in 60 % sea water up to 14 h. This scatter was interpreted as indicating that, when transferred to the dilute medium, some animals had remained closed longer than others and therefore the estimation of the freezing-point of their blood did not represent the result of a maximal exposure of their tissues to the lowered external salinity over the time stated. These readings would therefore be higher than those of animals that opened almost immediately. Furthermore, the mean of the readings for 1 and 2 h was higher for the specimens in 60 % than in 80 % sea water, and only very slightly lower after 4 h. The greater concentration difference to which the animals in 60 % sea water were exposed should result in a much faster decrease in the tonicity of the blood. The time taken for complete equilibration should not in fact be appreciably longer than in 80 % sea water (Jacobs, 1935).

In order to compare the rates of equilibration of open and closed specimens the previous experiment was repeated, but the equilibrating specimens were observed throughout the experiment and only those which had been open or closed continuously were selected for measurement of the freezing-point depression of their blood. The results are presented in Figs. 2 and 3.

These results show that the rate of equilibration of open animals is much more rapid than suggested by the previous experiment. Open animals equilibrate to 80 % sea water in 4–5 h and to 60 % sea water in 5–6 h. The very rapid rate of dilution of the blood over the first 15 min is particularly noticeable. In contrast the blood of closed animals changed very slowly. Those in 60 % sea water, where the concentration gradient is greatest, show an average drop in their blood osmotic pressure of only 1·5 % per hour, whereas those in 80 % sea water show a drop of about 4 % per hour.
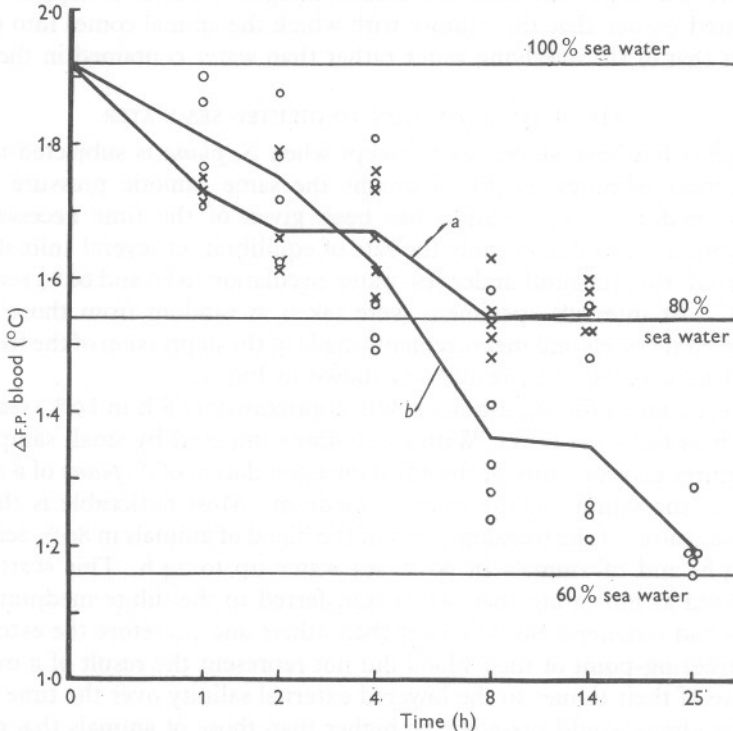


Fig. 1. Depression of the freezing-point of the blood of randomly chosen specimens of *Scrobicularia plana* after varying times of exposure to 80 % (×) and 60 % (o) sea water. The time scale is abbreviated by being expressed geometrically. *a*, line joining the means of the 80 % readings; *b*, the same for the 60 % readings.

### EFFECT OF DILUTION OF THE EXTERNAL MEDIUM ON RATE OF OPENING

In the previous section it was shown that the average drop in blood osmotic pressure of a population of *S. plana* exposed to 60 % sea water was slower over the first few hours than that of a population exposed to 80 % sea water, whereas animals selected as having been continuously open showed a more rapid decrease in their blood osmotic pressure over this period in 60 % than in 80 % sea water. In view of the very small change in osmotic pressure of the

blood of animals that remained closed, it seemed possible therefore that animals suddenly exposed to 60% sea water opened less rapidly than those exposed to the lesser dilution. It was indeed observed when selecting specimens that had been open or closed continuously that very few of the animals in 60% sea water opened soon after being transferred to this medium, whereas very few of those in 80% sea water remained closed throughout the experiment. The results given in the previous section also showed that specimens
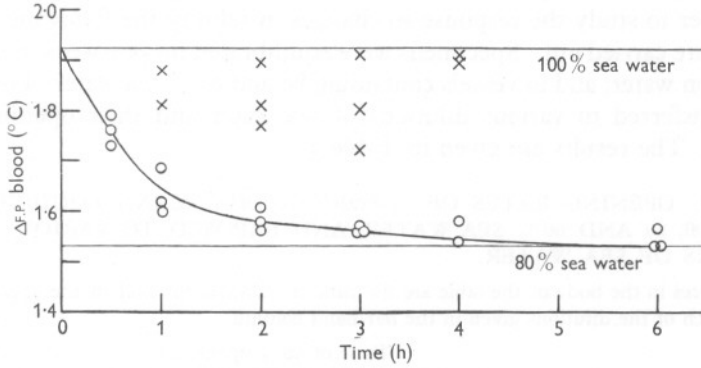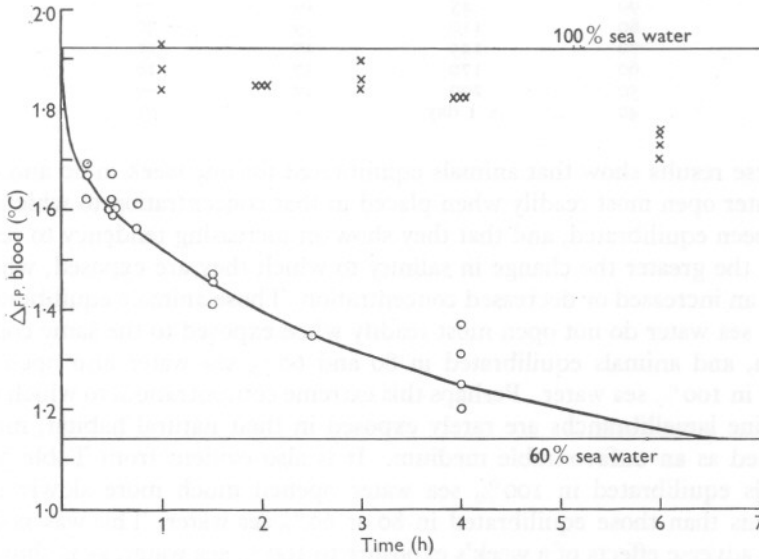


Fig. 2. Depression of the freezing-point of the blood of open and closed specimens of *Scrobicularia plana* after varying times of exposure to 80% sea water. ○, open animals; ×, closed animals.



Fig. 3. Depression of the freezing-point of the blood of open and closed specimens of *Scrobicularia plana* after varying times of exposure to 60% sea water. ○, open animals; ×, closed animals.

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that were selected as being closed in 60% sea water showed less change in their blood osmotic pressure than those in 80% sea water. This indication that closed animals in 60% sea water were more completely insulated from changes in external osmotic pressure than those in 80% sea water suggested that specimens remained more tightly closed in the more dilute medium. These differences might mean that 80% sea water could be regarded as a more favourable medium for *S. plana*, or that this species responds to the sudden large change in salinity from 100 to 60% sea water by remaining closed.

In order to study the response to changes in salinity the following experiments were carried out. Specimens were equilibrated for one week in running circulation water, and in vessels containing 80 and 60% sea water. They were then transferred to various dilutions of sea water and their opening rates recorded. The results are given in Table 3.

TABLE 3. OPENING RATES OF *SCROBICULARIA PLANA* EQUILIBRATED IN 100, 80 AND 60% SEA WATER, AND EXPOSED TO VARIOUS DILUTIONS OF SEA WATER.

The figures in the body of the table are the time in minutes for half of the specimens to open in each of the dilutions given in the left-hand column.

| | Time of 50% opening | | |
|---|---|---|---|
| Experimental concentration, % sea water | Equilibrated in 100% sea water | Equilibrated in 80% sea water | Equilibrated in 60% sea water |
| 100 | 110 | 27 | 57 |
| 90 | 45 | 19 | — |
| 80 | 110 | 9 | 30 |
| 70 | 145 | 17 | 18 |
| 60 | 170 | 17 | 18 |
| 50 | 290 | 12 | — |
| 40 | > 1 day | — | 65 |

These results show that animals equilibrated for one week in 80 and 60% sea water open most readily when placed in that concentration to which they have been equilibrated, and that they show an increasing tendency to remain closed the greater the change in salinity to which they are exposed, whether this is an increased or decreased concentration. Those animals equilibrated in 100% sea water do not open most readily when exposed to the same concentration, and animals equilibrated in 80 and 60% sea water also open very slowly in 100% sea water. Perhaps this extreme concentration, to which these estuarine lamellibranchs are rarely exposed in their natural habitat, may be regarded as an unfavourable medium. It is also evident from Table 3 that animals equilibrated in 100% sea water opened much more slowly in all dilutions than those equilibrated in 80 or 60% sea water. This was not due to any adverse effects of a week's exposure to 100% sea water, as is shown by the following experiment. Some specimens were equilibrated in an open bowl under splashing circulation water, others were allowed to burrow in mud

which was kept under circulation, and others were placed in a vessel of still 100% sea water. They were allowed to open in 100% sea water and the time for 50% of them to open was recorded. Those equilibrated in still water opened in 27 min, those under mud in 46 min, and those under splashing circulation in 120 min. It appears therefore that the very slow opening rate was not due to any difference in salinity of the water in which the animals were equilibrated, but rather to some other condition of pre-treatment. Those equilibrated in an open bowl under splashing circulation had necessarily been subjected to slight, constant vibration not experienced by those in still water or burrowed under mud, and therefore the effect of vibration was investigated in the following experiment. Specimens were equilibrated under splashing circulation and in still water and their opening rates measured in 100% sea

TABLE 4. THE EFFECT OF VIBRATION AND EQUILIBRATION CONDITIONS
ON THE OPENING OF *SCROBICULARIA PLANA*

All specimens equilibrated in 100% sea water. Opening rates expressed as time in minutes for 50% opening.

|  | Equilibrated under splashing circulation | | Equilibrated in still water. |
|  | Experimental concentration 100% | Experimental concentration 50% | Experimental concentration 100% |
|---|---|---|---|
| Control | 175 | 250 | 53 |
| Vibrated | 55 | 129 | 17 |

water under conditions where some of them were exposed to constant vibration and others allowed to open under control conditions. Also, in order to determine the interaction between the effects of vibration and those of exposure to diluted sea water, the opening rate was measured in 50% sea water.

Table 4 shows that all the vibrated animals opened more rapidly than those not vibrated. It also shows that the animals equilibrated in still water opened more rapidly than those equilibrated under splashing circulation under both control and vibrated conditions. The vibration acts as a stimulus to open which is superimposed on the control opening rates. Of the animals equilibrated under splashing circulation, those exposed to 100% sea water opened more rapidly than those in 50% sea water, emphasizing the effect of concentration of the medium in influencing opening rate, but those vibrated in 50% sea water opened more rapidly than those not vibrated in 100% sea water. Thus, under these conditions, the effect of concentration of the medium can be completely over-ridden by the effect of vibration.

#### EFFECT OF DILUTION OF THE EXTERNAL MEDIUM ON OPEN ANIMALS

In previous experiments the effect of changing the concentration of the external medium has been studied only on closed animals, but it is probable that, when burrowed in the mud in its natural habitat, *S. plana* would be

exposed to a changed salinity only when its siphons were protruding into the overlying water. To determine the response of animals with extended siphons to changed salinity, two groups of animals were allowed to open in 100% sea water and when a convenient number were open the water was siphoned out and replaced by 50% sea water in one bowl and by 100% sea water in the other. The effect of this change on the number of animals open is shown in Fig. 4.

This shows that the process of replacing the water did not in itself affect the number of animals open, but the sudden change from 100 to 50% sea water caused more than half of the animals to close.
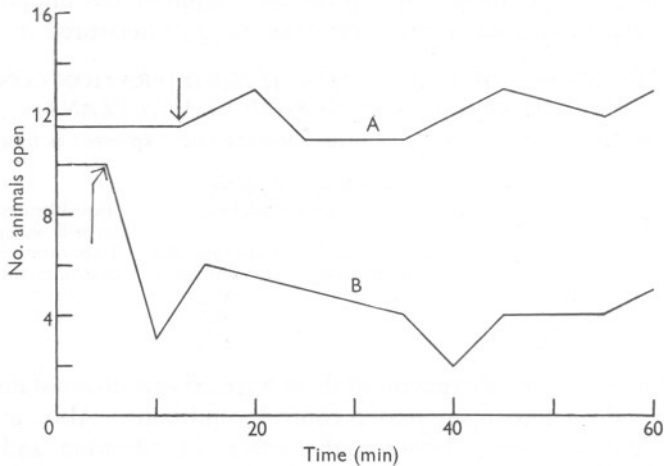


Fig. 4. Effect of dilution of the medium on numbers of open *Scrobicularia plana*. 100% sea water replaced by (A) 100% sea water, (B) 50% sea water, at points marked by arrows.

In this, as in previous experiments where the effect of changed concentration of the external medium has been studied, the medium has differed from that to which the animal was equilibrated both in concentration of individual ions and also in total osmotic pressure. To test which of these factors was significant in influencing the behaviour of *S. plana*, closed specimens were exposed to 100% sea water, 50% sea water, and sea water to which was added an equal volume of 0·9 M sucrose solution. This latter medium is equivalent to 50% sea water in ionic concentration and to 100% sea water in total osmotic pressure. The opening rates are compared in Fig. 5.

These results show that animals in the 50% sea water containing 0·45 M sucrose behave almost identically with those in 100% sea water and very differently from those in 50% sea water. The factor that tends to keep *S. plana* closed when suddenly exposed to media of different concentration is evidently an alteration in total osmotic pressure.

Some measurements were also made of the depression of the freezing-point

of the blood of animals that had been exposed to the 50 % sea water containing 0·45 M sucrose. Those that had opened showed signs of very considerable dehydration and with many it was found impossible to obtain enough blood for estimation. Samples from three animals each gave a depression of the freezing-point of 1·83° C, as compared with 1·91 and 1·92 for the blood of the only two specimens that did not open at all during the experiment. Presumably under these conditions the initial tendency is for the blood to lose ions to
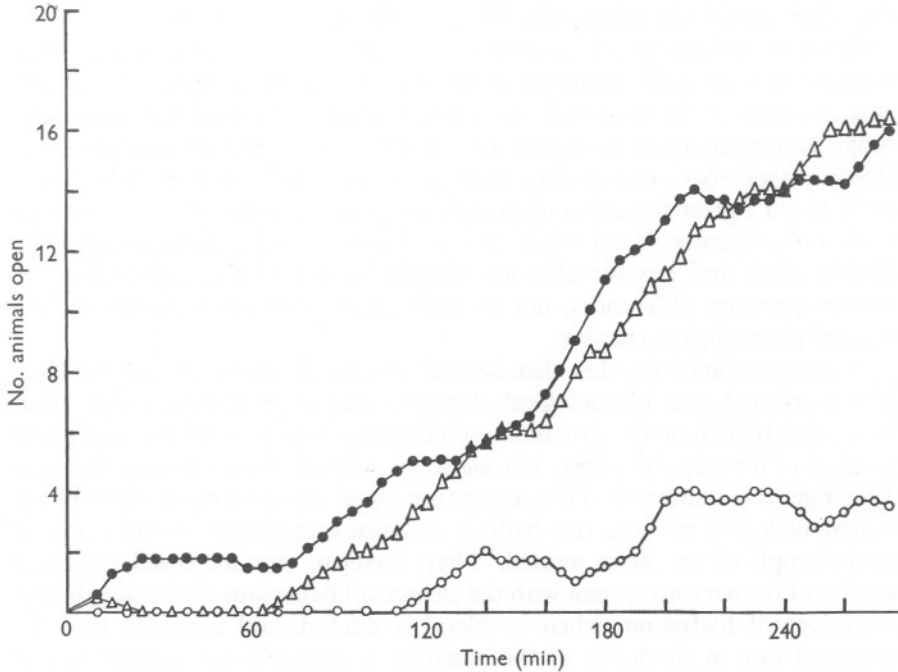


Fig. 5. The opening rates of *Scrobicularia plana* in sea water, 50 % sea water, and 50 % sea water made isotonic with an equal volume of 0·9 M sucrose solution. ●—●, 100 % sea water; △—△, 50 % sea water containing 0·45 M sucrose; ○—○, 50 % sea water. (The numbers plotted are the moving average of three readings.)

the medium, resulting in a decreased internal osmotic pressure, with a consequent outward movement of water. The blood might therefore be expected at any one time to show a slightly lower osmotic pressure than the external medium. It was noted that severely dehydrated specimens returned to sea water recovered to an apparently normal condition, although there would be no osmotic gradient tending to bring about a re-entry of water.

## DISCUSSION

*Scrobicularia plana* is similar to other estuarine and marine lamellibranchs that have been studied in showing no osmo-regulatory ability, except at very low external salinities. However, it reacts to extreme changes in external salinity by remaining closed, or, if open, by retracting its siphons, and thus protects its blood from extreme changes in concentration. These responses are to the stimulus of an alteration in total osmotic pressure of the external medium, which the animal can assess even while in the closed condition.

Variations in behaviour in response to varying external salinities have been observed in some other lamellibranchs. Maloeuf (1938) observed the maintained closure of *Mytilus edulis* in distilled water and found it necessary to wedge specimens open to expose the tissues to the external medium, and Morton, Boney & Corner (1957), working on the small lamellibranch *Lasaea rubra*, noted a greater number open after 5 min in sea water as compared with dilute and concentrated sea water. It is not known whether these responses of *Mytilus edulis* and *Lasaea rubra* are directly related to the appreciation of osmotic pressure differences, nor to what extent they are accompanied by internal physiological changes.

In groups of animals other than lamellibranchs, changes in behaviour have been correlated with physiological changes. Hughes & Kerkut (1956) have shown that there is an increase in the spontaneous activity of the isolated pedal ganglion of the slug *Agriolimax reticulatus* on dilution of the medium in which the ganglion is immersed. They argue that, in an animal with an open blood system such as a mollusc, this bathing medium corresponds to the blood or haemolymph of an intact animal. They correlate this increased electrical activity of the nervous system with the changes of behaviour of the slug during conditions of hydration, when its blood is diluted, and conclude that the increased rate of discharge of the ganglion is probably not related to any particular change in behaviour but represents a difference in the 'tonus' or 'vigilance' of the central nervous system against which reflex activity will be expressed. In an extension of this work, Kerkut & Taylor (1956) have shown that the effective change to which the ganglion responds is one of osmotic pressure, and that the limiting rate producing an increase in ganglionic activity is a change of $1\%$ in 4 min. They discuss these results in the light of other osmoreceptors known, particularly in mammals where there is a similar phenomenon of part of the central nervous system being sensitive to osmotic pressure changes in the blood.

The situation in *Scrobicularia plana* differs in one important respect from that in the slug. On exposure to external media of lowered osmotic pressure *S. plana* shows a greater reluctance to open than in sea water, there being a greater tendency to remain closed the greater the decrease in osmotic pressure. These changes in behaviour are accompanied by a much smaller rate of change

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