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# SOME ASPECTS OF THE PHYSIOLOGY OF *ARENICOLA MARINA* (POLYCHAETA) EXPOSED TO FLUCTUATING SALINITIES

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(Figs. 1–17)

The behaviour of *Arenicola marina* was studied under laboratory simulated estuarine conditions; changes in coelomic fluid and tissue constituents were also monitored.

Lugworms responded to abrupt dilution of the water overlying their burrows by becoming inactive and compressing themselves at the bottom of the burrow; they 'sampled' the overlying water about once every hour. Normal activity was resumed when the salinity returned to its initial high value. In sinusoidal salinity regimes, fluctuating between 100% and 30% sea water, it was found that activity stopped when the external sea-water concentration had fallen to about 55% s.w. Values derived from animals held in glass tubes rather than sand burrows were substantially higher, activity ceasing at about 70% s.w., suggesting that *Arenicola* derives a proportion of its water for irrigation from interstitial rather than surface water.

The combination of behavioural response and exploitation of interstitial water was shown to be extremely effective in maintaining coelomic fluid and tissue constituents at a constant level. This was in marked contrast to the situation found for non-burrowed worms exposed to the full salinity change; these exhibited the fluctuations in coelomic fluid and tissue constituents expected from a highly permeable osmoconformer.

## INTRODUCTION

*Arenicola marina* (L.) is one of the most common members of the outer estuarine sandy and muddy shores. A moderately euryhaline osmoconformer, this polychaete cannot survive in water of less than about 10‰ constant salinity and then only in the Baltic; however, in the outer portions of estuaries, it is not uncommon for the overlying water to be practically fresh at some stages of the tide. Wells (1949*b*) postulated that parts of the complex burrowing behaviour of the animal prevents irrigation of the burrow with noxious water, but the advantages to the lugworm of its periodic inactivity have never been fully investigated.

Davenport, Gruffydd & Beaumont (1975) have developed an apparatus to deliver water of fluctuating salinity to experimental animals and it is now possible to study the behaviour and physiology of organisms exposed to idealized salinity regimes which mimic those of the estuaries. It has been possible to establish the sea-water concentrations which trigger opercular flap closure in barnacles (Davenport, 1976) and shell valve closure in a variety of bivalves (Bettison, unpublished). Much information has also been gained about haemolymph and tissue constituent changes in bivalves and echinoderms exposed to such conditions (Shumway, 1977*a*, *b*, *c*, *d*).

Since the ability to become quiescent in its burrow might, for *Arenicola*, be con-

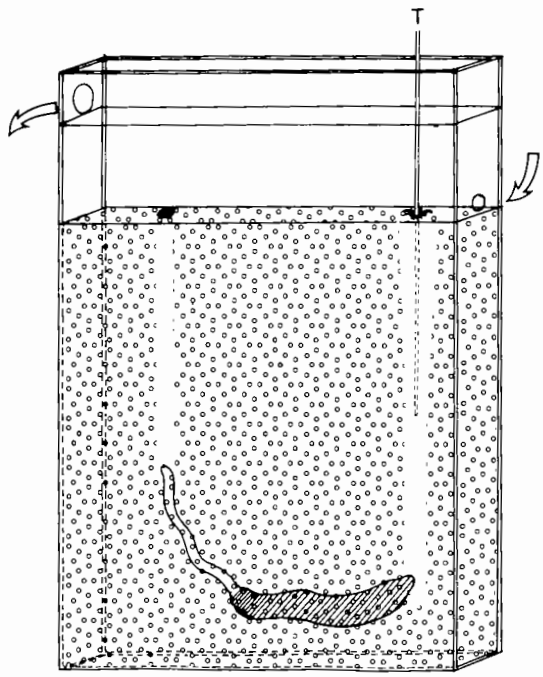


Fig. 1. Sand-filled box with *A. marina* in burrow and glass capillary, T, positioned in anterior end of burrow. Arrows indicate direction of water flow.

sidered to be an analogous response to the closure mechanisms of cirripedes and bivalves, the lugworm was expected to be a most interesting subject for investigation in laboratory simulated estuarine conditions. The results reported here show, in detail, the behavioural responses of *Arenicola* to such regimes by use of a new recording technique. Data is also presented which compares the coelomic fluid and tissue constituents of burrowed and non-burrowed worms, and demonstrates the efficacy of the burrowing habit in allowing the lugworm to cope with fluctuating environmental salinities.

## MATERIALS AND METHODS

Specimens of *A. marina* were collected locally from the Anglesey coast and kept at 15 °C in aquaria supplied with running sea water pumped from the Menai Strait (salinity approximately 32‰).

The apparatus used to produce fluctuating salinity regimes has been described by Davenport *et al.* (1975). Animals used in burrowed experiments were placed in sand-filled boxes similar to that illustrated in Fig. 1. *A. marina* burrowed within minutes of being placed on the surface of the sand. Animals were subjected to both gradual (sinusoidal) and abrupt (square-wave) salinity changes as shown in Fig. 2. To simulate idealized near-tidal fluctuations, the programmes ran for 24 h. The maximum sea-water concentration was always 100% sea water; the minimum sea-water concentration was 30% sea water. All experiments were carried out at 15 °C.

A total of 200 *A. marina* were examined. Specimens were taken for analyses initially and then every 3 h for 24 h. The animals were blotted dry, weighed and the coelomic fluid collected. Coelomic fluid pH values were measured immediately using a PHM 63 Digital pH meter (Radiometer: Copenhagen) and a Russell micro pH electrode. The worms were then frozen, freeze-dried and re-weighed to determine tissue water content, ninhydrin positive substances

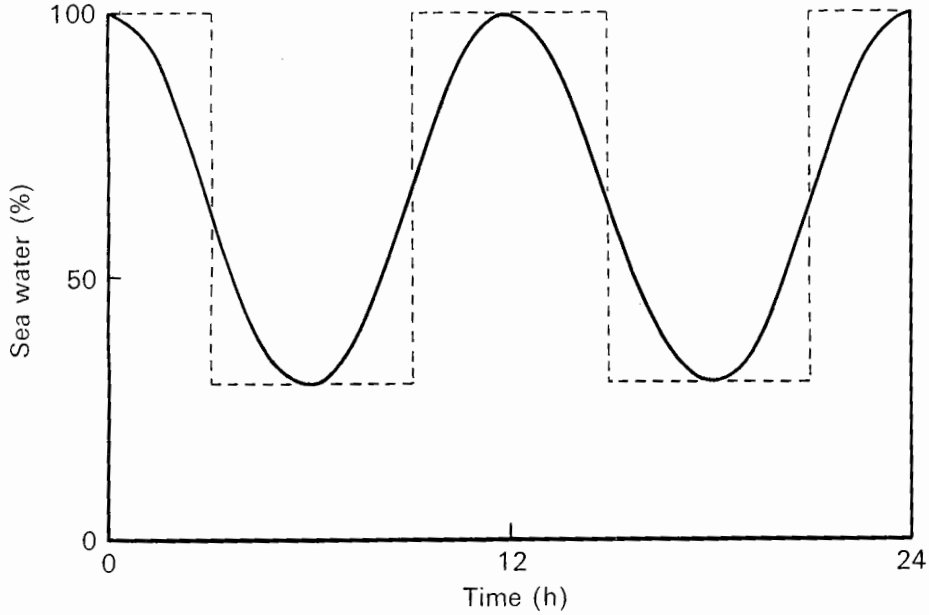


Fig. 2. Patterns of salinity changes.

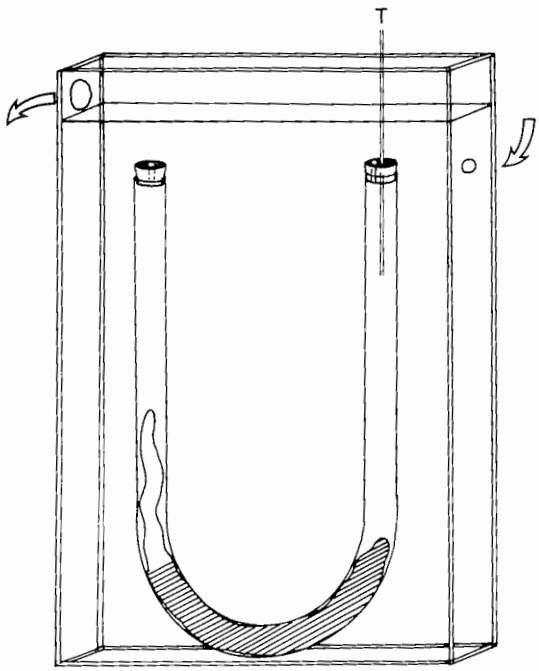


Fig. 3. *A. marina* in glass U-tube within a box and glass capillary, T, positioned in anterior end of tube. Arrows indicate direction of water flow.

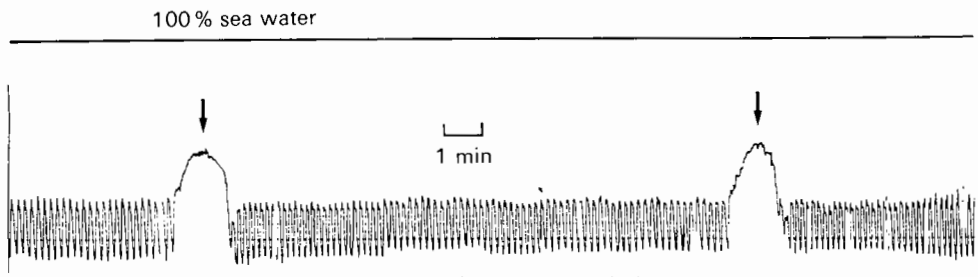


Fig. 4. Activity trace from *A. marina* in 100 % sea water. Peaks indicated by arrows are the result of tailward locomotion.

(NPS) and free amino acids (FAA) as described previously (Shumway, 1977*a*, *b*, *c*). Coelomic fluid samples were then centrifuged for 2 min at 12000 *g* and osmotic concentrations were measured using a Halbmikro Osmometer. Coelomic fluid samples were then frozen for later determination of $Na^{+}$, $K^{+}$, $Mg^{2+}$, $Ca^{2+}$ and $SO_4^{2-}$ as described previously (Shumway, 1977*a*, *b*, *c*). For comparative purposes, analyses were made of the coelomic fluid of worms which had been kept for at least 1 week in Menai Strait sea water.

*Activity*

Fig. 1 shows *Arenicola* burrowed in sand. In order to record movements within the burrow during salinity fluctuations a glass capillary tube was inserted in the sand at the anterior end of the burrow. The capillary tube was then connected to an SE laboratories SEM4-82 pressure transducer and the pressure changes recorded on a Servoscribe twin channel recorder which also displayed the salinity changes (see Figs. 4, 5A, 6A, 7A). To observe the animals' position in the burrow during salinity changes a worm was placed in a glass U-tube and the tube placed in a transparent box (Fig. 3). The glass capillary was then positioned in the anterior end of the tube. Activity patterns were recorded as in burrowed worms with the further advantage of being able to observe the worm's position in the burrow.

*Buffering capacity of the coelomic fluid*

The buffering capacity of a sample of coelomic fluid was determined by electrometric titration according to the method of Clark (1964).

## RESULTS

*Activity*

Figs. 4–7 show typical traces of *A. marina* activity patterns and the animals' position in the burrow during these activity periods. An upward movement of the pen corresponds to a tailward movement by the worm, or a decrease in pressure at the pressure sensor. The activity traces are taken from animals placed in sand burrows; the positional drawings are taken from animals observed in U-tubes. Similar activity patterns to those recorded from sand burrows were recorded from the animals in glass tubes.

Fig. 4 shows an activity trace from an animal in 100 % sea water. As long as the external concentration remained at 100 % this pattern of activity continued. Fig. 5A shows the trace recorded during defaecation. Each spike represents a thrust of the worm's tail (shown in Fig. 5B) during the deposition of faeces. Defaecation occurred sporadically, sometimes only twice in a 24 h period, and not at all under condition of decreased salinity.

Fig. 6A shows the effect of altering the external salinity on the activity of *A. marina*. During exposure to the square-wave salinity profile the animals were active while in

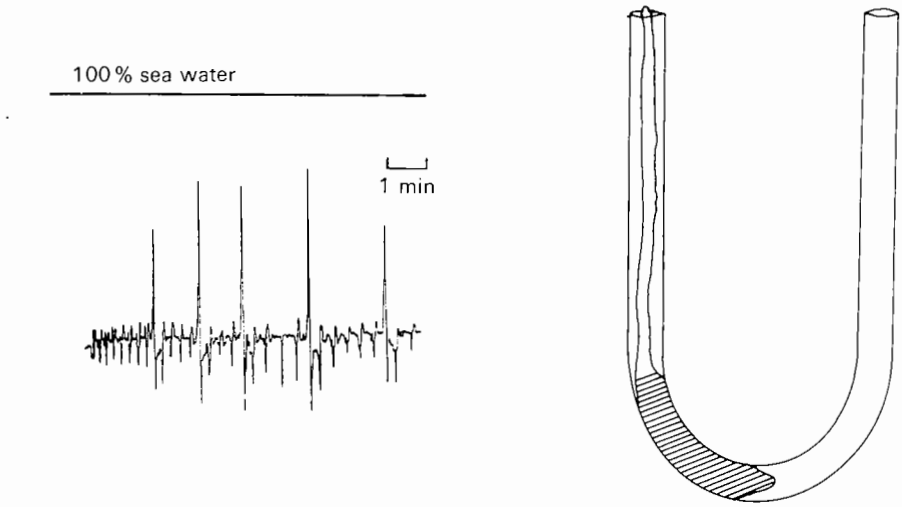


Fig. 5. Trace recorded from *A. marina* during defaecation (A) and the animal's position in the U-tube at the time of defaecation (B).

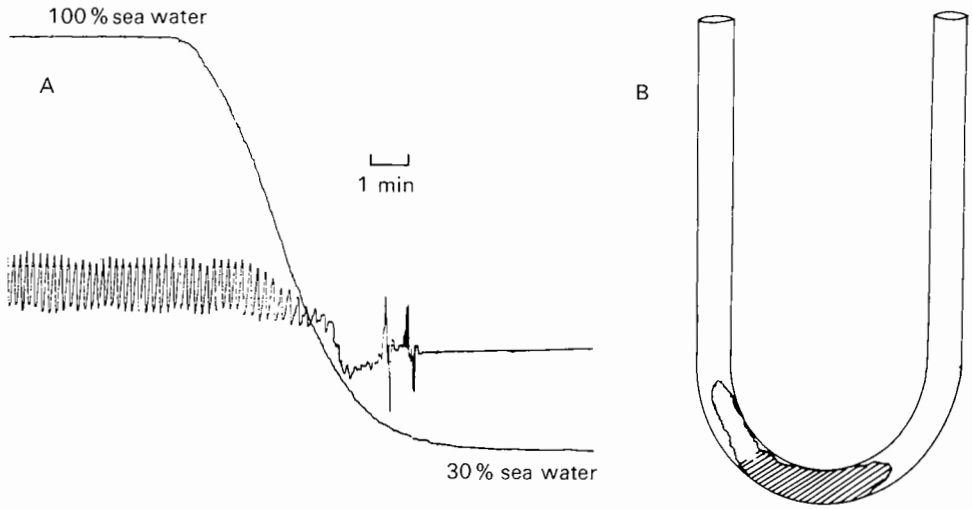


Fig. 6. Changes in *A. marina* activity when exposed to a 30 % sea-water minimum abrupt salinity change, and the animal's position in the U-tube after activity had ceased.

100 % sea water, but within approximately 3 min of the salinity decrease activity ceased and the worm compressed itself at the bottom of the tube (Fig. 6B). During periods of exposure to low salinity the worms 'sampled' the overlying water approximately once every hour (Fig. 7A, B). With the exception of these intermittent samplings of the surface water the worms remained inactive until the salinity increased as can be seen in Figs. 8A and B which show the % time active for worms exposed to both gradual (8A) and abrupt (8B) salinity changes. Although it appears that the worms were at least partially active during periods of low salinity, this activity was due solely to the sampling motion and at no time during exposure to low salinities did animals show the normal activity patterns as seen in Fig. 4.

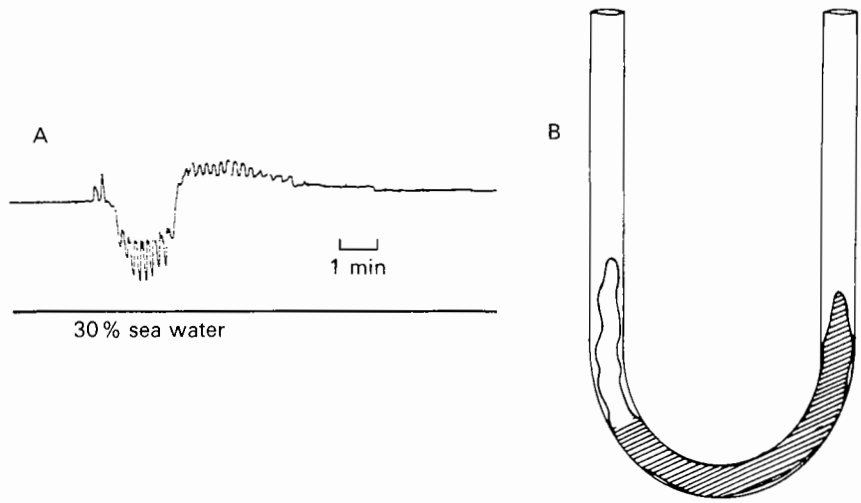


Fig. 7. Trace recorded during sampling excursion of *A. marina* exposed to decreased salinity and the position of the worm in the glass U-tube at the time of sampling.

Table 1 compares the activity cessation and onset concentrations in *A. marina* exposed to a 100–30% sea-water sinusoidal salinity regime in sand burrows with animals exposed to the same regime, but in glass U-tubes. It can be seen from the table that cessation and onset of activity occurred at higher concentrations of surface sea water in animals placed in U-tubes than in animals placed in sand burrows.

*Osmotic and ionic concentrations*

Table 2 shows the $Na^+$, $K^+$, $Ca^{2+}$, $Mg^{2+}$ and $SO_4^{2-}$ and NPS concentrations together with the osmolality and pH values for Menai Strait sea water and the coelomic fluid of *A. marina*. The osmotic and monovalent ion concentrations were higher than those of the surrounding sea water, while the divalent ions were slightly less concentrated in coelomic fluid than in the surrounding sea water. The coelomic fluid is more acidic than the sea water.

Figs. 9 and 10 show the changes in osmotic and ionic concentrations of *A. marina* coelomic fluid exposed to gradual and abrupt salinity changes respectively. Both burrowed and non-burrowed animals were used in the experiments and it can be seen that only exposed, non-burrowed animals showed fluctuations of the osmotic and ionic concentrations of their coelomic fluid. During fluctuating salinities, the coelomic fluid was always hyperosmotic and hyperionic to the external medium. Burrowed animals showed no significant change throughout the salinity profiles.

*Ninhydrin positive substances and free amino acids*

Figs. 11 and 12 show the changes of concentration of NPS in muscle tissue of *A. marina* exposed to 30% sea-water minimum gradual and abrupt salinity regimes respectively. Figs. 11 (gradual salinity change) shows a marked decrease in NPS initially followed by a steady increase in non-burrowed animals Fig. 12 (abrupt salinity change) however, shows a rapid decrease in NPS followed by an increase during the 6 h exposure

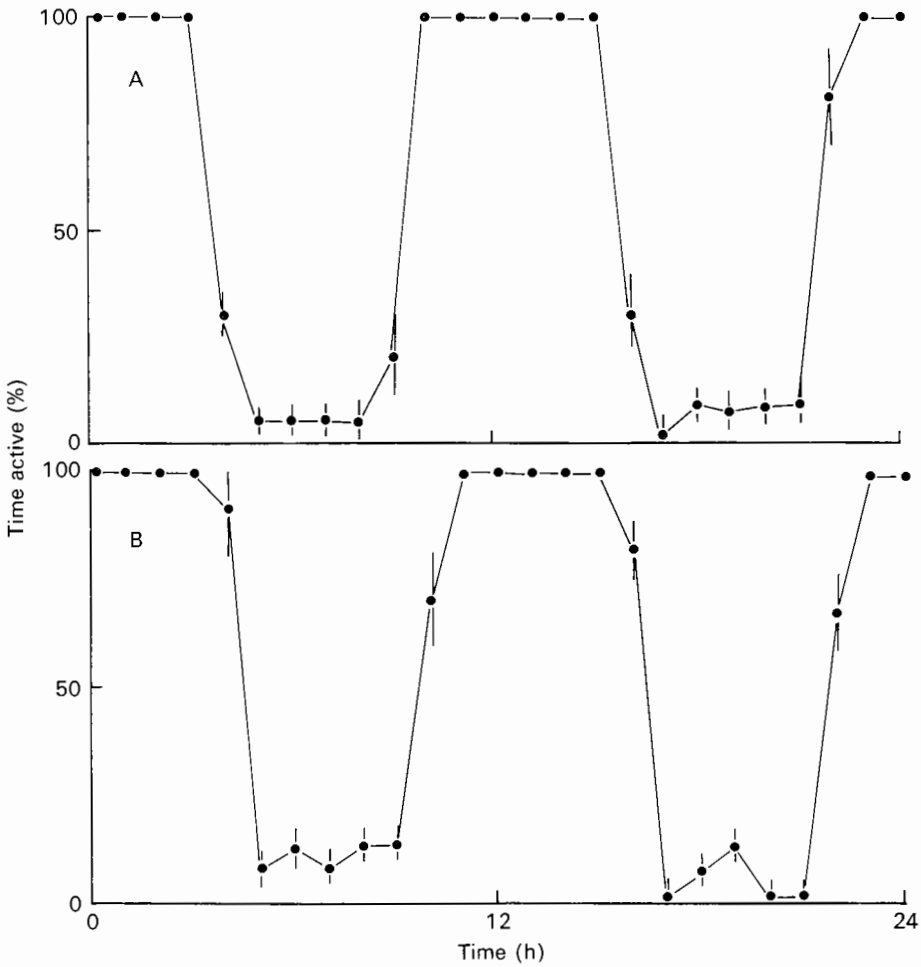


Fig. 8. Changes in the % time active of *A. marina* exposed to gradual (A) and abrupt (B) salinity fluctuations. Each point is a mean of six animals. Error bars represent 95 % confidence limits.

Table 1. *A comparison of activity cessation and onset concentrations in* A. marina *exposed to different salinity regimes*

| Regime | 1st cessation | 1st onset | 2nd cessation | 2nd onset |
|---|---|---|---|---|
| 100–30 % sinusoidal (sand burrow) | 58·6 | 64·0 | 54·3 | 50·4 |
| 100–30 % sinusoidal (glass U-tube) | 68·4 | 70·1 | 66·0 | 72·3 |

to 100 % sea water, again in non-burrowed animals. This is followed again by a decrease during low salinity exposure. It should be noted that in neither regime did the NPS concentration return to its original value after the animals had been exposed to dilute sea water. There was no change in the concentration of NPS in burrowed *A. marina* exposed to either the gradual or the abrupt regime.

The FAA composition of normal *A. marina* muscle and coelomic fluid together with

Table 2. *The $Na^+$, $K^+$, $Ca^{2+}$, $Mg^{2+}$, $SO_4^{2-}$, osmotic and NPS concentrations and pH of Menai Strait sea water and the coelomic fluid of* A. marina*

| | m-osmole | $Na^+$ | $K^+$ | $Ca^{2+}$ | $Mg^{2+}$ | $SO_4^{2-}$ | pH | NPS |
|---|---|---|---|---|---|---|---|---|
| Menai Straits | 965 ± 5 | 465 ± 7 | 9·8 ± 0·6 | 10·0 ± 0·4 | 52·1 ± 1·0 | 28·1 ± 1·0 | 7·90 ± 0·03 | — |
| *A. marina*, coelomic fluid | 1047 ± 11 | 468 ± 9 | 10·0 ± 0·4 | 9·9 ± 0·3 | 51·9 ± 1·1 | 27·3 ± 1·1 | 6·70 ± 0·05 | 61·30 ± 5·30 |

* Osmolality values, m-osmole/kg $H_2O$; ionic values, mM; NPS values, μM/ml.

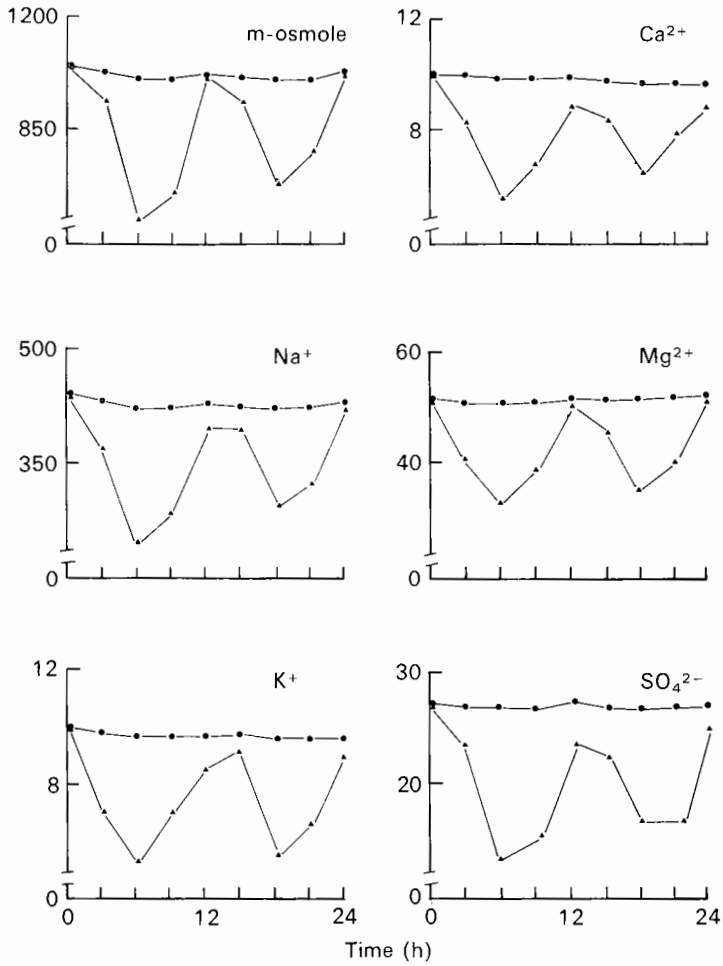


Fig. 9. The changes in coelomic fluid osmolality and $Na^+$, $K^+$, $Mg^{2+}$, $Ca^{2+}$ and $SO_4$ concentrations of burrowed (●) and non-burrowed (▲) *A. marina* exposed to a 30% sea-water minimum sinusoidal salinity regime. Each point is a mean of five animals. Error bars at the 95% confidence level are smaller than the actual points.

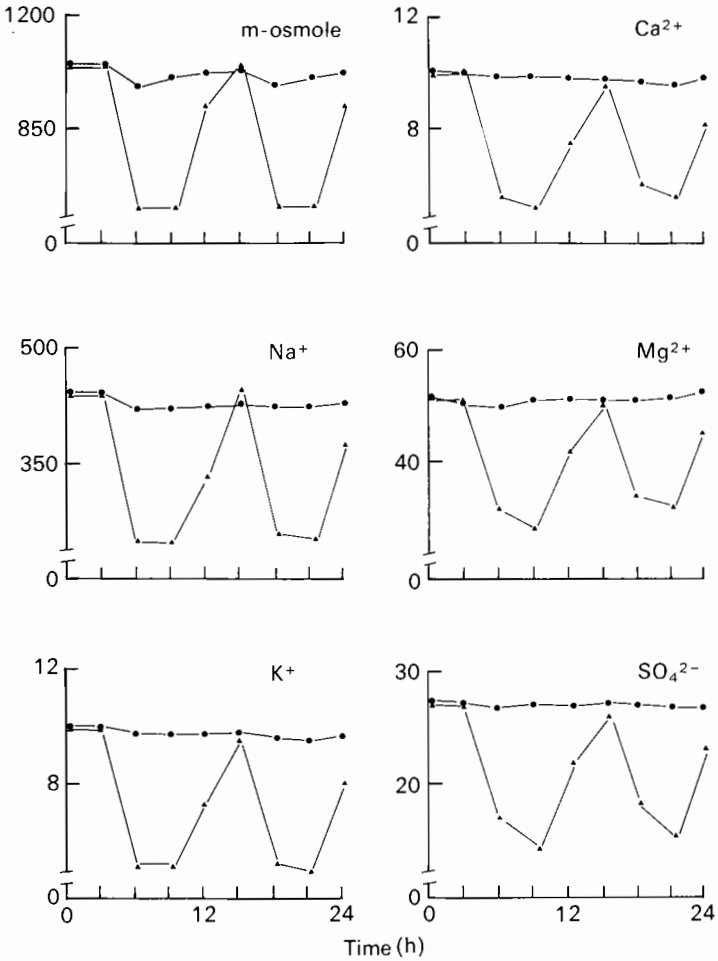


Fig. 10. The changes in coelomic fluid osmolality and $Na^+$, $K^+$, $Mg^{2+}$, $Ca^{2+}$ and $SO_4$ concentrations of burrowed (●) and non-burrowed (▲) *A. marina* exposed to a 30% sea-water minimum abrupt salinity regime. Each point is a mean of five animals. Error bars at the 95% confidence level are smaller than the actual points.

the FAA composition of animals exposed to fluctuating salinity are given in Table 3. It can be seen that alanine and glycine together make up 80% of the FAA pool in the muscle in both normal and experimental animals and only 39% of the FAA pool in the coelomic fluid. After 6 h in the 30% sea-water minimum sinusoidal salinity regime the FAA concentration decreased by 30% and this decrease was due primarily to a loss of alanine and glycine.

*Tissue water*

Non-burrowed *A. marina* exposed to both gradual (Fig. 13) and abrupt (Fig. 14) salinity fluctuations showed significant changes in tissue water content. During periods of decreasing salinity there was an increase in tissue water content and likewise, a decrease in tissue water during periods of increasing salinity. Burrowed animals, how-

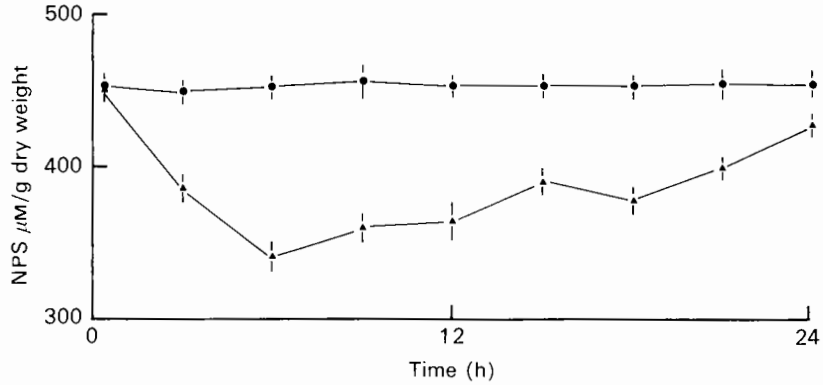


Fig. 11. Changes in muscle tissue NPS in burrowed (●) and non-burrowed (▲) *A. marina* exposed to a 30% sea-water minimum sinusoidal salinity regime. Each point is a mean of three animals. Error bars represent 95% confidence limits.

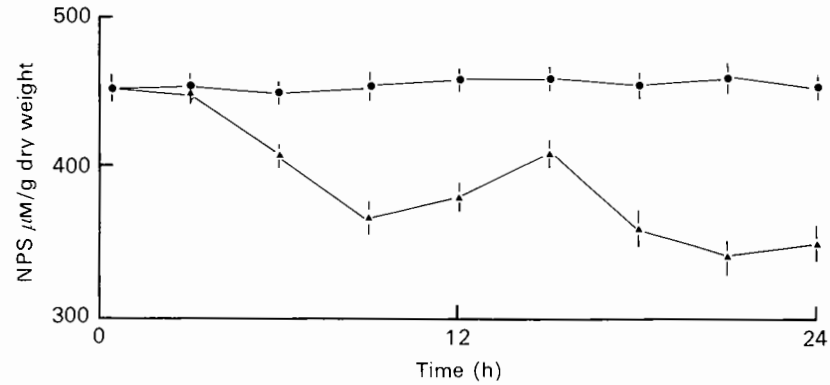


Fig. 12. Changes in muscle tissue NPS in burrowed (●) and non-burrowed (▲) *A. marina* exposed to a 30% sea-water minimum abrupt salinity regime. Each point is a mean of five animals. Error bars represent 95% confidence limits.

ever, showed no significant changes in tissue water content in either the gradual or the abrupt profile.

*pH and buffering capacity of the coelomic fluid*

There was no significant difference between the pH value of the coelomic fluid of burrowed and non-burrowed animals in 100% sea water (6·65–6·75).

Figs. 15 and 16 show the changes in *A. marina* coelomic fluid pH during exposure to gradual and abrupt salinity fluctuations respectively. There were significant changes in pH in the burrowed and the non-burrowed animals. The highest pH values occurred during periods of low salinity.

The curve of buffering capacity of *A. marina* coelomic fluid is given in Fig. 17. The buffering capacity between pH 4·5 and 7·5 is about 8 m-equiv/l/pH unit. This value is the same as that obtained by Clark (1964) for the coelomic fluid of *Nephtys hombergi*.

Table 3. *Free amino acid and ammonia levels in non-burrowed* A. marina

Results are expressed as $\mu$M/g dry weight of tissue.

| | Initial Body wall | Coelomic fluid | Hour 6* Body wall |
|---|---|---|---|
| Lys | 2·51 | 3·31 | 1·46 |
| His | 1·48 | 0·79 | 1·75 |
| Arg | 18·30 | 1·79 | 5·25 |
| Tau | 21·26 | 0·40 | 17·37 |
| Asp | 3·89 | 1·94 | 15·17 |
| Thr | 2·03 | 1·18 | 1·05 |
| Ser | 2·58 | 1·52 | 3·29 |
| Glu | 2·20 | 1·76 | 2·60 |
| Pro | 1·72 | 0·87 | 1·02 |
| Gly | 193·50 | 14·50 | 139·82 |
| Ala | 112·87 | 6·43 | 66·74 |
| Cys | — | — | — |
| Val | 2·06 | 2·46 | 0·41 |
| Met | — | 1·02 | — |
| Ileu | 1·27 | 1·67 | 0·28 |
| Leu | 1·68 | 3·35 | 0·65 |
| Tyr | 0·79 | 1·35 | — |
| Phe | 1·31 | 1·72 | — |
| $NH_3$ | 13·86 | 7·16 | 11·14 |
| Total | 383·31 | 53·22 | 268·00 |

* Sample taken at hour 6 of 30 % s.w. minimum sinusoidal salinity regime.

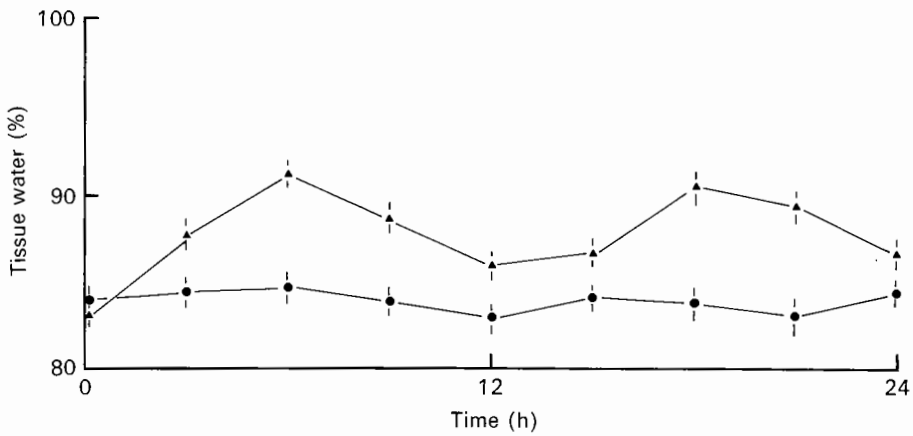


Fig. 13. Changes in tissue water content of burrowed (●) and non-burrowed (▲) *A. marina* exposed to a 30 % sea-water minimum sinusoidal salinity regime. Each point is a mean of five animals. Error bars represent 95 % confidence limits.

## DISCUSSION

### *Activity*

Behaviour patterns of *A. marina* have been examined and discussed in great detail by Wells (1944, 1949*a*, *b*, 1961). He studied respiratory movements of worms housed in glass tubes and found that periods of irrigation alternated regularly with periods of rest. Irrigation outbursts were shown to consist of three phases: (*a*) tailward locomotion, (*b*) headward irrigation and slow headward creeping, (*c*) tailward irrigation. Wells found

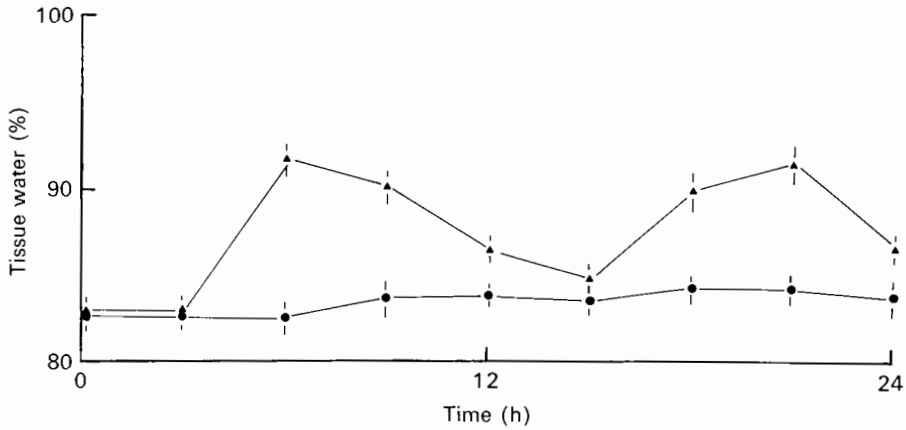


Fig. 14. Changes in tissue water content of burrowed (●) and non-burrowed (▲) *A. marina* exposed to a 30% sea-water minimum abrupt salinity regime. Each point is a mean of five animals. Error bars represent 95% confidence limits.

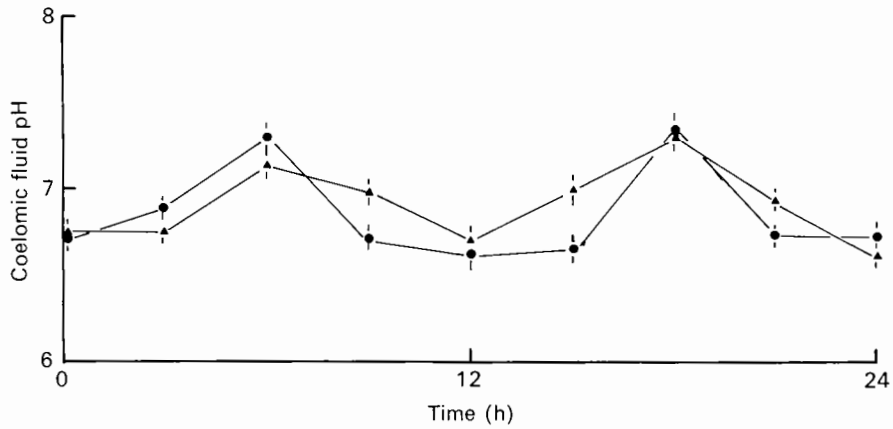


Fig. 15. Changes in the coelomic fluid pH of burrowed (●) and non-burrowed (▲) *A. marina* exposed to a 30% sea-water minimum sinusoidal salinity regime. Each point is a mean of five animals. Error bars represent 95% confidence limits.

that these three phases always follow each other in that order, but points out that their relative prominence is variable, the second stage being the most conspicuous in well oxygenated water. Wells also suggested that the intermittance was intrinsic, i.e. produced solely by conditions internal to the worm. He further states that the pacemaker for irrigation cycles is in the ventral nerve cord and that the presence of a pacemaker might have survival value if, at low tide, the burrow was covered by surface water which was uncongenial to the worm.

A typical trace of the normal irrigation pattern of *A. marina* as measured by the pressure transducer is shown in Fig. 4. The two large peaks, indicated by arrows, are the result of tailward locomotion followed by small, regular peaks representing the headward irrigation and slow headward creeping previously described by Wells (1949). Each small peak represents a muscular contraction. These were observed in the animals placed in U-tubes and found to be extremely regular (approximately 7 contractions/min). In

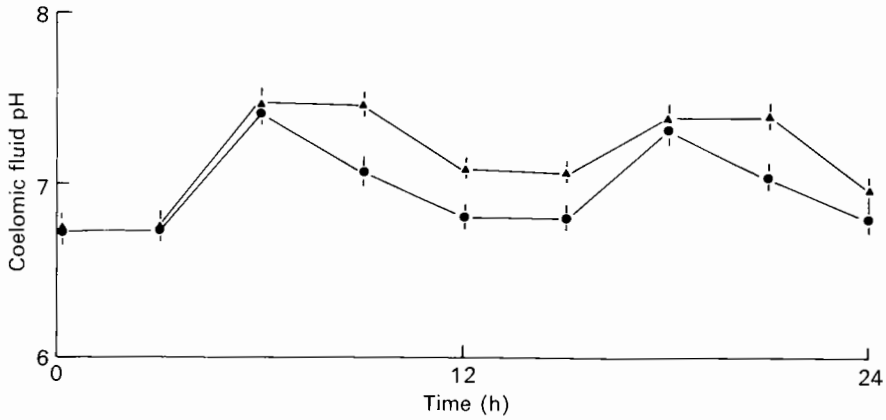


Fig. 16. Changes in the coelomic fluid pH of burrowed (●) and non-burrowed (▲) *A. marina* exposed to a 30 % sea-water minimum abrupt salinity regime. Each point is a mean of five animals. Error bars represent 95 % confidence limits.

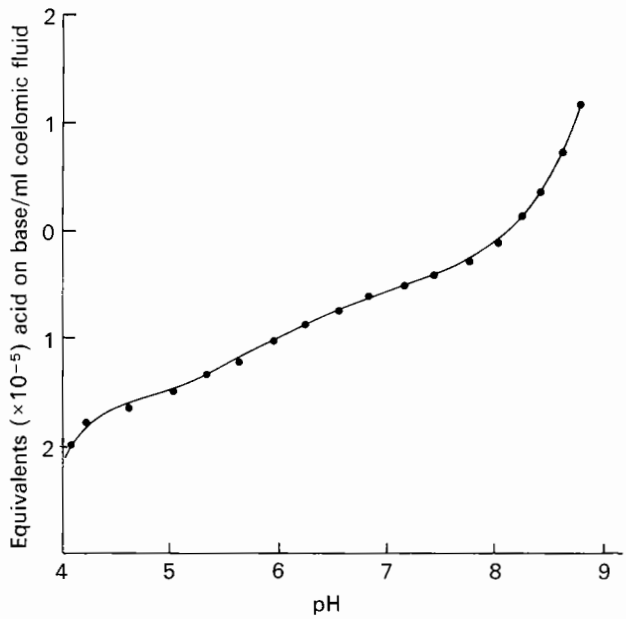


Fig. 17. Curve of buffering capacity of *A. marina* coelomic fluid. 100 $\mu$l diluted in 10 ml 0·5 M-NaCl and titrated with $10^{-3}$ N-HCl and NaOH.

contrast with Wells' work, there were no periods of 'rest', the pattern of activity remaining as long as the animals were exposed to aerated, full strength sea water. The behaviour patterns exhibited during the fluctuating salinity regimes are in line with the findings of Wells & Ledingham (1940) who also found that all activity ceased in low salinity conditions.

Wells (1949*b*) states that under conditions which may arise from time to time in the field, intermittent irrigation cycles based on a 'clock' would have a greater survival

value than those based on reflex responses to respiratory needs. If the respiratory movements of *A. marina* were governed solely by a 'clock', it is conceivable that the animal could be pumping water of a noxious nature simply because the 'clock' was turned on. It seems more likely that activity during periods of exposure to a favourable external medium is probably governed by a pacemaker, but that this pacemaker can be 'turned off' if the animals are exposed to unfavourable external media. The ability to detect and avoid a noxious external medium behaviourally is of great importance to an animal such as *A. marina* which is likely to be exposed to great variations in salinity in its natural, estuarine environment and which has no physiological means of regulating the osmotic or ionic concentration of its internal medium. The act of compressing itself at the bottom of the burrow and waiting for more favourable external conditions is akin to the mechanism of shell valve closure employed by bivalve molluscs exposed to a similar, noxious environment (Shumway, 1977*a*).

*Osmotic and ionic composition*

Several authors (Schlieper, 1929; Beadle, 1937; Zenkevich, 1938; Robertson, 1949) have shown that *A. marina* body fluids are isotonic with the external medium in full-strength sea water. Table 1 shows this to be the case in the present study as well. Leersnyder & Glaçon (1974) and Freeman & Shuttleworth (1977) have confirmed that *A. marina* is an isosmotic conformer in dilute sea water. It is not surprising, therefore, that in this study non-burrowed *A. marina* exposed to fluctuating salinities showed no evidence of ionic or osmotic regulation. The osmotic and ionic concentration of the coelomic fluid from non-burrowed worms exposed to fluctuating salinities followed the same pattern of change as that of the external medium, whereas the osmotic and ionic composition of the coelomic fluid from burrowed worms exposed to the same salinity fluctuations remained constant.

Clearly the main mechanism for protecting the lugworm against severe osmotic changes is its habit of remaining quiescent at the base of its tube. A secondary factor might arise by admixture of, and/or diffusion of salt from, the saline interstitial water into the lumen of the tube. This is suggested by the fact that animals in glass tubes, where no such admixture can occur went into the quiescent state at surface salinities higher than for the animals in sand burrows.

All previous studies on the osmotic and ionic tolerance of *A. marina* in dilute sea water have been carried out using exposed worms. This is not a situation likely to be experienced by the worms in their natural habitat and as seen in Figs. 9, 10, 13, 14 burrowed worms are not affected by osmotic and/or ionic changes of the external medium. The results indicate that *A. marina* is an osmotic and ionic conformer, but that it can control the concentration of its body fluids by remaining inactive at the bottom of its burrow during periods of stress.

*Ninhydrin positive substances and free amino acids*

The available information on the amino acid level of annelids is sparse and contradictory. Clark (1968*a*, *b*) gives values for the amino acid nitrogen (AAN) levels in the body wall of several species of polychaetes ranging from 186·3 mg AAN/100 g $H_2O$ in

*Aphrodite japonica* to 682·2 mg AAN/100 g $H_2O$ in *Thelepus crispus*. This is a range of 88·8 $\mu$M/g $H_2O$ to 327 $\mu$M/g $H_2O$. Abbott & Awapara (1960) give a value of 41·68 $\mu$M/g fresh weight for *A. marina* while Virkar (1966) gives a value for *Golfingia gouldii* of 550 $\mu$M/g tissue $H_2O$. Yet another value is given for *A. marina* by Duchâteau-Bosson, Jeuniaux & Florkin (1961) of 2795 mg AA/100 g fresh tissue. This is equivalent to 13·35 $\mu$M/g $H_2O$. Cowey & Shaw (unpublished) give still another value for *A. marina* of 431 mM/kg water (431 $\mu$M/g $H_2O$). Thus for one species alone (*A. marina*) reported values range from 41·68 $\mu$M/g fresh weight to 431 $\mu$M/g $H_2O$. If we assume a tissue water content of approximately 85 %, this is a range of approximately 280–2875 $\mu$M/g dry weight of tissue. Clark (1968) reported that she found 'considerable' variation within individual species, but did not give the actual range of values. In this study, the NPS concentration of the body wall was found to be 449 $\mu$M/g dry weight, while that of free amino acids (independent analysis done at IMB) was 383·31 $\mu$M/g dry weight. This discrepancy between NPS and FAA is probably accounted for by the presence of other substances such as betaine, trimethylamine oxide, homarine, glycine betaine, $\gamma$-butyrobetaine, carnitine and trigonelline, all of which have been shown to occur in annelids, sometimes in considerable quantities (Ackermann, 1955*a*, *b*; Beers, 1967).

Table 3 shows the free amino acid and ammonia levels of *A. marina*. It can be seen that in muscle tissue glycine + alanine make up 80 % of the total pool, while arginine + taurine + ammonia make up another 14 %. This is interesting, as Duchâteau-Bosson *et al.* (1961) report that taurine is absent in *A. marina*. Glycine + alanine make up only 39 % of the total pool in the coelomic fluid.

Clark (1968) found that most of the polychaetes she studied showed only a limited ability to lower their tissue AAN levels during osmotic stress, at least during the first few days. As seen in Figs. 11 and 12 such was not the case in this study. Animals exposed to decreasing salinities lost FAA within hours. Although non-burrowed *A. marina* did show a decrease in the concentration of NPS and in the FAA pool when exposed to salinity fluctuations, it is not likely that the animals ever show this response in the natural environment, as seen in Figs. 11 and 12, where it can be seen that burrowed animals showed no changes in NPS concentration during fluctuating salinity changes.

*Tissue water*

In both the gradual and abrupt salinity regimes, exposed *A. marina* showed a return to a hydration level higher than the original 100 % sea-water control level after a period of exposure to dilute sea water but there were no significant changes in the tissue water content of burrowed animals subjected to similar treatments.

*pH and buffering capacity of the coelomic fluid*

The coelomic fluid pH of both burrowed and non-burrowed *A. marina* exposed to gradual and abrupt salinity profiles showed an inverse relationship to the salinity change. The value for animals in full strength sea water is 6·65–6·75. This is slightly lower than the value of 7·43 reported for *A. cristata* (Mangum & Shick, 1972). They found that the pH of *A. cristata* decreased with time to a value of 6·58 after 45 min and apparently complete deoxygenation. Although it is possible that the coelomic fluid in this study was

deoxygenated before the readings were taken, it is unlikely since all readings were taken immediately after sampling.

The buffering capacity for the coelomic fluid of *A. marina* is shown in the curve of Fig. 17. Clark (1964) measured the buffering capacity of the coelomic fluid of *Nephtys hombergi* and found it to be about 8 m-equiv/l/pH unit. The same value was obtained for the coelomic fluid of *A. marina* in this study. It has been shown (Jones, 1955) that during long periods of tidal exposure the oxygen supply is insufficient to maintain respiration and that *Nephtys* accumulates lactic acid when kept at low oxygen tensions in the laboratory (Clark, 1964) indicating active glycolysis. Thus, the high buffering capacity of the coelomic fluid is to be expected (Clark, 1964). From the activity data presented here and preliminary oxygen consumption studies with *A. marina* exposed to fluctuating salinities, it is probable that *A. marina* does not maintain respiration during periods of low salinity. This could account for a decrease in pH of the coelomic fluid in both burrowed and non-burrowed animals during periods of low salinity and nor activity since acidic end products of anaerobic metabolism would accumulate. However, in this study it has been shown that the pH increases, indicating increased alkalinity of the coelomic fluid. One possible explanation for this phenomenon is an increase in the $NH_3$ concentration as a result of NPS and FAA being extruded from the muscle tissue into the coelomic fluid. This would account for the rise in pH of non-burrowed worms, but as previously pointed out there was no significant loss of NPS during low salinity in burrowed worms.

Duchâteau-Bosson *et al.* (1961) have pointed out that, in more or less euryhaline marine invertebrates, the degree of euryhalinity is accounted for, either by an adjustment of the intracellular concentration, a regulation in which free amino acids are involved or an osmoregulation of the blood and of an adjustment of the intracellular concentration. They cite *Mytilus* and *Arenicola* as examples of the first category. Shumway (1977*a*, *b*, *c*) has shown that in bivalve molluscs ranging from the sublittoral to the highly estuarine environment the behavioural mechanism of shell valve closure and/or siphon retraction is by far the more important factor in limiting the distribution of these animals. If a physiological mechanism for osmoregulation is absent, it is necessary for the animals to have developed a behavioural mechanism if they are to survive in constantly changing environments such as estuaries. It is shown here that for the annelid, *A. marina*, the burrowing habitat provides adequate protection against a cyclical changing external environment and that ionic, osmotic and intracellular regulation are processes which the worm probably never employs in its normal environment.

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